INFLAMMATORY CYTOKINE PROFILES IN HIV/AIDS PATIENTS WITH HYPERTENSION COMORBIDITY AT KENYATTA NATIONAL HOSPITAL COMPREHENSIVE CARE CENTER.

ANGELINE CHEPCHIRCHIR

Reg. /No. W80/49941/2015

A THESIS SUBMITTED TO THE UNIVERSITY OF NAIROBI, INSTITUTE OF TROPICAL AND INFECTIOUS DISEASES, IN PART FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF THE UNIVERSITY OF NAIROBI.

DECEMBER 2019

DECLARATION

I Angeline Chepchirchir, declare that this is my original work and has not been presented anywhere for academic or other awards.

Sign_____

Date_____

SUPERVISORS' APPROVAL.

This thesis has been written and submitted as a true account of the candidates' own original work. The candidate through our supervision and mentorship support carried out research and thesis writing with due diligence.

1. Dr. Joshua Nyagol, PhD

   Senior Lecturer,

   Department of Pathology,

   University of Nairobi.

   Email: Joshua.nyagol@uonbi.ac.ke

   Signature_____ Date _____

2. Prof. Walter Jaoko, MBCHB, M.Med, PhD

   Director,

   KAVI Institute of Clinical Research (KAVI-ICR)

   University of Nairobi

   Email: Wjaoko@KAVIUON.org

   Signature._____Date._____

DEDICATION

This work is dedicated to my family: Gideon and our children; Debra, Diana, Daisy and Allan for their patience and encouragement during the entire period of these studies.

Also, to my parents, Magdalena and late grandmother (Martha), who belabored to educate me in my formative years and for not giving up even in the most trying moments.

May the Almighty God bless you all.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to all those who supported me in the course of these studies; Course work and Research. Special thanks go to:

1. My Supervisors; Dr. J. Nyagol and Prof. W. Jaoko for their unraveled academic advice and mentorship throughout the PhD programme.
2. University of Nairobi for granting me tuition fees waiver for the entire period of study
3. Consortium for Advanced Research Training in Africa (CARTA) for sponsoring academic seminars and subsistence support through stipend and partial sponsorship for research work
4. BD laboratory support center in Nairobi, for their professional handling of procurement process for laboratory reagents and direct expert support in bench work.
5. Research assistants that included Vincent Mayaka for their time and dedication
6. Laboratory Staff of CCC, KNH.
7. Desmond for data analysis
8. My Cohort 3 CARTA fellows for moral support
9. School of Nursing Sciences, UON, for professional support.

TABLE OF CONTENTS

List of Tables

List of figures

List of abbreviations.

HIV	Human immunodeficiency virus
ACEI	Angiotensin Converting enzyme Inhibitor
AIDS	Acquired immunodeficiency syndrome
ARB	Angiotensin II receptor blockers
BB	Beta Blockers
BMI	Body Mass Index
cART	Highly Active Antiretroviral Therapy
CCB	Calcium Channel Blocker
CD3	T- Cell Receptor Complex Component
CD4	a marker of Helper T Lymphocytes
CD8	a marker of Cytotoxic T Lymphocytes (CTLs)
CRP	C-Reactive protein
CVA	Cardiovascular Accident
CVD	Cardiovascular Disease
DASH	Dietary Approaches to stop Hypertension
DBP	Diastolic blood pressure
HBPM	Home blood pressure measuring

HCT	Hematocrit
HTN	Hypertension
IF	Interferon
IG	Immunoglobulin
IL	Interleukin
IRIS	Immune Reconstitution Syndrome
PLWH	People Living with HIV infection
SBP	Systolic Blood Pressure

Definition of terms

HIV-a retrovirus that attacks the CD4+ T lymphocytes resulting in immunodeficiency

AIDS- Immunosuppressive state marked by low CD4+ cell count that is less than five hundred (<500) copies per ml of blood

Hypertension- disorder marked by increased diastolic pressure more than 90mmHg and Systolic pressure that is more than 140mm/Hg.

Cytokines- chemical messengers of the immune system released by activated macrophages in response to infection or chemical injury.

Selected cytokines in this study refers to: (IL -17A, IL-2, IL-4, IL 6, IL-8, IL-10, TNF-α and INF- γ)

Inflammation – a series of reactions that bring cells and molecules of the immune system to sites of infection or damage.

Co-morbidity- the occurrence of two ailments concurrently in an individual giving rise to mixed presentation of signs and symptoms and deterioration of body functions.

Biomarker- a protein molecule released into blood as an indicator of inflammation.

Interleukin- a molecule that controls the immune system responses in the body.

Interferon- a biomolecule that triggers inflammatory reaction in tissues

Reactive protein- a biomolecule that is released by endothelial walls of the blood vessels and triggers inflammatory response in tissues

Immunoglobulin-a neutralizing molecule released by the B lymphocytes in response to an antigen

Cardiovascular Disease-any form of disorder that manifests with altered cardiovascular function(s).

Cardiovascular Events- includes; Coronary heart disease, non- fatal myocardial infarction, Stroke

Atherosclerosis-narrowing of the blood vessels due to factors such as arterial hardening and lipid deposition

Stroke- the rupture of blood vessels in the brain tissue resulting in increased intracranial pressure due to hematoma formation

Abstract.

Background. Hypertension is the leading risk factor of Cardiovascular complications in HIV/AIDS patients and poses a threat to their survival, quality of life and economic productivity. The high predisposition to developing hypertension is associated with complex pathology that is linked to HIV virus inflammation. Chronic inflammation is responsible for most of the pathophysiology and is maintained by resurgence of the immune mechanisms following viral suppression a resultant effect of antiretroviral therapy. Inflammation has been linked independently as a potential causative factor of hypertension and driver of the disease pathophysiology. Less than half of HIV/AIDS patients with hypertension are diagnosed and further few are on treatment and well controlled. Some patients are diagnosed late with asymptomatic hypertensive urgency with very high readings e. g (BP>180mmhg/>110mmhg). Efforts to combine HIV treatment with vascular disease risk assessment are urgently needed to address hypertensive co-morbidity in HIV-positive persons. Thus, there is need to identify potential markers of inflammation that could be used to diagnose increased risk of developing hypertension in this population for effective prevention and control of the disorder.

Objective: To determine the risk indicators of hypertension and variation in selected plasma cytokines in hypertension comorbidity in HIV/AIDS patients

Methods: A total of 297 adult patients were recruited using systematic sampling. This was a cross-sectional study and data collection was carried out using questionnaires and desktop review of medical records as well as flow cytometry assay for selected cytokines, (IL-2, IL-4, IL-6, IL-8, IL-10, IL-17A, IFN-y and TNF-α) in participants' blood samples. The study was carried out between January 2015 to August 2016. Ethical approval was obtained from Joint University of Nairobi and Kenyatta National Hospital Ethics and Research Committee (UON-KNHERC). Informed consent was obtained from participants with assurance of confidentiality

in handling of data. Data was analyzed using STATA ^{TM} version 22 to establish inferential statistical correlations between the independent and dependent variables. Statistical significance was set at 95% confidence interval with 0.05 α level of significance

Results: Out of the total 297 participants, 89 (30%) were male, while 208 (70%) were female. The age of the participants ranged from 30 – 57 years (M = 42.97, SD = 6.21). An independent-sample t test revealed that the average age of males (M = 44.56, SD = 6.05) was higher than females (M = 42.29, SD = 6.16), t (295) = 2.922, p < .01.

The prevalence of hypertension was 69(23.2%)**.** A high CD4+ cell count level above 250 showed a positive association with BMI values and the same CD4+ cell count within the range of 200 and below presented a negative association with BMI indicating wasting syndrome. This study showed that gender may be a key factor that influences secretion patterns and levels of IL-17A in HIV/AIDS patients

The relation between CD4 counts and creatinine was statistically significant, F (1, 270) = 6.684, R^2 = .024, β = -0.155, n = 272, p < .01. Also, the positive association between CD4 counts and BMI using linear regression was statistically significant, F (1, 295) = 9.321, R^2 = .031, β = 0.175, n = 297, p < .01. Regression between gender and IL-17A showed a positive association (r_s(124) = 0.417, p < 0.001). From this study female participants were likely to register increased IL-17A expression than male counterparts. Regression between CD4+ cell counts, creatinine and IL-6 showed a mild, negative and statistically significant association(r_s(124) = -0.268, 0.285; p < 0.05) respectively. Regression between IL-6 and IL-8 showed a strong, positive and statistically significant association (r_s(124) = 0.917, p < 0.001). However, the association between alcohol, tobacco use and IL-8 among participants was moderate, (r_s(124) = 0.360, p < 0.01). INF γ levels between hypertensive and non-hypertensive participants were different with the latter showing higher values, p=0.003.

Conclusion

The HIV positive patients had a high prevalence of hypertension associated with statistically significant risk factors which include prolonged use of ART and increased body mass index. Hypertension is associated with HIV progression marked by lowered CD4+cell counts.

The present study showed that interleukins IL (17A, 8) and INF ɣ), are highly expressed in patients with hypertension comorbidity in HIV/AIDS patients. IFN-γ levels may independently predict the risk of hypertension for HIV/AIDS patients on treatment and may also be considered as a potential risk indicator.

Where possible, integrating specific cytokine assessment as part of screening for risk of developing hypertension while on HIV/AIDS care, may go a long way in the prevention efforts. Therefore, routine measurement of these cytokines may increase prediction of risk of developing hypertension in patients on HIV care resulting in early interventions and reduced complications.

Key words: HIV/AIDS, Hypertension, Cytokines, HIV/AIDS Progression, Comorbidity.

CHAPTER ONE: BACKGROUND INFORMATION

1.1. Hypertension and HIV/AIDS interaction

Hypertension is the leading cardiovascular disorder in HIV/AIDS patients [1]. Some studies have documented its prevalence ranging from 16% to 36% [1,2,3]. It is a non- communicable disease of immense significance in the world today associated with high morbidity and mortality [4]. It contributes to high prevalence of cardiovascular complications among the HIV/AIDS patients and is associated with premature deaths [1]. Compared to the general population, inflammatory sequelae and side effects of ARV's are additional factors of association in HIV seropositive population. However, changes have been made on the composition of ART to reduce side effects that contributed to lipodystrophy and metabolic syndrome, but still the HIV/AIDS population remains largely predisposed to hypertension. It is a consequence of atherosclerotic cardiovascular pathology [5]. This observation is attributed to a combination of known risk factors compounded by chronic HIV/AIDS related inflammation [2]. HIV is a primary disorder that is treated, but not cured, leaving underlying pathogenic processes to continue and increase vulnerability to other comorbidities. Lifestyle factors too play a role as the infected patients are more worried of body wasting and their general physical appearance. Most of them take high quantities of high nutrient value foods such as organ meats and take less physical activity. This has seen most of them present with high BMI ranges that predispose them to development of hypertension [6]. Less than half of HIV/AIDS patients with hypertension are diagnosed and fewer are on treatment and well controlled. Some may be diagnosed late with asymptomatic hypertensive urgency with BP readings as high as (>180mmhg/>110mmhg) [5].

1.2. Chronic inflammation and HIV/AIDS

HIV infection is associated with chronic inflammation due to persistent HIV viral replication and immune reconstitution following initiation of ART. This contributes to pathophysiologic changes in arterial blood vessels giving rise to atherosclerosis. This is further compounded by uncontrolled inflammation marked by elevated cytokines that cause dysfunction in many tissues and organs as seen in chronic diseases [6-8]. Uncontrolled inflammation is associated with destruction of affected mucosal linings and arterial walls by activated macrophages. For example, pulmonary hypertension presents with infiltration of blood vessels with inflammatory cells and increase in proinflammatory cytokine expression [9]. The lymphoid tissue is infiltrated with interleukin 1, a pro-inflammatory molecule whose effects in tissues worsen with the use of antiretroviral treatment [10]. This phenomenon is associated with lack of T cell activation and immune reconstitution. Introduction of corticosteroids to counter adverse effects of inflammation due to IRIS may be beneficial to the HIV patient as part of care [5, 2].

1.3. Cytokines and HIV inflammation

Cytokines are important molecules that regulate the homeostatic balances in relation to immune response and metabolism [24]. High levels of inflammatory markers highly predict increased mortality and other adverse events in a disease state [8]. A detailed cytokine analysis showing different types and levels in plasma is predictive of long term HIV disease progression and prognosis [8]. Chronic inflammation marked by infiltration of inflammatory cells on the vascular endothelium, increased proinflammatory cytokine levels and growth factors have been documented in pulmonary arterial hypertension [11]. Persistent inflammation results in complications and occurrence of adverse cardiovascular events that contributes to non-AIDs related deaths in HIV/AIDs patients [12]. The presence of certain biomarkers such as CRP and

other vascular endothelial adhesion molecules predict the risk and prognosis of cardiovascular disease [13].

However, HIV infection is known to trigger and facilitate chronic interleukin 6 production that sustains inflammation despite antiretroviral therapy [14]. HIV structural proteins induce chronic polyclonal B cell activation resulting in the release of several pro-inflammatory cytokines whose persistent effects predispose the patients to development of arteriosclerosis [15]. The HIV virus infection deregulates the immune functions and alters the cytokine profiles [16]. The changes result in cytokine dysregulation that can be measured to establish the level of inflammation and predict the progression of HIV disease [17]. The sustained HIV replication and immune suppression in the infected patients in turn significantly contributes to elevated levels of pro-inflammatory cytokines which target viral suppression. Low pro inflammatory cytokine levels reduce the target points for the anti-retroviral agents and enables the persistence of the virus in lymphoid tissues leading to high viral load [18].

Co-morbidities are a common feature with HIV infection due to its pathophysiology that is marked by chronic systemic inflammation and immune reconstitution syndrome [19]. The common link between HIV and hypertension is the expression of high levels of inflammatory biomarkers associated with the two chronic diseases [20]. There is the risk of drug resistant hypertension that is induced by anti-HIV agents, and this is thought to drive high levels of pro-inflammatory cytokines [21]. Inflammatory comorbidity in HIV positive patients is less documented [22]. Inflammation with increased immune response that is marked by increased levels of interleukin 6 in circulation, correlates positively with HIV infection and markers of immune dysfunction [23].

Overweight also may present because of metabolic syndrome and together with hypertension contribute to cardio metabolic disease states [25]. Assessment of body mass index in hypertensive cases is important in primary care of HIV/AIDS patients [9]. The average Body

Mass Index (BMI) in HIV/AIDS patients is generally low compared to the general population but progressive BMI changes is associated with occurrence of chronic disorders such as hypertension [19].

Chronic inflammation in HIV disease is associated with endothelial dysfunction of the cardio vasculature giving rise to reduced elasticity and subsequently high blood pressure [5-7]. This state is marked by increased IL6 cytokine expression as manifested in patients with type 2 diabetes and hypertension [8, 9]. Low dose of omega 3 fish oil use by HIV positive clients have not shown any benefits in reduction of IL6 expression [10]. Elevated IL6 expression in chronic conditions is associated with poor progression and clinical outcomes of patients [11, 12]. Evolution of IL-6 gene polymorphism has been shown to increase risk of cardiovascular disease [13]. It is a marker of endothelial injury and damage to the vasculature [14]. Th17A has been implicated in Large vessel vasculitis (e.g. aorta and its high level expression is a risk factor for vascular damage and associated complications) [15]. Determination of levels of expression of these cytokines for example, IL-6 is useful as a diagnostic marker for risk of Myocardial infarction [16]. Pro-inflammatory cytokines IL-6 and IL-8 measures may be used as an indicator of severity of cardiovascular disease complications such as chronic heart failure [17, 18]. Trigger factors of cardiovascular disease include alcohol abuse which is prevalent with HIV seropositive patients [19]. Interventions that target inhibition of IL-6 have shown benefits of reduced cardiovascular events [20]. Individuals with prehypertension have been shown to have gradual increase in IL-6 expression over a 3 year period with increasing risk of arterial stiffness and arterial fibrillation [21, 22].on the other hand expression of IL-17A may offer a compensatory adaptation of dilated cardiomyopathy to improve function in the event of viral myocarditis [23]. This study sought to establish variation in selected plasma cytokine levels in HIV seropositive patients with hypertension comorbidity and associate with HIV/AIDS progression at Comprehensive Care Center of Kenyatta National Hospital. We

hypothesized that chronic inflammation and presence of hypertension comorbidity presents higher levels of plasma cytokines such as IL-6, IL-8 and IL-17A.

1.4. Problem statement

The effective use of antiretroviral therapy (ART) has reduced AIDS related morbidity due to decreased opportunistic infections, thus prolonging life. However, these patients remain at high risk of non-communicable cardio metabolic disorders, hypertension being the most common. Hypertension often progresses unnoticed in a subset of HIV/AIDS patients with severe complications. Inflammation, which results from IRIS (immune reconstitution syndrome) in patients on ART has been hypothesized as a potential risk factor in development of hypertension in HIV positive patients. Traditionally HIV patients on care have been monitored using CD4+ cell count measurements and Viral load. However, the risk of occurrence of metabolic disorders is not predicted by these clinical parameters. The present study sought to determine risk indicators and variation in plasma cytokines levels to inform the future approach in HIV/AIDS patients' care.

1.5. Study Justification.

As increasing numbers of those infected with HIV receive long-term treatment and are confronted by rise in NCDs, evidence-based intervention strategies are critical to ensure that health gains in these populations made over the past decade are not eroded. One such way is to identify reliable indicators of increased risk to facilitate early detection of hypertension in HIV infected population.

The population with HIV related cardiovascular disease are younger with relatively low cardiovascular event rates of deaths on average compared to the general population. The assessment of risk using the traditional risk factors may not reliably distinguish individuals at risk of cardiovascular disease.

Cardiovascular ultrasound assessment too has shown progressive increase in the carotid intima thickness and amount of plaque in carotid arteries in HIV patients. However, there is lack of consistency in results depending on the geographical setting, study design and ultrasound technique used.

Coronary artery calcium (CAC) scoring approach too does not reliably compare risk of cardiovascular disease in infected patients versus the general population. CAC scoring has been shown to underestimate the amount of atherosclerotic plaque in HIV patients.

Elsewhere, clinical data have shown association between viremia and cardiovascular disease with ART use leading to effective control of HIV replication and improvement of vascular functions. However, ART does not eradicate HIV virus from the body and residual viremia continues to stimulate the immune system and thus persistent inflammation with continued endothelial vascular damage. The immunobiology of atherosclerosis implicates immune activation and actions of macrophages that triggers release of nitric oxidase on mucosal linings of vessels and other chemo attractants. The net effect is the thickening of endothelium of affected vessels giving rise to increased peripheral pressure and eventually hypertension

Currently, there are available therapies that may be used to control inflammation in chronic diseases and by extension reduce occurrence of cardiovascular disease. Use of statins has been proven to reduce risk of developing CVD and control subclinical condition in HIV patients.

However, assessment of risk of cardiovascular disease is not clearly documented and especially in the HIV positive population. The use of cytokines profiles may be a gateway to identify patients at risk and initiate them on statin therapy and other measures to prevent hypertensive disease.

This study sought to determine risk indicators of hypertension and assess and identify variation in cytokines' expression in HIV/AIDS patients with hypertension comorbidity that could be used to assess risk of hypertension, a cardinal factor in cardiovascular disease.

1.6. Scientific questions:

1) What are the risk indicators of hypertension co-morbidity in HIV/AIDS patients on care at KNH CCC?

2) Are the variations in inflammatory cytokines expressions associated with hypertension co-morbidity among HIV/AIDS patients on care at KNH CCC?

1.7. Broad objective

To determine variations in levels and group of plasma cytokines in HIV/AIDS' patients with hypertension comorbidity and explore the risk indicators of hypertension in HIV/AIDS patients at CCC, KNH.

1.8. Specific objectives

i. To establish variation in selected plasma cytokines' levels (IL-2, IL-4, IL-6, IL-8, IL-10, IL-17A, IFN-y and TNF-α) and hypertension co-morbidity in HIV/AIDS patients.

Hypothesis: We hypothesized that hypertensive patients will have higher individual and group mean values of plasma cytokines' levels in comparison with the non-hypertensive patients.

ii. To identify risk indicators of hypertension co-morbidity in HIV/AIDS patients on care.

Hypothesis: We hypothesized that participants with hypertension comorbidity will have higher levels of pro-inflammatory cytokines namely;(IL-17A, IL-6, IL-8, IFN-y & TNF-α), high BMI, high creatinine, low hematocrit and low CD4 count.

1.9. Study variables

i). Independent variables;

- Hypertension status
- CD4+ cell count
- Creatinine levels
- Hematocrit levels
- Body Mass Index (BMI)
- Pro-Inflammatory cytokines

ii). Dependent variable(s);

- HIV/AIDS progression

iii). Interaction variables

- Patients' gender
- Treatment
- Patients' age
- Socio-economic support

1.10. Study benefits

This study sought to establish association between inflammatory cytokines secretion in hypertension comorbidity and HIV/AIDS progression. The findings of this study lay the basis for further research especially in diagnosis of risk of hypertension disorder in HIV/AIDS population. Most hypertensive patients were observed to be in advanced WHO HIV/AIDS

staging. This observation may be associated with interaction between HIV infection and hypertension comorbidity. The study findings established an association between hypertension status and HIV AIDS progression with hypertensive patients being more immunosuppressed clinically and immunologically compared to non-hypertensive counterparts.

1.11. Study duration

The study was carried out for a period of 24 months, between September 2014 to August 2016.

CHAPTER TWO: LITERATURE REVIEW

2.1.HIV and Hypertension Interaction

Hypertension is a disorder of immense significance in the world today associated with high morbidity and mortality [10, 26]. Most people in the tropics live with high blood pressure that remain undetected for long and often seek for care too late when presenting with complications of the disorder [10]. The prevalence has been low in the rural communities but the trends have been changing due to adoption of western lifestyles and inflammation associated with chronic diseases such as HIV/AIDS [27].

HIV/AIDS patients present with increased co-morbidity and mortality due to other ailments which are either communicable or non-communicable. The co-morbidity trends have been changing due to anti- retroviral therapy with non- communicable diseases such as cardiovascular and diabetes taking the biggest proportion of non-AIDS deaths since HIV seropositive population live longer [12].

The odds of HIV/AIDs patient developing a secondary disorder increases significantly in the range of 1.3 to1.99 [51, 52,53]. Most of these secondary disorders are often diagnosed too late at onset of complications making interventions less effective [54]. AIDs related deaths have declined significantly due to ART treatment while non-AIDS related deaths have been increasing [55, 56]. The percentage of deaths due exclusively to non-AIDS causes rose from 13.1% in 1996 to 42.5% in 2004, of which the most frequent cause is cardiovascular ailments [35, 36]. The risk of developing acute myocardial infarction is increased by 50% in HIV disease compared to the general population whose pathology is attributed to the Framingham risk factors [3].

The known risk factors of hypertension have been documented regarding the general population, but new trends have emerged with HIV seropositive population. This sub-group in the population are presenting with higher prevalence of hypertension and cardiovascular related conditions. [28,29,34,35]. This observation is attributed to a combination of known risk factors as in the general population as well as additional effects of persistent inflammation and metabolic imbalance. [7].

Hypertension disorder is documented as a significant contributor of non-AIDS related morbidity and mortality in HIV seropositive patients [30, 31]. Occurrence of hypertension in HIV/AIDS patients in different regions of the world varies between 25 and 36.5 % [32, 33].

As life expectancy increases with the use of ART, there are inevitably more untreated and later disabled victims of hypertension [29, 37]. Most of the patients succumb to adverse cardiovascular events which include myocardial infarction, cerebrovascular accident and cardiac failure, which is often preceded by hypertension due to lack of effective diagnostic approaches[38].

The ART formulations in use currently are more potent, less toxic, and appear to have less adverse effect on the cardiovascular system [39]. This has shifted the focus to inflammatory sequel as the main contributory factors to hypertension in HIV/AIDS patients [40]. However, immune reconstitution syndrome is responsible for enhanced inflammation in HIV patients on treatment [41].

Chronic inflammation marked by infiltration of inflammatory cells on the vascular endothelium, increased cytokine levels and growth factors have been documented in pulmonary arterial hypertension [11]. HIV proteins is known to induce chronic polyclonal B cell sensitization that in turn releases several pro-inflammatory cytokines whose persistent effects predispose the patients to premature development of arteriosclerosis [15].

HIV deregulates the immune functions and alters the cytokine profiles [16]. Cytokines can be measured to assess levels of inflammation and associated damage on the endothelial linings of blood vessels [42, 43]. Some of the elevated pro inflammatory cytokines associated with chronic inflammation include; IL-8, IL-1β, IL-6, IL-17A, tumor necrosis factor (TNFα), IL-12. Interleukin 6, interleukin 8 levels reflect the intensity of atherosclerotic plaques and may help to predict risk of atherosclerosis, while interleukin 10 exerts anti-inflammatory effects which include suppression of nitric oxide synthase [44, 45].

Hypertension in HIV/AIDS can be controlled with proper timely diagnosis and effective treatment interventions [48]. Late diagnosis is associated with irreversible complications, some of which are fatal. The management of patients with adverse cardiovascular events is a very expensive undertaking with cases of stroke and heart failure that require intensive care and clinical support [49]. The resources for specialized clinical care are expensive and not readily available in the developing countries often resulting in high mortality of patients with cardiovascular disease complications [50]. Determination of pro-inflammatory cytokine levels in addition to known cardiovascular disease risk factors may contribute significantly to timely interventions in HIV/AIDS patients [46, 47].

2.2.Chronic inflammation in HIV/AIDS disease

Inflammatory reactions following invasion by microorganisms are vital in host defense, but uncontrolled inflammation results in tissue damage, fibrosis and loss of function especially in chronic states. HIV disease has low grade inflammation that is aggravated by continued attraction of macrophages to lymphoid tissues [57]. The macrophages secrete several chemokines, some of which act on the endothelial linings of the vasculature and contribute to the development of atherosclerosis [58-60]. Several studies have shown that HIV infected patients have a significantly increased risk of cardiovascular disease linked to HIV/AIDS progression and its direct and indirect effects on the arterial structures [11-16,24]. One such

effect is dysregulation of lipid metabolism and inflammatory cytokine networks linked to vascular damage [61].

Biomarkers of inflammation are increased in patients with significantly higher viral concentration and low CD4+ cell count. This is attributed to rapid HIV replication and immune depletion [23].The persistent HIV inflammation as a result contributes to atherosclerosis that increases risk of developing hypertension and cardiovascular disease [7]. Infiltration by inflammatory cells results in the remodeling of vascular vessels contributing to increase in peripheral resistance [11].Figure2.2 .shows the interrelationship of factors associated with the endothelial tissue pathophysiology[24]

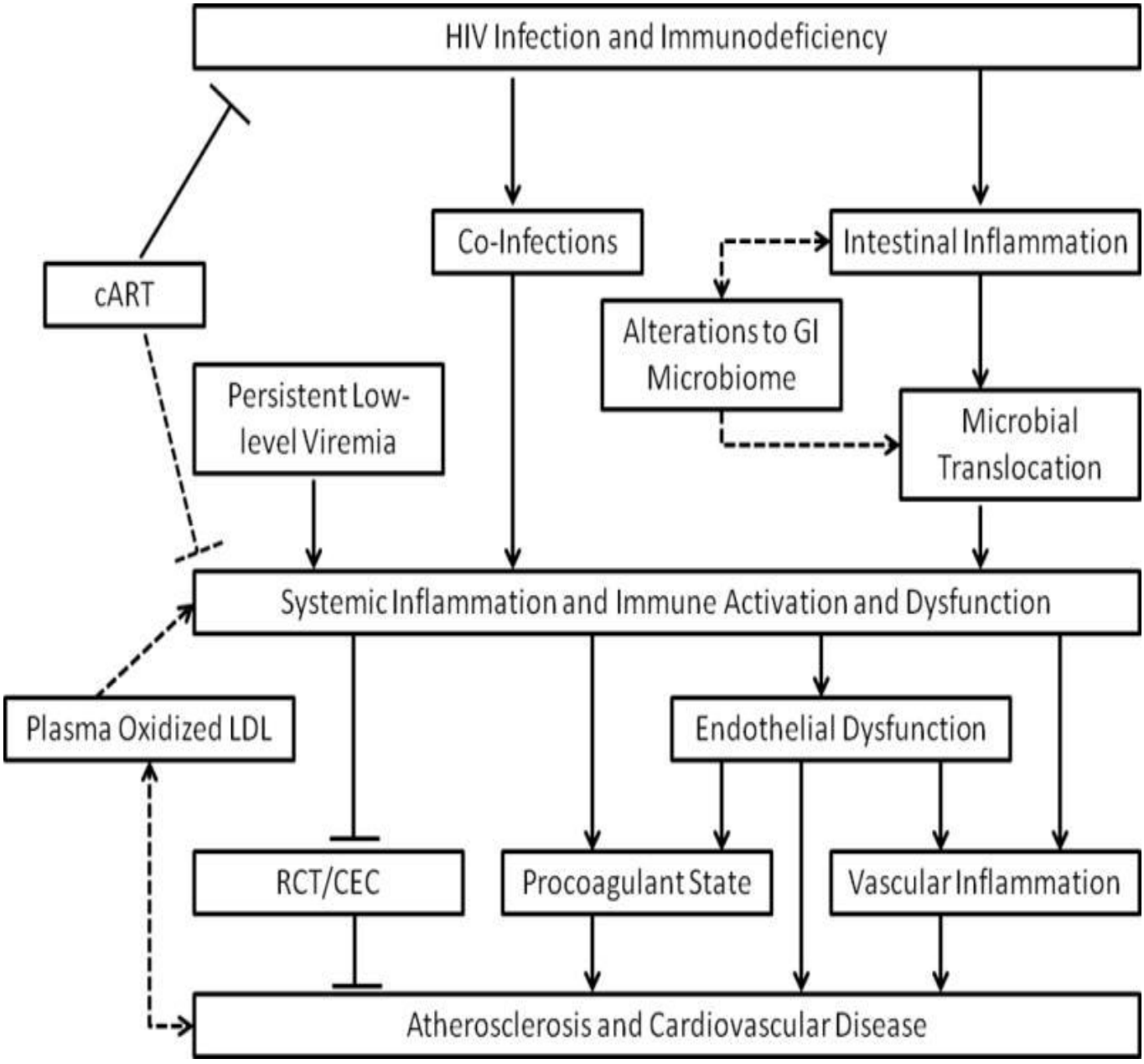

Figure. 2.2**.** Pathways involved in the development of Immune Activation and Atherosclerosis in HIV. Arrows shows a contributory effect. Terminal lines indicate an inhibitory effect. Dotted lines indicate a potential yet uncertain relationship. cART= combined antiretroviral therapy. RCT=reverse cholesterol transport, CEC=cholesterol efflux capacity, GI=Gastrointestinal, LDL=low density lipoprotein. [20]

2.3.Cytokines

Cytokine production is a cell mediated mechanism that is initiated by several factors which include foreign antigens, chemicals or cancer cells. The excretion of cytokines may result immune-pathological consequences. Inflammation due to chronic diseases triggers sustained production of high levels of cytokines in the body. Pro-inflammatory cytokines which include C-reactive protein, IL-6, IL-8, IL-10 and TNF-α are associated with disorders of metabolism such as obesity, hypertension and diabetes [20, 64,65].

Monocyte chemo-attractant protein 1 and IL-8 attracts macrophages to the site of inflammation, triggering atherosclerosis. On the other hand interleukin 6 and C-reactive protein determine the intensity of occult plaque formation and likelihood of rapture [7, 66]. High levels of pro-inflammatory cytokines such as L-8 have been associated with development of hypertension in pregnant women [67]. The HIV/AIDS patients are known to have increased IL-6 and coagulation factors as compared to the seronegative group [71]. Most multiple comorbidities in HIV-seropositive patients are unrecognized and untreated [51], and therefore the assessment of bio markers may be key in monitoring and clinical follow up for these patients [72]. Also, increased risk factors for cardiovascular disease in HIV infection require timely diagnosis and control [73].

Besides, serological biomarker assessment has been utilized to diagnose and determine the prognosis and outcome of neoplastic, osteoarthritis, malaria and other non- communicable diseases [60,74]. This study sought to assess the relationship between hypertension status and levels of inflammatory cytokines in serum in HIV/AIDS' patients, and to explore the effects of hypertension on HIV/AIDS progression as well as identify potential inflammatory cytokine(s), marker(s) for predicting risk of hypertension in HIV/AIDS patients[1].

Table.2.3. Showing some selected cytokines and their roles in inflammation process [74]

Serial no.	Cytokine	Role in inflammation	Associated diseases
1.	IL1	A potent mediator in response to infection	Alzheimer's, Arthritis
2.	IL 2	Induces "bursts" of HIV viremia in patients not on effective ART	Increase in AIDS related opportunistic infections
3.	IL4	Regulates T and B cell responses, differentiation of naive T cells into the TH2 phenotype, promoting B cell proliferation, antibody isotype switching	Allergic inflammation & Asthma
4.	IL6	Inflammation & infection responses. regulation of metabolic, regenerative, and neural processes	Atherosclerosis
5.	IL8	Activates neutrophils	Ischaemia, Trauma, myocardial infarction
6.	IL10	Important for immune regulation	Rheumatoid arthritis, inflammatory bowel disease, psoriasis, organ transplantation, and chronic hepatitis C
7.	tnfα	Cell signalling protein (cytokine),	Acute phase reactions & Systemic inflammation

8.	Ifn ϒ	activator of macrophages	Promotes cellular immunity cytotoxic t cell activation hence used to treat Kaposi's sarcoma, autoimmune diseases, insulin resistance,
9.	IL-17A	Pro-inflammatory cytokine expression and chronic inflammation, which lead to tissue damage and autoimmune disease	Multiple sclerosis (MS), Rheumatoid arthritis (RA), inflammatory bowel disease and psoriasis

2.4.Treatment of HIV Infection

Combination antiretroviral therapy is used to suppress HIV virus replication. It comprises of a minimum of two active drugs from two classes. Anti-retroviral therapy has significantly improved overall health of infected patients. However, their prolonged lives are associated with increased co-morbid conditions such as hypertension [75]. These non-AIDS related conditions are a setback that threatens to reverse the successes that have been realized with the use of antiretroviral therapy [51, 76]. The consequences of multi morbidity are considerable and include poor health maintenance marked by reduced quality of life, increased healthcare utilization and costs, and reduced survival. Therapeutic interventions to reduce the immunological effects of HIV may go a long way to reduce morbidity and mortality [53, 77].

Arteriosclerosis in HIV disease is significantly associated with hyper cholesterol but neither with duration nor type of antiretroviral therapy [64, 78]. cART has an indirect contribution to risk of cardiovascular disease [79]. Metabolic imbalances such as hyperlipidemia and low glucose uptake resulting in diabetes have been established in anti-retroviral therapy [34].

The complications are triggered by inflammatory syndrome as seen in the severe reactions that follow cART in patients with TB-HIV comorbidity [80]. cART use predisposes young HIV positive patients to metabolic complications that lead to coronary and cardiovascular disease [37, 81].

2.5.HIV/AIDS and Hypertension

HIV infected persons live longer with the use of anti-retroviral therapy. However, their life expectancy remains shorter compared to that of the general population, as a result of increased secondary illnesses and risk of death from both communicable and non-communicable conditions including cardiovascular complications [82]. The development of arterial hypertension is commonly established in HIV disease, although the risk indicators are not clearly documented [83].`deposition of low density lipoproteins and smooth muscle hardening associated with age[36]. Importance of diagnosis and treatment is clear with the use of existing treatment options like renin-angiotensin-aldosterone system inhibitors as prophylaxis in clients at high risk of development of hypertension [32, 36, 87].

HIV infection has long been associated with increased risk of development of hypertension [89]. Prevalence of hypertension in HIV/AIDS patients is significantly higher compared to the general population but little information exists on the management strategies [90, 91]. Management of hypertension follows the standard use of antihypertensive drugs which fall into four major classes, namely; blockers of the renin-angiotensin system, calcium channel blockers, diuretics and beta-blockers as well modification of lifestyle[92]. Dietary modifications target reduction of salt intake as a way of reducing water retention in the body [89, 93].

Overweight too may present because of metabolic syndrome and together with hypertension contribute to cardio metabolic disease states [25]. Assessment of body mass index in

hypertensive cases is significant in primary care of HIV/AIDS patients [9]. The average Body Mass Index (BMI) in HIV/AIDS patients is generally low compared to the general population but progressive BMI changes is associated with occurrence of chronic disorders like hypertension [19].

The sketch diagram that follows shows the interrelationship between factors that control hypertensive disease inflammation, [94].

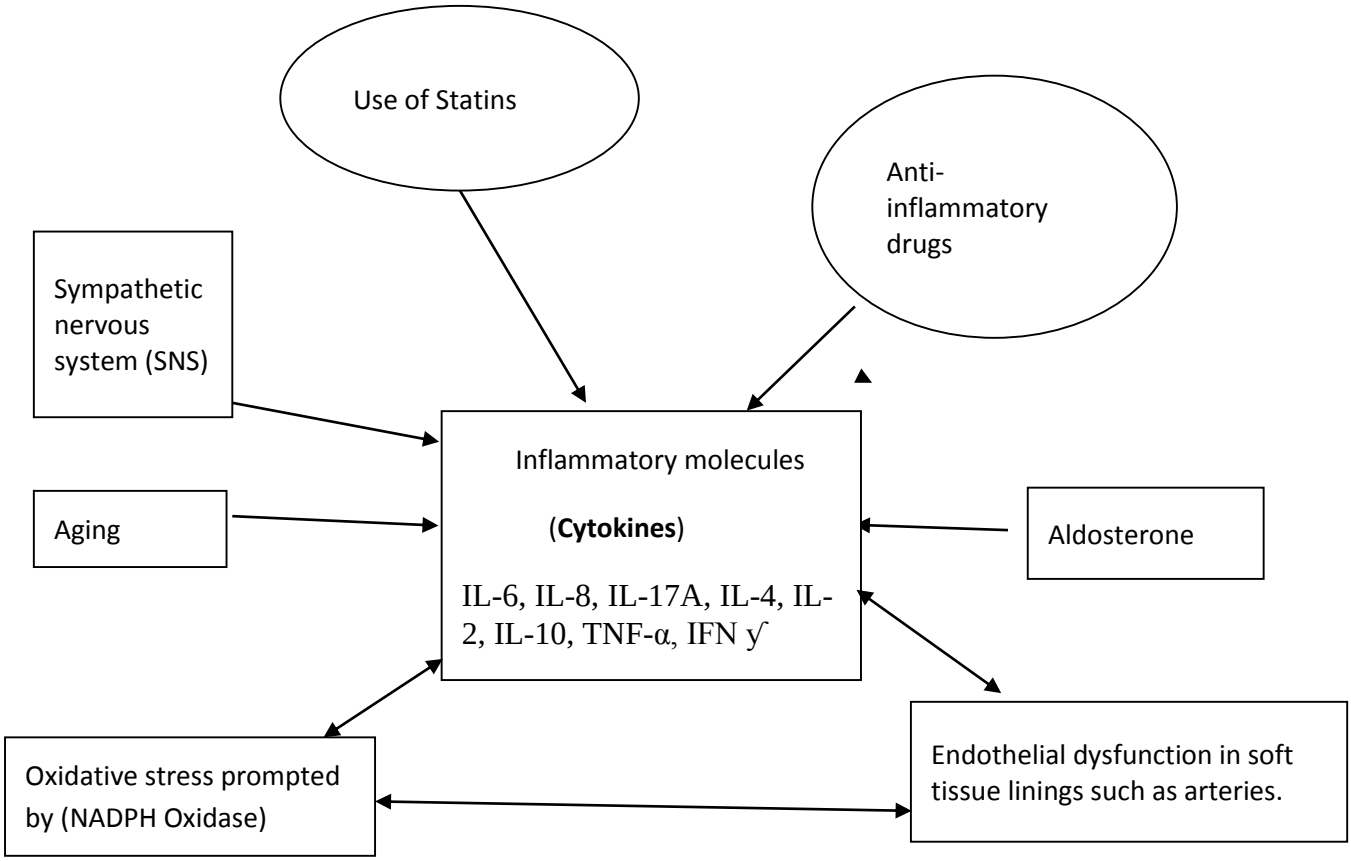

Figure: 2.5. Conceptual framework showing pathological pathways of HIV induced inflammation

CHAPTER THREE: RESEARCH METHODS

3.1. Study design

This was an observational study that adopted a cross sectional descriptive design to assess and compare plasma cytokine levels in hypertensive versus non hypertensive HIV/AIDS patients. As an exploratory study, the study also sought to assess association of hypertension with HIV/AIDS progression by obtaining and comparing the mean CD4 + cell counts between the two comparative groups. This design was chosen to generate hypothesis on the potential cytokine markers for diagnosing risk of developing hypertension in HIV/AIDS patients as a basis for future research.

3.2. Study Site

This study was carried out at Kenyatta National Hospital HIV Comprehensive care Centre in Nairobi. The center offers comprehensive HIV/AIDS care services to a wide range of clientele drawn from the city of Nairobi and its environs. A larger proportion of patients are referrals from HIV testing facilities and hospitals located within Nairobi County and beyond. The center has been credited for quality operating systems with good record keeping and follow-up of clients which offers a credible platform with complete patient documentation. The Centre has an enrolment of about 10,000 registered patients but only 6,000 are actively on care. The clinical return dates vary between one month and four months with a mode of 3 months. Most patients have been on care for more than four years. The patients report to the clinic as early as 5.30am and most of them leave the Clinic by 8.30am. They patients are booked for clinic on specific days of week except Fridays when Centre staff hold review meetings for the week.

3.3. Study population

Study participants were drawn from patients who are enrolled for HIV/AIDS care at Kenyatta National Hospital, (*figure 3.2*). The HIV/AIDS Care Centre is located within Nairobi city and

is the pioneer of such facilities in Kenya, having served patients for over 10 years, with majority having been on follow up for at least 5years. Since its inception, it has enrolled about 10,000 patients but only 6,000 are actively on follow up. The facility serves patients from Nairobi county and its environs. The population is diverse since Nairobi county is a cosmopolitan city. There are inhabitants of diverse cultural, religious and sociodemographic characteristics.

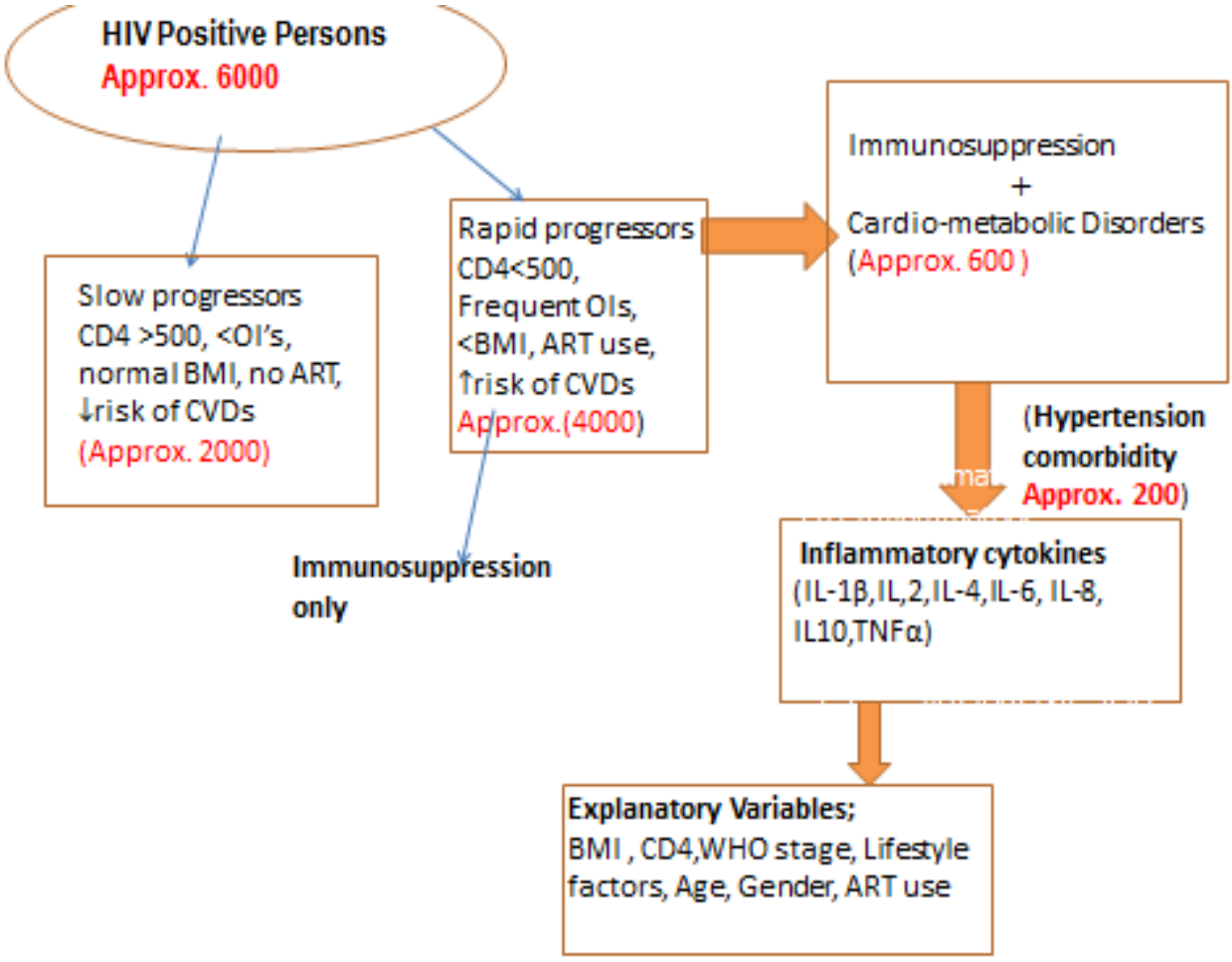

Fig.3.3. Diagram showing characteristics of study population, at CCC, KNH. Annual records December 2014.

3.4. Sample Size

Taking into consideration a prevalence of 6% for hypertension in the general population [4], and a 95% confidence interval, a sample of 300 participants was computed using comparison of proportions formula according to Fisher et al.1998;[95]

n= (2× (Z_α+ Z)^2× 〖2×σ〗^2)/((μ_1−μ_2)^2)

Z_α = 1.96 (95% CI)

Z_β = 0.84 (80% power)

n= (2× (1.96+ 0.84) ^2×2× 〖3.1〗^2)/ ((7.5−6.5)^2) = 150 for each group; ratio 1:1.

3.5. Sampling.

Kenyatta National Hospital Comprehensive care center was Purposively sampled for this study. A total of 300 adult patients were then recruited and categorized into hypertensive and non-hypertensive groups. A total of 70 hypertensive patients and 230 non hypertensives patients were recruited. They were recruited using systematic sampling where every 10^{th} patient who came to the clinic on routine care between Monday and Thursday was recruited into the study. The booked number of patients ranged from 60 to 100 per day. On average, a total of between 4-10 patients were recruited daily. The recruitment drive ran for a period of three months to attain the desired sample size.

3.6. Inclusion and exclusion criteria

3.6.1. Inclusion Criteria

All patients who were scheduled for follow up at the care center during the period of data collection were eligible for recruitment and constituted the sampling frame. The following characteristics were considered for inclusion into the study:

i. Patients aged 18-55 years, both ages included.

ii. Patients who consented to participate.

3.6.2. Exclusion Criteria

i. Pregnant women

ii. Patients from correctional facilities

iii. Minors below 18yrs

iv. Elderly patients above 55 years of age

v. Patients with arthritis, TB, Diabetes and Hepatitis B

vi. Users of statins or aspirin therapy

3.7. Ethical Considerations

The protocol was reviewed and approved by the joint University of Nairobi and Kenyatta National Hospital (UON/KNH) research ethics Review Committee (ERC). The study participants were educated on the research process, study objectives and their individual roles as participants. Informed consent was sought from each participant. Confidentiality was assured and granted to the participants during data collection through observation of privacy during interview, anonymity of data collection forms, safe handling of data and storage.

3.8. Data collection

Demographic, clinical and cytokine data was obtained from the selected participants. The data were collected using a structured researcher administered questionnaire whereas checklists were used to capture clinical data abstracted from medical records. Data on social and lifestyle variables were obtained too, from the participants.

Vital signs which included pulse as well as the primary outcome; blood pressure, were measured in each participant. To ensure reduced variability in individual blood pressure

measurements, each participant had their blood pressure taken three times in a series at an interval of 15 minutes and an average of the three blood pressure readings was documented. The normal cut off BP was lower (90/60mmHg) and upper (130/90mmHg).

The questionnaire was then administered for an average of 15 to 25 minutes with each participant. Information obtained covered areas which included; individual demographics, social habits history, clinical care, treatment, clinic revisits, and social support practices.

Physical measurements for weight and height were obtained after questionnaire administration. The two measurements were applied in the computation of individual participant's BMI. Desktop review of patients' files was carried out to obtain data on CD4 count, creatinine, hematocrit and treatment characteristics. The participants' clinical files were used to cross check information that was collected from participants regarding their clinical care. The clinical tests result obtained within the last three months of care were accepted as valid for this study. These tests include; CD4+ cell count, Viral load, Creatinine, and hematocrit.

3.9. Cytokine data

Cytokines were obtained from the analysis of plasma samples obtained from participants. The plasma was extracted from routine blood samples and analyzed for selected cytokines that included; IL- 2, IL-4, IL-6, IL-8, L-10), IL-17A, TNF-α and IFN-γ.

The BD™ CBA Human Th1/Th2/Th17 and IL-8 Flex Cytokine Kits were used to measure the cytokine levels in participants' single plasma samples. The kit performance is optimized for analysis of physiologically relevant concentrations of specific cytokine proteins in tissue culture supernatants, EDTA plasma, and serum samples. Refer to detailed procedure in *appendix 5.*

3.10. Data analysis

A total of 297 questionnaires were complete and data entered for analysis. The hypertensive participants were 69 while non hypertensive group participants were 129. The assumption from figure 3.3, page 22, is that the 69 were recruited out of the 200 suspected to have hypertension comorbidity while the 128 non hypertensive participants were drawn from the 2000 with reduced risk of cardiometabolic disease.

STATA^{TM} version 22 and "R" version 3.5.1 were used in analysis of data. The author used descriptive and inferential statistics to generate inferences between variables under study. Tables and figures were used to present the results. Measures of central tendency were obtained as well as proportions for binomial data. Univariate and bivariate analysis was adopted to explore the variations in expression of pro-inflammatory cytokines in hypertension Co-morbidity. The hypothesized association between plasma cytokines' levels and hypertension status was entered in the multiple regression model. The hypothesized mean difference in cytokine levels between hypertensive and non-hypertensive participants was mean of at least +/- 0.001pg/ml in participants' plasma. P values <0.05 were considered statistically significant. During analysis cytokine data was transformed because of non-normality distribution. Student 't' and logistic regression tests were carried out to obtain inferences from the data.

CHAPTER FOUR: RESULTS

4.1. Participants' characteristics

This study was conducted to determine the prevalence of hypertension, identify risk indicators and establish variation in selected plasma cytokines in hypertensive versus non hypertensive HIV/AIDS patients. An alpha level of 0.05 was used for all statistical tests.

Out of the 297 participants, 89 (30.0%) were males while 208 (70.0%) were females. The age ranged between 30 – 57 years with a mean of 42.97$\pm$6.21 SD, and a median of 42 years. Independent Sample t-test showed a statistically significant difference in participants' age between males (M = 44.56, SD = 6.05) and females (M = 42.29, SD = 6.16), t (295) = 2.922, p = 0.004. The association of age group and hypertension status was statistically significant ($X^2(2) = 15.736$, $p<0.001$). Those above 45 years of age had a higher predisposition to development of hypertension, with the average age of the hypertensive patients being 42 years. Education level of participants was distributed as follows; secondary 138(46.9%), primary 64(21.7%) and tertiary/college 92(31.2%), as shown in, Table 4.1.

Table 4.1. Descriptive characteristics of participants

Study participants co-variates	Hypertensive	Non- Hypertensive	P value
Marital status (%)			
Married	48 (69.6%)	135 (59.2%)	
Single	13 (18.8%)	59 (25.9%)	
Widowed	3 (4.3%)	14 (6.1%)	0.645
Gender (Male)	22 (31.9)	67(29.4)	0.805
Age (mean (SD))	42.16(6.12)	45.67(5.77)	<0.001**
Education level (%)			
Secondary			0.291
Primary	14(20.3%	50(21.9)	
BMI (mean (SD))	28.15 (5.33)	26.70 (4.68)	0.029**
WHO HIV/AIDS stage (mean (SD))	2.08 (1.12)	1.80 (1.02)	0.066
Creatinine ug/dl (mean (SD))	93.25 (40.15)	86.89 (51.39)	0.371
HCT (mean (SD))	36.50 (9.28)	39.08 (6.69)	0.028**
Art use (%)			
No	4 (5.8%)	15 (6.6%)	1.000
CD4 (Mean (SD))	499.41 (218.46)	515.06 (275.70)	0.666
Septrin treatment (%)			
Yes	55(79.7)	194 (85.1)	
Self-reported level of stress (%)	18(26.1)	94(41.2)	0.0531
Moderate	43(62.3)	123(53.9)	
Severe	8(11.6)	11(4.9)	
Member of social support group (%) (yes)	0(0.0)	12(5.3)	0.151
Opportunistic infections reported (%)			
Integumentary	0 (0.0)	2 (0.9)	0.621
Others	2 (2.9)	4 (1.8)	
Respiratory	20 (29.0)	54 (23.7)	

ART, antiretroviral therapy; **BMI**, Body mass index, **HCT**; hematocrit concentration, ** statistically significant

4.2. Association of Hypertension status and participants' variables.

Hypertension prevalence established in this study was n=69(23.2%). High stress levels and CD4 + cell range were statistically associated with the occurrence of hypertension in HIV/AIDS patients (χ^2) = 7.686*, p = 0.042 and (χ^2) = 7.863*, p = 0.020).

Table 4.2a. Association of CD4$^+$cell range and Stress levels with Hypertension Status.

	Hypertension status	
Characteristics	**Hypertensive**	**Non-hypertensive**
CD4+ cell range		
Normal immunity (≥500)	29	113
Mild immunodeficiency	34	75
Moderate immunodeficiency	5	26
Severe immunodeficiency	1	14
	$\chi^2(3) = 7.686^*$, p = 0.042	
	Phi = 0.161*, p = 0.042	
	Cramer's V = 0.161*, p = 0.042	
Stress levels		
Mild	17	91
Moderate	43	123
Severe	8	11
	$\chi^2(2) = 7.863^*$, p = 0.020	
	Phi = 0.164*, p = 0.020	
	Cramer's V = 0.164*, p = 0.020	

Chi-Square Test for Independence (Pearson Chi-Square) was used.

Phi and Cramer's V Test was used.

*p<0.01

The simple linear regression of age and hematocrit against hypertension status demonstrated that the two variables were statistically significant in association with hypertension status. A relatively, younger age group with an average age of 41years, OR=1.1,95%CI [1.05,1.15], p<0.001, were presenting with hypertension compared to the general population. Higher

hematocrit levels were highly associated with development of hypertension in participants, OR=0.96,95%CI [0.92,1.00], p<0.028, Table 4.2b. BMI had a positive and statistically significant association with hypertension status in the study population, OR=1.06,95%CI [1.01.1.13], p<0.03, Table 4.2b.

Most participants were largely within WHO stage 1 of HIV progression, which is marked by heightened immune response and reconstitution syndrome. Logistic regression of HIV AIDS stage and hypertension status was not statistically significant, OR=1.28,95%CI [0.98,1.67], p=0.068, Table 4.2b.

A logistic regression was performed to ascertain the effects of hypertension on the likelihood that participants have low CD4+ cell range. The logistic regression model was statistically significant, $\chi 2$ (2) = 8.171, p = 0.017. The odds of hypertensive participants having low CD4+ cell range was 1.912 (95% CI, -0.877 to -0.175) times that of non-hypertensive participants, a statistically significant effect,

p = 0. 003,Table 4.2b.

Table 4.2b: Simple regression of the factors associated with Hypertension disorder among Participants.

Study participants co-variates	OR	95% CI	p-value
Marital status			
Married	1.778	(0.203, 15.604)	
Single	1.102	(0.119,10.240)	
Widowed	1.071	(0.0895,12.831)	0.815
Gender (male)	1.125	(0.629,2.011)	0.692
Age	1.098	(1.049, 1.149)	<0.001**
Education level			0.103
Secondary	0.163		
Primary	0.140	(0.011,1.513)	
BMI	1.064	(1.006,1.125)	0.030**
WHO HIV/AIDS stage	1.280	(0.982,1.670)	0.068
Creatinine	1.002	(0.997,1.008)	0.379
HCT	0.958	(0.921,0.996)	0.032**
Art use (Yes)	1.144	(0.367, 3.569)	0.816
CD4	1.000	(1.000,1.001)	0.665
Septrin treatment (Yes)	0.689	(0.345,1.374)	0.290
Self-reported level of stress	0.560	(0.055,5.712)	0.625
Moderate	1.049	(0.106,10.353)	0.967
Severe	2.182	(0.190,25.021)	0.531
Member of a social group	0.0957	(0.189, 4.855)	0.958
Yes	1.917	(0.000, inf)	0.982
Opportunistic infections reported			
Skin	6.21 (-07)	(0.000, inf)	0.989
Others	1.787 +00	(0.318,10.060)	0.510
Respiratory	1.324 +00	(0.722, 2.428)	0.365

Antiretroviral therapy (**ART**); Body mass index (**BMI)**, Hematocrit concentration (**HCT). ** statistically significant**

Further analysis adjusting for possible confounding, demonstrated that age and hematocrit levels were independently linked to hypertension in HIV/AIDS population. The two factors qualified as precision variables in the model of determinants of hypertension in HIV disease, Table 4.3.

Table 4.3: Logistic Regression analysis for Gender, Age, BMI, Creatinine, CD4 , HCT and Hypertension status

Participants co-variates	**OR**	**95% CI**	**p-value**
Female	Ref	Ref	Ref
(male)	1.11	(0.504,2.025)	0.32224
Age	1.09	(1.053,1.165)	**0.00279 ****
BMI	1.05	(0.992,1.129)	0.16258
CD4	1.00	(0.9984,1.001)	0.77911
Creatinine	1.00	(0.995,1.007)	0.88831
HCT	0.94	(0.903,0.984)	**0.00730 ****

BMI, Body mass index, **HCT**; hematocrit concentration

4.3. Distribution of participants' Clinical variables.

The variables described here include; CD4+ cell count, creatinine, hematocrit and BMI. These four variables are usually measured at baseline before commencement of treatment and later as part of monitoring of patients' clinical progress. These factors have some collinearity hence analyzing their levels independently and collectively is useful in associations with patients' possible clinical outcome(s).

4.3.1. Distribution of CD4+ Cell Count in participants.

The mean CD4 +cell count among participants was 511.42+_263.25. Female participants had higher CD4+ cell count values on average compared to male participants. Gender was one of the key demographic variables that showed significant statistical association with CD4+ cell

count among the study participants, p=0.013,95%CI [-149.23, - 35.64]. The age group had some variation but no significant statistical association, p=0.974.

CD4 + cell count was correlated with past medical history of participants which showed a statistically significant influence on the average CD4+ cell count, p=0.049, 95%CI [-229, -2.53]. Those participants who reported past illnesses without prompt treatment had lower average CD4+ cell counts, compared to those who reported no past medical illness or who were diagnosed and treated promptly.

Among clinical care variables studied, use of Cotrimoxazole emerged as the only factor with significant statistical association on the CD4+ cell count among study participants, p=0.047, 95%CI [-162.81, -0.26]**.** The participants, who were using Cotrimoxazole prophylactic treatment had on average a higher CD4+ cell count compared to the non-users.

The CD4+ cell count range and WHO HIV/AIDS disease stage showed significant statistical associations with the average CD4+ cell count, (p <0.001,95%CI [36.22, – 64.71], p=0.009) respectively.

The general characteristics of participants were correlated with CD4 + cell counts, and this included; BMI range, frequency of clinical review, manifestation of wasting, stress levels and social behavior. Of these, BMI range, frequency of clinical review and manifestation of wasting had significant statistical association with the average CD4+ cell count among participants, (p <0.007, 0.001 and 0.003), respectively.

4.3.2. Distribution of Creatinine levels in participants

The mean creatinine level among participants was 88.34+49.06mmol/dl. This study observed a significant difference in levels of creatinine by participants' gender, p=0.001. Variation was also observed between the age groups p=0.016.

Regression of selected clinical characteristics that included past medical illness, hypertension status, and blood pressure range, use of antihypertensive drugs, type and duration of antihypertensive treatment as well as its side effects did not demonstrate any statistically significant association with creatinine levels, $p>0.05$. The hypertensive participants did not have any statistically significant difference in creatinine values with the non-hypertensive group.

HIV/AIDS care characteristics analyzed which included ART use, Septrin use, duration of ART use and support group membership did not show any statistically significant association with creatinine regulation among participants, $P>0.05$.

The participants' creatinine levels were compared with the CD4+ cell count range, WHO HIV/AIDS disease stage, and HCT range and cytokine levels. There was a statistically significant association between CD4+ cell count, WHO HIV/AIDS disease stage and the creatinine levels. Low CD4+ cell count and advanced WHO HIV/AIDS disease stage presented with a higher creatinine level ($p=0.081$, 0.092), respectively. This observation is a sign of severe inflammatory state that results in over secretion of creatinine. On the other hand, there was no significant statistical association between HCT and cytokine levels and the regulation of creatinine among the study participants.

It was observed that the level of creatinine was associated with some of the participants' general characteristics which included BMI range, frequency of clinical reviews, and manifestation of wasting, stress levels and social behavior. Among the five factors analyzed, low BMI an indication of wasting had a positive and statistically significant association with creatinine levels in participants, $p = 0.022$, 95%CI [-37.3, – 7.66].

4.3.3. Distribution of Hematocrit (HCT) in participants

Hematocrit is a measure of percentage of red blood cells in a blood volume. It ranges between 40-45% in men and 36-40% in women. However, elevated levels are been associated with hypertension, independent of known confounders [93]. The observed mean of hematocrit level was 38.43 + -7.49.

The association between gender and HCT was statistically significant in this study ($p<0.001$, 95%CI [2.60 ,6.83]). On average the participant HCT ranges were within the normal limits, [35-42mml/l]. Participants aged 50 years and above had on average higher hematocrit values. Though not statistically significant, severe hypertension status and prolonged ART therapy of more than four years among participants was associated with increased hematocrit levels above 40 unit mark. Use of first line or second line ART did not influence hematocrit values among participants.

However, ART and Septrin use, duration of ART use and being a member of a social support group did not show any significant statistical association with hematocrit levels among participants, p= (0.343, 0.500, 0.261,0.595), respectively.

The manifestation of wasting is the other parameter that showed a positive and statistically significant association with the HCT levels, OR=0.94, p=0.001,95%CI [1.56, 9.62]. Participants with wasting syndrome had lower average levels of hematocrit and low risk of developing hypertension, $p>0.05$

4.3.4. Body Mass Index (BMI)

The mean BMI among participants was 27.04+4.87. This observation indicates an overweight measure for majority of participants and a known risk factor associated with cardio metabolic disorders that are linked to cardiovascular disease. The relationship between BMI categories and CD4 + cell count of participants was determined using Spearman's rank-order correlation.

The test elicited a mild, positive and statistically significant association, ($p < 0.05$), which showed that the BMI category of the patients is associated with their CD4 + cell counts and patients with higher BMI categories were more likely to have higher CD4+ cell counts than patients with lower BMI categories, Table 4.1.1a**.** High BMI value is associated with improved health status with balanced metabolic rate and physiological function marked by less wasting.

There was a mild, positive correlation between ART duration and BMI values, which was not-statistically significant (r_s (272) = 0.033, p = 0.590). Participants' creatinine levels had a mild positive correlation with BMI values (r (270) = 0.008, p = 0.890), although statistically insignificant. However, it showed a likelihood of increase in creatinine levels with increase in BMI values.

CD4+ cell counts, and BMI values showed a positive correlation which was statistically significant, p=0.002.

The age of the patient had a moderate, positive correlation with BMI (r (295) = 0.113, p = 0.052). As the participant's age advances, the BMI values were likely to increase. This is supported by other studies that have reported reduced metabolic rate as a result of age related physiological changes [88,106].

Most participants presented with mild to moderate stress levels which was positively associated with higher BMI values, although with no significant statistical association (Fisher's Exact test=5.38, p=0.50). Absence of wasting in participants was directly related to higher BMI values, with a strong statistical significance (Fisher's exact test =39.852**, $p < 0.001$).

Most of the clinical variables which include ART duration and BMI showed a positive, non-statistically significant association, p=0.590. WHO HIV/AIDS stage had a negative but statistically significant association with the CD4+ cell count, p = 0.009.

4.3.5. Correlation between Participants' CD4 + Cell Count, Creatinine, HCT and BMI.

Pearson product-moment correlation model showed the association between the four explanatory variables which include CD4, Creatinine, HCT and BMI. The figure shows near perfect convergence of measurements of the four variables under analysis depicting a strong positive association between them. Creatinine and HCT variables did not depict collinearity except for CD4 + cell count and BMI. The CD4+cell count also showed a positive correlation with BMI that was statistically significant r(295) = 0.175**, p = 0.002). The CD4+cell count had a negative correlation with Creatinine that was statistically significant (r (270) = -0.155, p = 0.010). Figure 4.3.5.

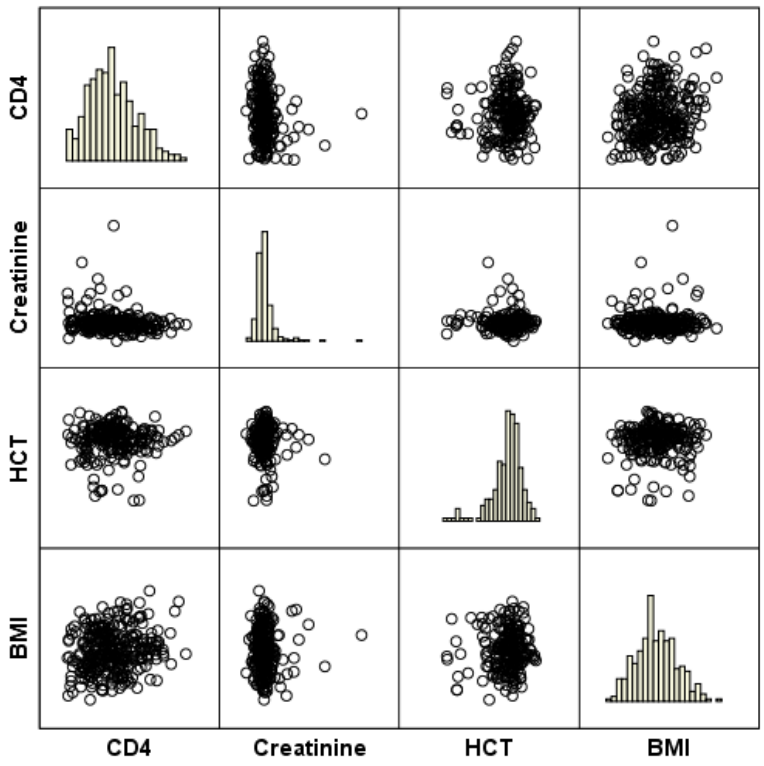

Figure 4.3.5. Collinearity plot showing the association between participants' CD4+ Cell count, Creatinine, HCT and BMI.

4.3.5a. Linear regression plot for participants' Creatinine and CD4+ cell count.

A Linear regression model plot too showed a statistically significant association between creatinine values and CD4+ cell counts, F (1, 270) = 6.684, R^2 = .024, β = -0.155, n = 272, p = 0.010**.**

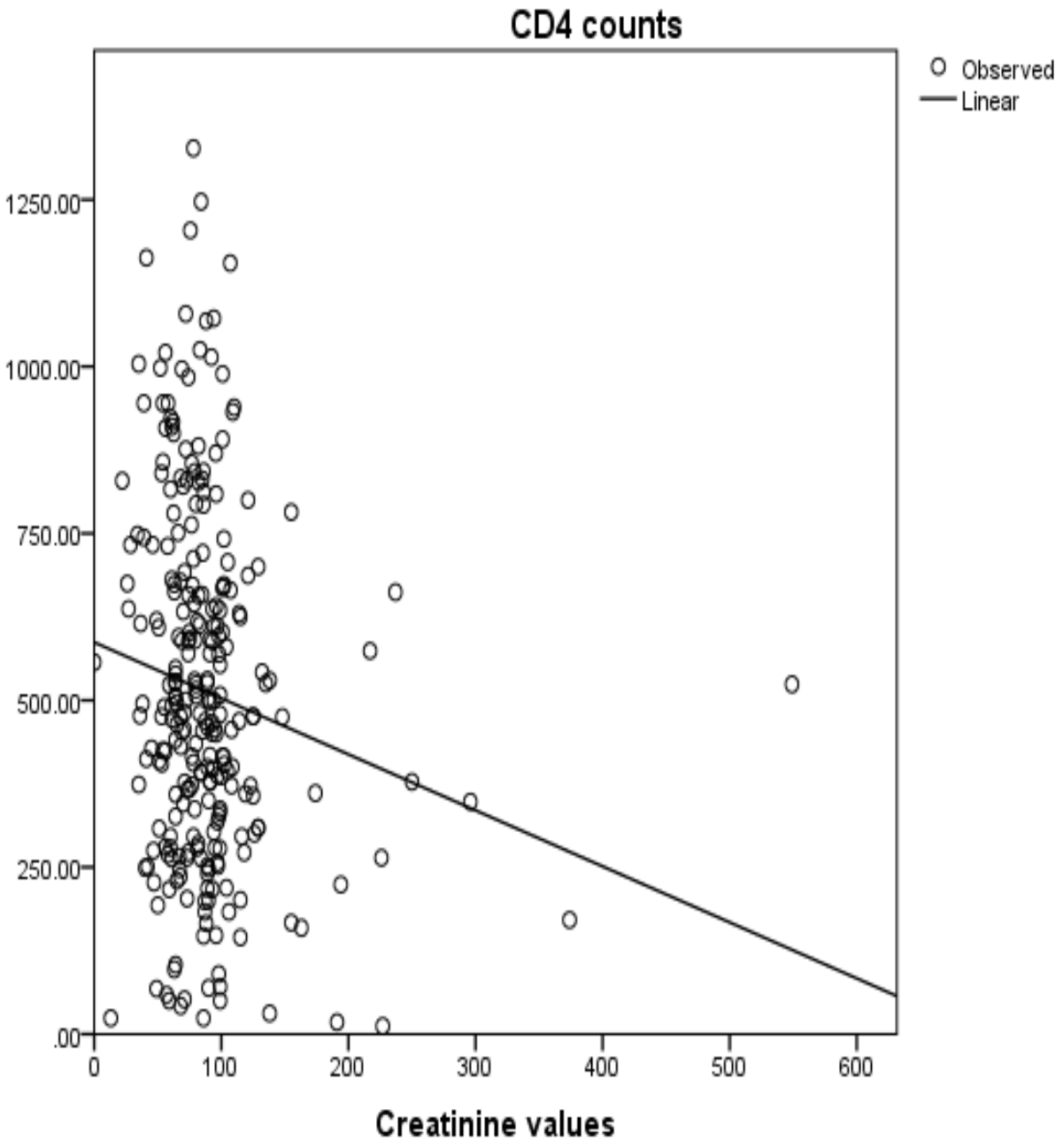

Figure 4.3.5a. linear regression Plot for participants' CD4+ cell count and creatinine values.

4.3.5b. Regression for participants' BMI and CD4+ cell count.

A Curve Estimation Linear Regression model demonstrated a statistically significant relationship between BMI values and CD4+ cell counts, F (1, 295) = 9.321, R^2 = .031, β = 0.175, n = 297, p = 0.002.

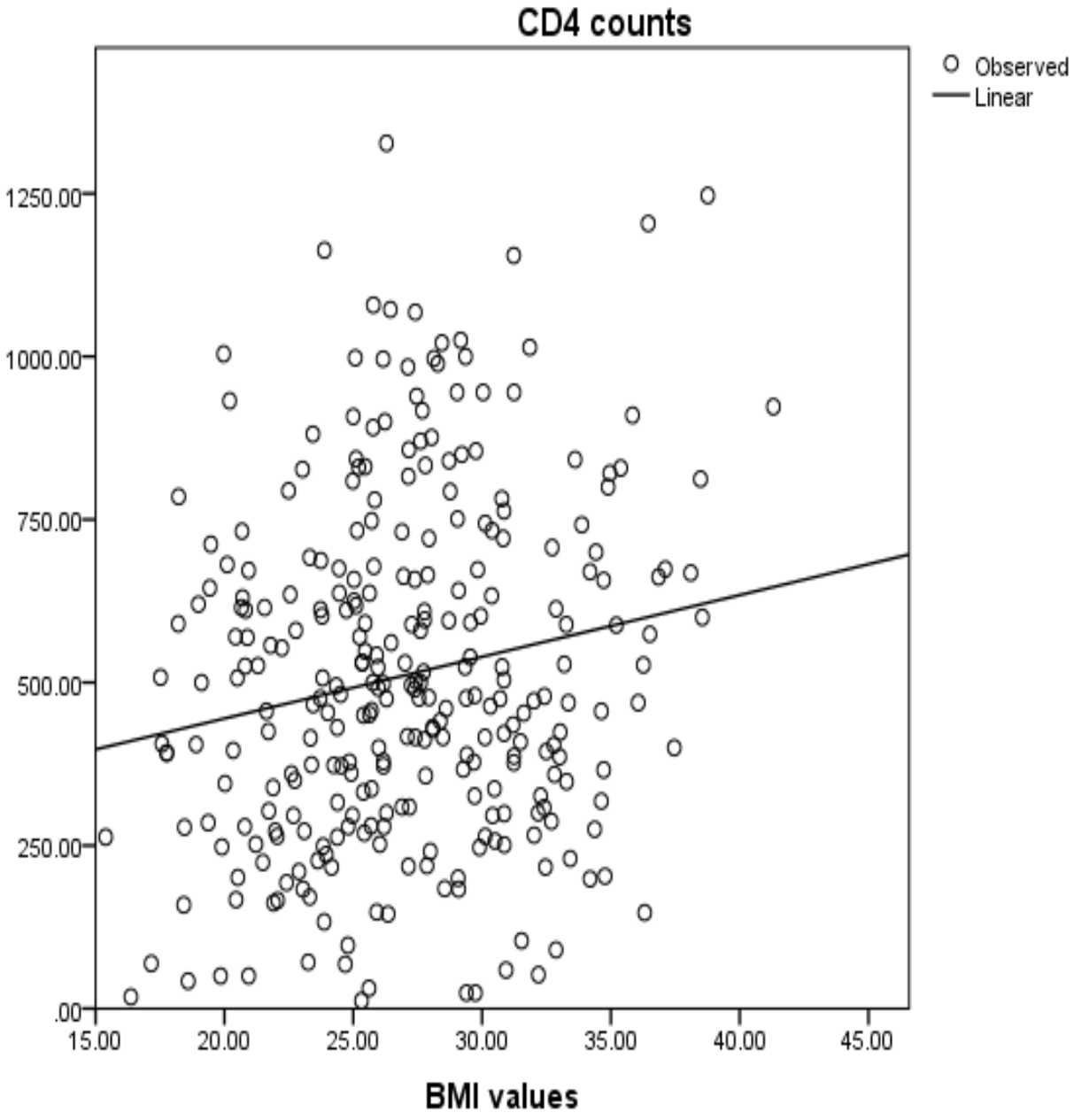

Figure 4.3.5b. linear regression plot for participants' BMI values and CD4+ cell counts.

4.4. Participants' Clinical and Socio-behavioral characteristics.

4.4.1. Description of participants treatment

Most participants, n=157(52.8%), had a three months frequency of follow up at the Comprehensive Care Centre. This was however not statistically significant in association with social behavior (Fisher's exact test=6.732, p=0.383).

Fishers' exact test showed no significant statistically association between manifestation of wasting and participants' social behavior (Fishers exact test =3.156, p=0.231). Absence of wasting among participants was highly associated with no tobacco and alcohol use.

The stress levels showed a positive correlation with social behavior, although no significant statistical association was obtained. Majority of the patients were non- alcohol and tobacco users and presented with mild and moderate stress levels as compared to proportion of tobacco and alcohol users (X2(4) =1.745, p=0.790).

Secretion of pro- inflammatory cytokines was associated strongly with both ART and Cotrimoxazole treatment among participants (X2=1.165, p=0.685). The two treatments also had a positive correlation with absence of wasting among participants, (X^2=0.274, p=0.685); (X^2=0.006, p=0.939) respectively. The strongly positive association of ART treatment and frequency of clinical reviews for participants was statistically significant (Fishers exact test=20.180, p<0.001). The positive association with Cotrimoxazole use and clinical reviews on the other hand was not statistically significant (X^2=0.006, p=0.939).

Table 4.4.1 Participants' ART and Cotrimoxazole use versus cytokines category, manifestation of wasting and frequency of clinical reviews parameters

Clinical parameter	ART use		Septrin use	
Manifestation of wasting				
	Yes	**No**	**Yes**	**No**
Absent	251	18	229	40
Category of cytokines				
Present	24	1	18	7
Pro-Inflammatory	98	8	84	22
	$\chi^2(1) = 0.274$, p = 0.717		$\chi^2(1) = 0.006$, p = 0.939	
Anti-Inflammatory	19	1	16	4
Frequency of clinical reviews	$\chi^2(1) = 1.165$, p = 0.685		$\chi^2(1) = 0.006$, p = 0.939	
Once monthly	18	8	18	8
Once in two months	13	0	11	2
Once in three months	155	4	138	21
Once in four months	90	7	80	17
	Fisher's Exact Test = 20.180**, p < 0.001		$\chi^2(1) = 0.006$, p = 0.939	

Chi-Square Test for Independence (Pearson Chi-Square) was used.
Fisher's Exact Test was used. **p<0.01, *p<0.05

4.4.2. Predictors of HIV/AIDS progression in the study population

In seeking to establish the predictors that influence HIV AIDS progression, multivariate logistic regression was used to analyze the association with clinical variables. Age of participant, creatinine, HCT and BMI did not show any statistically significant association with HIVAIDS progression. CD4+cell count was the only parameter that was statistically significant r_s (275) = -0.157), p = 0.009. It was inversely proportional to the HIV/AIDS stage.

A logistic regression was performed to ascertain the effects of hypertension on the likelihood that participants demonstrated low CD4+ cell count. The logistic regression model showed positive and statistically significant values, χ^2 (2) = 8.171, p = 0.017. The odds of hypertensive

participants having low CD4+ cell count range was 1.912 (95% CI, [-0.877, -0.175] times that of non-hypertensive participants, a statistically significant association, (p = 0.003).

4.5. Distribution of selected cytokine levels in participant plasma samples.

Interleukin type	Mean values (Hypertensive N=65)	Mean values (Non-Hypertensive N=61	Mean diff	95% CI	P value
IL17a	75.51369	42.22738	33.29	(6.59, 59.99)	0.015**
Il2	0.6549231	0.1160656	-0.10	(-0.10 1.18)	0.097
Il4	0.03	0.01311475	-0.15	(-0.04, 0.07)	0.522
IL6	3.589846	1.94377	0.75	(-0.94,4.23)	0.211
IL8	29.66985	16.59262	11.73	(-7.43, 33.59)	0.287
IL10	0.2103077	0.7221311	-6.53	-15.13, 2.07)	0.242
TNFα	0.1006154	0.07557377	-0.10	(-2.38, 0.39)	0.123
IFN γ	6.871385	-	-	-	-

Il17a, Interleukin 17a, **IL2** -Interleukin2, **IL4**-Interleukin 4, **IL6**- Interleukin 6, **IL8**-interleukin 8, **IL10**-Interleukin 10, **TNFα**-Tumor necrosis alpha, IFNγ-interferon gamma.

Plasma samples from one hundred and twenty six participants were analyzed for selected cytokines as shown in table 4.5.1a**.** The mean difference by hypertension status for most of the selected cytokines were not statistically significant except for IL17a, mD=33.29ug/mml, 95%CI [6.59,59.99], p=0.015.

4.5.1. Association between plasma cytokines' levels and selected clinical parameters of participants

Pearson product-moment correlation coefficient and Spearman's rank-order correlation coefficient were performed to determine the associations for Participants' age, gender and cytokines values. The participants' gender had a significant statistical association with production of IL-2 and IL-17-A. Age had no significant statistical association with any of the measured cytokines, Table 4.5.2. The relationship between hypertension status and IL-17A in participants was determined using Spearman's rank-order correlation. The test showed a mild, negative and statistically significant association ($r_s(124) = -0.248$, $p < 0.05$). This finding showed that hypertension status may be associated with variation in the release of IL-17A. Those known hypertensive HIV infected patients were more likely to have higher IL-17A levels than non-hypertensive patients.

When the association between gender and IL-17A was determined using Spearman's rank-order correlation, the test showed a moderate, positive and statistically significant association ($p < 0.001$).

When the association between gender and IL-2 was determined using Spearman's rank-order correlation, the test showed a strong, negative and significant statistical association ($p < 0.05$), demonstrating too that gender influences the release of IL-2 and males are more likely to have higher IL-2 levels than females in HIV positive patients.

When the association between social behavior of the patients and IL-17A was determined using Spearman's rank-order correlation, the test showed a moderate, negative and significant statistical association ($p < 0.05$), showing that the social behavior of patients influences the release of IL-17A and both smokers and alcoholic patients were more likely to have lower IL-17A levels than non-smokers and non-alcoholic patients. When the association between social behavior of the patients and IL-6 was determined using Spearman's rank-order correlation, the

test showed a moderate, positive and statistically significant association ($p < 0.05$), meaning that the social behavior of patients too influences the release of IL-6 and both smokers and alcoholic patients were more likely to have higher IL-6 levels than non-smokers and non-alcoholic patients. The association between social behavior of the patients and IL-8 was determined using Spearman's rank-order correlation and the test showed a moderate, positive and statistically significant association, $p < 0.01$.

Table 4.5.2. Simple regression model of mean difference of cytokines and participants' covariates

Covariate		**IL17A**	**IL2**	**IL4**	**IL6**	**IL8**	**IL10**	**tnfα**	**Ifnϒ**
Gender	**Female**	ref	ref	ref	ref	ref	ref	ref	ref
	Male	-39.00 P=**0.004**	0.639 P=0.058	-0.034 P=0.208	-0.242 P=0.860	0.698 P=0.949	2.829 P=0.540	-0.001 P=0.989	-5.58 P=0.243
Age group (yrs)	**30-39**	ref	ref	ref	ref	ref	ref	ref	ref
	40-49	7.39 P=0.68	0.013 P=0.411	-0.005 P=0.87	0.213 P=0.904	-0.196 P=0.989	5.415 P=0.384	-0.042, p=0.659	0.035 P=0.575
	≥50	8.53 P=0.697	0.018 P=0.112	0.031 P=0.451	-1.280 P=0.540	-4.651 p=0.779	6.448 P=0.355	0.109 P=0.338	0.067 P=0.362
BMI	**Normal Weight**	ref	ref	ref	ref	ref	ref	ref	ref
	Obese	17.67 p=0.339	0.105 p=0.809	0.009 p=0.775	-1.69 p=0.337	-17.94 p=0.197	-4.74 p=0.435	-0.040 p=0.680	0.091 p=0.878
	Overweight	11.57 p=0.501	0.279 p=0.492	0.010	-2.420 P=0.141	-22.27	-4.74	0.028 P=0.751	0.045

				P=0.751		P=0.087	P=0.435		p=0.414
	Under-Weight	52.47 P=0.203	1.32 p=0.713	0.335 **P<0.0001**	-3.230 P=0.409	-21.65 P=0.483	-	0.238 P=0.267	0.146 **P=0.0006****
WHO HIV/AIDS stage	(Continuous variable)	-11.28 P=0.107	0.250 P=0.151	0.011 P=0.438	0.419 P=0.543	4.22 P=0.451	-4.78 P=.069	-0.016 P=0.698	0.203 P=0.935
Creatinine	,, ,,	-0.095 P=0.479	-0.001 P=0.749	-0.0001 P=0.840	0.036 **P=0.001****	0.207 **P=0.032****	-0.010 p=0.611	0.0003 P=0.675	-0.018 P=0.699
HCT		-0.643 P=0.467	0.039 P=0.106	-0.001 P=0.968	-0.033 P=0.864	-0.664 P=0.355	0.157 P=0.518	-0.0002 P=0.965	0.402 P=0.245
CD4+ cells		0.065 **P=.029****	0.0 P=0.614	-0.0007 P=0.911	-0.007 **P=0.017*****	-0.043 P=0.055	-0.008 P=0.464	-0.0002 P=0.266	0.004 P=0.661
Septrin treatment		8.29 **P=0.628**	0.315 p=0.434	-0.028 P=0.398	0.809 P=0.619	2.637 P=0.838	2.943 P=0.555	-0.112 p=0.021	4.463 P=0.433

4.5.2. Association of selected Cytokines and participants' Clinical Parameters

The type of cytokine whether proinflammatory or anti-inflammatory was not statistically significantly associated with gender or age group of participants; Chi square test $X^2(1) = 0.023$, $p = 0.879$ and $\chi^2(2) = 2.716$, $p = 0.257$.

Some individual cytokines were influenced by specific clinical parameters of participants. The association between $CD4^+$ cell counts of the participants and IL-6 was determined using Pearson product-moment correlation coefficient and the test showed a mild, negative and statistically significant association, $p < 0.05$) Table 4.5.3.

The association between participants' creatinine levels and IL-6 and IL-8 was determined using Pearson product-moment correlation coefficient, whereby the test showed a mild, positive and statistically significant association, $p < 0.05$, Table 4.5.3.

The association between WHO HIV/AIDS stage of the patients and IL-10 was determined using Spearman's rank-order correlation, the test elicited a strong, negative and statistically significant association, $p < 0.05$), Table 4.5.3.

Table 4.5.3. Logistic regression for mean difference of cytokines and participant covariates.

Interleukin	Covariables	Mean diff	P value	95% CI
IL17a				
	Hypertension	37.98	**0.0264 ***	(5.059,70.894)
	Gender(male)	-44.54	**0.0175 ***	(-80.518, -8.551)
	Age	0.268	0.858	(-2.660, 3.196)
	CD4	0.070	0.064	(-0.003, 0.144)
	BMI	-3.020	0.010	(-6.619,0.577)
	Hct	0.417	0.653	(-1.397,2.233)
	Creatinine	0.108	0.674	(-0.395,0.613)
IL-2	**Hypertension**	0.638	0.177	(-0.281,1.557)
	Gender(male)	0.827	0.110	(-0.177,1.832)
	Age	0.044	0.297	(-0.038,0.126)
	CD4	-0.001	0.608	(-0.003,0.002)
	BMI	0.007	0.890	(-0.093,0.108)
	HCT	0.035	0.183	(-0.016,0.085)
	Creatinine	-0.006	0.404	(-0.020,0.008)
IL-4	**Hypertension status**	0.050	0.133	(-0.015,0.115)
	Gender(male)	-0.088	**0.01653 ***	(-0.160, -0.018)
	Age	0.004	0.19614	(-0.002,0.010)
	CD4	0.00005	0.47966	(-0.0001,0.000)
	BMI	-0.0124	**0.00102 ****	(-0.019, -0.005)
	HCT	0.0023	0.218	(-0.001,0.006)
	Creatinine	0.0005	0.366	(-0.001,0.002)
IL-6	**Hypertension status**	1.929	0.211	(-1.068, 4.927)
	Gender(male)	0.260	0.778	(-3.043,0.004)
	Age	-0.176	0.209	(-0.448, -0.009)
	CD4	-0.007	0.056	(-0.014, 0.000)
	BMI	0.005	0.975	(-0.324,0.013)
	HCT	-0.019	0.824	(-0.190, -0.015)
	Creatinine	0.012	0.620	(-0.036, -0.006)
	Hypertension status	19.728	0.164	(-7.827, 47.2)

IL-8	**Gender(male)**	3.069	0.842	(-27.051,33.190)
	Age	-1.859	0.141	(-4.310,0.592)
	CD4	-0.041	0.185	(-0.103,0.020)
	BMI	-0.680	0.659	(-3.692, 2.332)
	HCT	-0.534	0.493	(-2.054,0.985)
	Creatinine	0.211	0.328	(-0.210,0.634)
IL-10	**Hypertension status**	-0.922	0.153	(-2.175,0.330)
	Gender(male)	0.0205	0.977	(-1.349, 1.390)
	Age	0.0922	0.109	(-0.019,0.204)
	CD4	-0.0018	0.200	(-0.005,0.001)
	BMI	-0.066	0.345	(-0.203,0.0706)
	HCT	-0.007	0.835	(-0.076, 0.062)
	Creatinine	-0.002	0.838	(-0.021,0.018)
TNFα	**Hypertension status**	0.079	0.426	(-0.115,0.274)
	Gender(male)	-0.054	0.620	(-0.267,0.159)
	Age	-0.0002	0.979	(-0.018,0.017)
	CD4	0.0002	0.328	(-0.001,0.000)
	BMI	-0.017	0.127	(-0.038,0.005)
	HCT	0.002	0.790	(-0.009,0.012)
	Creatinine	0.002	0.226	(-0.001,0.005)
IFN γ	**Hypertension status**	12.22	0.066	(-0.655,25.097)
	Gender(male)	-17.93	**0.0146***	(-32.003, -3.853)
	Age	0.824	0.162	(-0.321,1.970)
	CD4	0.014	0.332	(-0.014,0.043)
	BMI	-1.983	**0.007****	(-3.390, -0.575)
	HCT	0.858	**0.020***	(0.148,1.568)
	Creatinine	-0.007	0.948	(-0.204,0.191)

IL-17a, Interleukin 17a, **IL-2** -Interleukin2, **IL-4**-Interleukin 4, **IL-6**- Interleukin 6, **IL-8**-interleukin 8, **IL-10**- Interleukin 10, **TNFα**- Tumor necrosis alpha, **IFNγ**-interferon gamma. ***Strong statistical significance, **** very strong statistical significance

4.5.3. Correlation between Interleukin-6 and Interleukin-8.

The association between IL-6 and IL-8 cytokines values was determined using Pearson product-moment correlation coefficient. The test showed a strong, positive and statistically significant association, $p < 0.001$. From the analysis, it is apparent that low values of one of the two cytokines in a participant mirrored similar observation for the other and vice versa for higher values. This observation presents collinearity between IL-6 and IL-8. Figure.4.5.2.

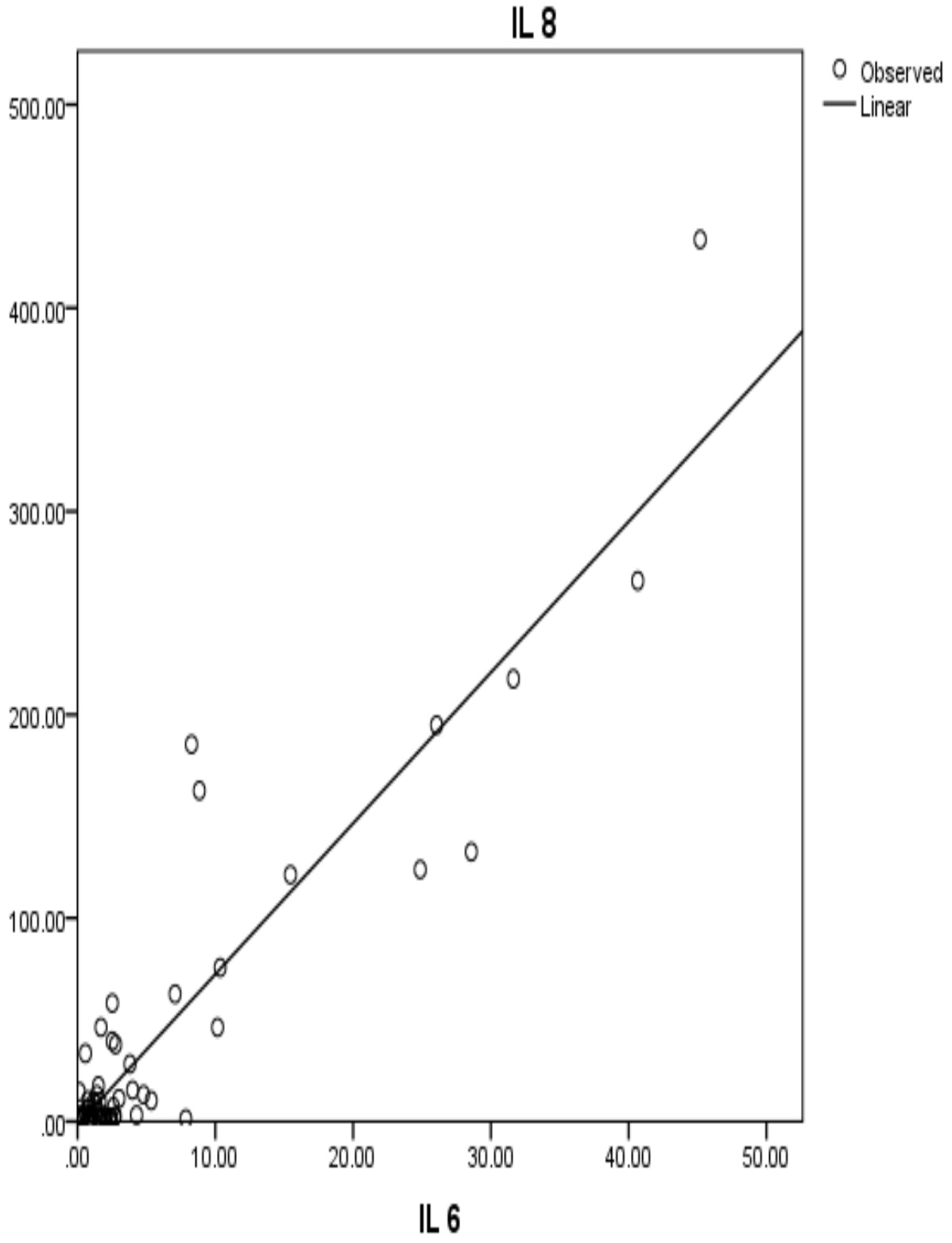

Figure 4.5.3. Regression plot model for IL-6 and IL-8.

4.5.4. Comparison between Participants' Hypertension status versus cytokines' category

The relationship between participant' hypertension status and IL-2 was determined using Spearman's rank-order correlation and the test showed a mild, negative and statistically significant association, $p < 0.05$).

The relationship between hypertension status and IL-6 was determined using Spearman's rank-order correlation, where the test showed a mild, negative and statistically significant association, $p < 0.01$).

The association between hypertension status of the participants and INF γ was determined using Spearman's rank-order correlation, where the test showed a mild, negative and statistically significant association, $p < 0.05$).

When a Chi-Square test of independence was used to determine the relationship between hypertension status, pro-inflammatory and anti-inflammatory cytokines measured in participants' plasma, the test showed a mild, negative and non-statistically significant association, $p = 0.228$, figure 4.5.4.

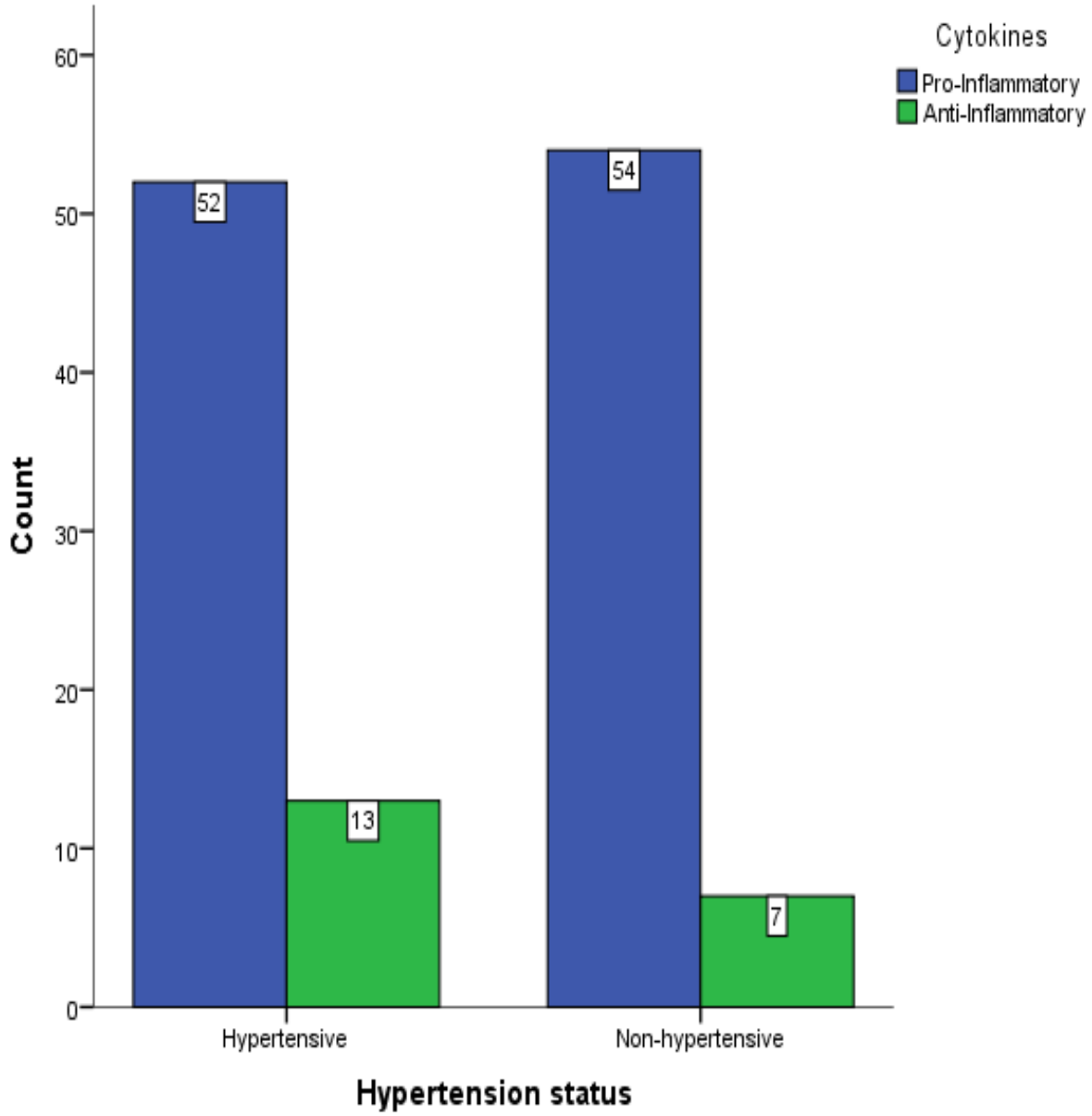

Figure 4.5.4. Histogram showing group of cytokines by hypertension status.

4.5.5. Potential independent cytokine marker for assessing risk of hypertension in participants

When determining the probable reliable cytokine to be monitored in determining risk of developing hypertension and hypertension status in participants', INF-γ was the only cytokine type that showed both statistically significant mean differences between hypertensive and non-hypertensive patients, $p > 0.05$. The mean values by hypertension status was statistically significant with a negative correlation, p(0.003,0.015). The mean values of IL-2 and IL-17A differed when analyzed by hypertension status of the participants, with a statistically significant association p (0.001, 0.001), respectively. In addition, IL-17A showed a negative correlation by hypertension status, $p = 0.052$. Additionally, IL-2 and IL-6 both demonstrated negative correlation when analyzed based on the hypertension status of participants, p= (0.038, 0.004) respectively.

CHAPTER 5: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

This study showed that hypertension is a highly prevalent comorbidity at 23.2% in HIV positive patients and occurs in early age with the average age of hypertensive group being 42 years in this study. This observation may be attributed to continued chronic inflammation as a result of HIV virus infection and use of combination therapy. Other predisposing factors include increase in stress levels and poor organ functions associated with HIV/AIDS disease. A higher CD4+ cell count (mean of 511 cells/ ml) was observed and this demonstrated good suppression of the virus in participants using of ART therapy and positive living. However, this level of CD4 + cell count presented a heightened immune activity which may pose a risk of self-destruction of soft tissue linings as a result of immune reconstitution syndrome. This syndrome may manifest as a net effect of ineffective elimination of the HIV virus with increased release of oxidative radicals within the soft tissue mucosa linings. The affected soft tissues include blood capillaries whose structural qualities change giving rise to atherosclerosis. This inference is similar to what other studies have documented [58, 67, 95]**.** This finding correlates with other studies that have shown that prolonged ART use results in impaired metabolism resulting in weight gain and increased risk of cardio metabolic disorders.

The high prevalence of hypertension was associated with individual participants' clinical characteristics, which included increased CD4+ cell count and BMI levels [92]. These two are significant risk factors that influence the metabolic functions of the body and indirectly contribute to cardio metabolic complications, including increased cholesterol levels in blood [96]**.**

A median BMI level above 27 is associated with impaired metabolism of lipids resulting in increased levels of low density lipoproteins [79]**.** The sustained CD4+ cell counts above 500cells/ml in most participants normalizes body's physiology and reduces occurrence of

opportunistic infections and loss of weight [97]. This may directly contribute to the high mean levels of BMI for HIV patients [98]. The hypertensive group had significantly increased mean BMI levels within overweight range of 26-30. The patterns observed on BMI were not linked to duration of treatment, contrary to known knowledge that linked prolonged ART treatment to metabolic changes and cumulative toxic effects on the kidneys [12]. This observation is associated with effectiveness of ART to restore immune function and not necessarily the duration of treatment. As CD4+ cell count values improved as a sign of wellbeing, the body's physiological functions were optimized. Thus, the high levels of Creatinine associated with poor immune strength are reversed, showing an inverse relationship.

A significant association of WHO HIV/AIDS stage and CD4+ cell count was observed, $p < 0.01$. This finding is supported by other studies which showed a decline in CD4+ cell count with advancing HIV/AIDS disease [105-108]. Stages one and two of HIV/AIDS progression cumulatively were associated with an increased mean of CD4+ cell count compared to the later stages three and four. WHO HIV/AIDS stage was positively associated with participants' creatinine levels, $p > .05$. This observation showed that advancing HIV disease may impair creatinine clearance thus giving rise to increased average levels which is an indicator of poor kidney functions [99, 100]. It may also be that advanced disease stage may increase the level of production of creatinine. The WHO HIV/AIDS stage too was negatively associated with participants' BMI level, ($p > .05$). This demonstrated that advancing HIV/AIDS disease may be associated with body weight loss and wasting in most participants.

The study findings showed a statistically significant gender difference in CD4+ cell counts, with females showing higher mean CD4+ cell count than males, $p < .01$. The association between gender and CD4+ cell counts was mild and statistically significant, ($p < 0.01$). This may be associated with good adherence and healthy seeking behavior by the females as compared to male patients [100]. The advancing HIV disease marked by the increased risk of

non-communicable diseases due to chronic inflammation adds to the already existing mortality and economic inequalities with the general population [101]. The cardio metabolic risk factors are being experienced in early age categories due to sustained chronic inflammation, resulting from HIV infection.

Stress is often associated with occurrence of hypertension and that hypertensive HIV/AIDS patients are more likely to have higher stress levels than non-hypertensive HIV patients. However, a $\chi 2$ test of independence performed to examine the difference in proportions and relation between participants' hypertension status and reported stress levels, showed that the difference in proportions for hypertensive and non-hypertensive HIV/AIDS patients was not statistically significant, $p = 0.053$.

The observed mean level of hematocrit in participants was below the lower limit of 40 for women and 42 for men. This observation is an indicator of inadequate synthesis of erythrocytes in the body that may be attributed to chronic inflammation in HIV/AIDS. An individual's hematocrit level may be influenced by the social behavior, environmental factors as well as metabolic syndrome. A high hematocrit value regardless of the clinical factors poses an increased risk of hypertension in participants [94]. HIV/AIDS is known to independently alter the body's biochemical processes as a result of chronic inflammatory reactions. The use of ART too has been associated with hyperlipidemia marked by high levels of low density lipoproteins. This factor may affect the elasticity and free flow of blood in the capillaries.

BMI levels above 25, showed a positive association with IL-6, which is commonly associated with atherosclerosis as demonstrated in other studies [105]. This observation suggested that an improved CD4+ cell count influences positive change in BMI values. This can be explained scientifically that a high CD4+ cell count is a measure of normal body function, improved wellbeing and increased possibility of positive weight gain. This observation showed a positive influence on weight gain when participants are initiated on ART therapy for a longer sustained

period with good adherence. Improvement on care for HIV/AIDS patients is often assessed by evaluating positive change in their BMI. However, unchecked increase in BMI may become detrimental to individuals' overall health due to associated complications that include hypertension. Identification of severe stages of immunosuppression due to HIV infection is dependent on the CD4+ cell count.

The use of ART and cotrimoxazole had a positive association with CD4 count. Use of ART restores the body's immune response in a process referred to as immune reconstitution. On the other hand, Cotrimoxazole suppresses the occurrence of opportunistic infections thus enabling the preservation of individual's immune response. This results in active immune response to invading virus resulting in sustained release of pro-inflammatory cytokines such as TNFα and INF γ.

Evaluation of cytokine expression previously has shown that IL-6 and IL-10 production is usually increased in both slow and rapid HIV/AIDS progressors [104]. This may indicate altered immune sensitivity and ineffective response to infectious agents. This allows the HIV replication, ease of transmission and rapid progression. The observed changes in levels of selected cytokines may be used to predict risk of development of hypertension among HIV infected participants.in HIV disease. Variation in TH1 and TH2 expression which is dependent on hypertension status may be used to indicate patients' level of predisposition to cardiovascular disease.

Creatinine level was observed to be higher in patients with low CD4 T cell count and high proinflammatory cytokines expression. This observation meant that creatinine levels of participants may influence the release of IL- 6 and IL-8; as participants with increased creatinine levels were more likely to have higher IL-6 and IL-8 levels than participants with

lower creatinine levels. High creatinine levels are an indicator of poor kidney function and a predisposing factor to development of hypertension in HIV/AIDS patients.

The statistically significant association between IL-8 and social behavior of participants observed shows that social behavior of patients influences the release of IL 8 and both smokers and alcoholic patients were more likely to have higher IL-8 levels than non-smokers and non-alcoholic patients.

This study showed statistically significant increase in levels of IL-6 and IL-17A in hypertensive participants, p(0.014, 0.004), respectively, an observation documented in other studies and linked to renal and vascular dysfunction resulting in blood pressure elevation [102]. This study showed that release of IL-10 was lower in hypertensive participants as compared to non-hypertensive participants, $p < 0.26$. This observation is like findings of a comparative study involving acute and chronic immunosuppressed patients [103].

Marked and progressive increase in IFN-γ, IL-6 and IL-8 for followed up patients is a strong indicator of predisposition to development of high blood pressure. With the highlighted cytokines and their potential, further scientific inquiry is key to establish their effectiveness in predicting risk of being hypertensive for a client with HIV infection. This will lay basis for primary diagnosis and effective interventions to control potential damage and hinder development of cardiovascular complications [107, 108,109].

It is apparent that hypertension is significantly associated with disability, inequality and poor quality of life among HIV infected patients. Association of hypertension status with clinical characteristics of participants showed increased risk of developing hypertension with median CD4+ cell count above 250 cells/ml of blood and median BMI of 27 and above. This means that severe stages of HIV/AIDs were marked by severe immunosuppression secondary to HIV viral invasion and destruction of CD4+ cells. Most patients on ART therapy maintain high levels of CD4+ cell counts and were more at risk of developing hypertension.

This study showed that hypertension develops early in age in those living with HIV disease. This observation is like what other studies established [110,111,112]. Accelerated aging is a significant factor seen in HIV infected persons and this may be associated with the early age development of chronic disorders like hypertension seen in the study population. The mean age for the hypertensive patients was demonstrated at 42 years with a mean deviation of +/- 6 years. [111]. The occurrence of multiple comorbidities simultaneously adds up to the increased burden of aging and deterioration of body functions accelerating HIV/AIDS progression [75]. Comorbidities associated with increase in age overlap with morbidity from HIV disease and are predicted by lower CD4+ cell counts and low hematocrit values. This is an indicator of the physiological strain on the body, resulting in changes in selected clinical parameters [112].

Gender showed a positive correlation with IL-17A expression showing that gender influences the release of IL-17A, and female patients are more likely to have higher IL-17A levels than male patients in HIV/AIDS population.

The care of HIV infected persons must adopt multiple comorbidity screening approaches, even if the patient does not present with obvious signs and symptoms of any of the known common comorbidities. This may help to diagnose and manage the disorders in time and reduce complications [58, 71, 113,114]. Additional risk of adverse cardiovascular events in HIV patients begins with the increased prevalence of hypertension in this population [115]. Hypertension develops early in age in those living with HIV disease [115].

This study showed that IL-6 and IL8 have a positive collinearity hence patients had similar patterns of cytokines' levels secreted by hypertension status. Monitoring of the levels in HIV infected patients may present a picture that can be used to detect risk of hypertension in patients with HIV infection. Therefore, routine measurement may be integrated in the primary care plans of HIV/AIDS patients. The variation in either TH1 or TH2 cytokine expression affects

the pattern of change in clinical parameters of HIV patients on care and overall response to ART. IFN-γ levels may independently predict the level of predisposition to development of hypertension in patients on HIV care.

This demonstrated that participants' hypertension status may positively influence secretion of IL-2 cytokines. Higher secretion of IL-2 in hypertensive HIV/AIDS patients demonstrates poor immune response to foreign antigens, a state of immune unresponsiveness in the body.

From the analysis, we inferred that INF-γ was the likely cytokine to be examined concretely to ascertain its usefulness in determining the risk of developing hypertension among HIV infected patients

5.2. Conclusion

This study established a high burden of hypertension in HIV/AIDS patients, its risk indicators and its association with HIV/AIDS the progression. Hypertension thus, remains a silent killer as a result of its prevalence in HIV positive population. Its complications are irreversible and increases non-HIV/AIDS morbidity and mortality. A higher CD4+ cell count and BMI values predisposes the patient to greater risk of developing hypertension for the HIV infected population. Majority of patients on ART therapy maintain high levels of CD4+ cell counts and BMI values and are therefore at risk of developing hypertension. The changes in the immune response that takes place in HIV disease, that is either cellular or antibody mediated brings out destructive effects on mucosal tissue linings. Management of HIV infected patients has always utilized the CD4 cells count and viral load as the key parameters to assess patients' progress on care. IFN-γ together with IL-6 and IL-8 are increased in hypertensive patients and could be diagnostic of increased risk of developing hypertension. In conclusion, the findings of this study showed independent associations of both IL-6 and IL-8 with risk of hypertension providing a hypothesis for further evaluation through research. This study also highlights the need for further robust cytokines' studies

to establish risk of hypertension and enhance our understanding of the biology of hypertension and lead to the discovery of novel treatments.

5.3. Recommendations.

1. The high prevalence of hypertension in HIV/AIDS patients calls for concerted efforts to educate patients on prevention and control interventions.

2. Intervention studies should be done to evaluate the effectiveness of using IL6, IL8 and IFN- γ as potential cytokine markers for diagnosing risk of developing hypertension in HIV /AIDS patients.
3. There is need for further research to explore use of anti-inflammatory agents together with ART to reduce effects of chronic inflammation in HIV/AIDS patients.

5.4. Study limitations.

1. This was a cross sectional study and was not possible to establish causal relationship between variables under study but only associations between study variables.
2. The study site was only one thus limiting external validity of the findings; limits generalizability of findings
3. The calculated sample size was small, owing to limitations in funding, therefore limiting the power of the study

REFERENCES

1. Ipp, H. and A. Zemlin,.(2013). The paradox of the immune response in HIV Infection: when inflammation becomes harmful.Clinica chimica acta;international journal of clinical chemistry 2013.416: p.96-9. doi:10.1016/j.cca.2012.11.025. Epub 2012 Dec 7
2. Ipp H (1), Zemlin AE, Erasmus RT, Glashoff RH, (2014). Role of inflammation in HIV-1 disease progression and prognosis. Critical Reviews in Clinical Laboratory Science, 2014. doi: 10.3109/10408363.2013.865702
3. Freiberg, M.S., Chang, C. C., Kuller, L. H., Skanderson, M., Lowy, E.,et al (2013). HIV Infection and the Risk of Acute Myocardial Infarction. JAMA internal medicine 2013: p.1-9. doi 10.1001/jamainternmed.2013.3728
4. El Chami, H. and P.M. Hassoun, (2012). Inflammatory mechanisms in the pathogenesis of pulmonary arterial hypertension. Progress in cardiovascular diseases journal. 1(4): p. 1929-41. doi. 10.1016/j.pcad.2012.07.006
5. Shive, C.L., et al., Inflammatory Cytokines Drive CD4 T cell Cycling and Impaired Responsiveness to Interleukin-7: Implications for Immune Failure in HIV Disease. J Infect Dis, 2014.
6. Hyle EP, Bekker LG, Martey EB, et al 2019. Cardiovascular risk factors among ART-experienced people with HIV in South Africa. International AIDS Society. 2019 Apr;22(4):e25274. doi: 10.1002/jia2.25274.
7. Rodríguez-Arbolí E, Mwamelo K, Kalinjuma AV et al 2017. Incidence and risk factors for hypertension among HIV patients in rural Tanzania - A prospective cohort study. PLoS One. 2017 Mar 8;12(3):e0172089. doi: 10.1371/journal.pone.0172089. eCollection 2017.
8. Hunt, P.W., (2012). HIV and inflammation: mechanisms and consequences. Current HIV/AIDS Reports journal, 2012. 9(2): p. 139-47. doi: 10.1007/s11904-012-0118-8.

9. Ito,T. and U. Ikeda.(2003). Inflammatory cytokines and cardiovascular disease. Current drug targets, Inflammation and allergy journal. **2**(3): p. 257-65. Accession no.14561160
10. Mateen FJ1, Kanters S, Kalyesubula R,etal (2013).Hypertension prevalence and Framingham risk score stratification in a large HIV-positive cohort in Uganda. Journal ofHypertension.Jul;31(7):1372-8;discussion1378.doi: 10.1097/HJH.0b013e328360de1c.
11. Roberts L1, Passmore JA, Williamson C, Little F, Bebell LM, Mlisana K, (2010). Plasma cytokine levels during acute HIV-1 infection predict HIV disease progression. AIDS, 2010. 24(6): p. 819-31. doi: 10.1097/QAD.0b013e3283367836.
12. Amberbir, A., Banda, V., Singano, V., Matengeni, A., Pfaff, C., Ismail, Z., … van Oosterhout, J. J. (2019). Effect of cardio-metabolic risk factors on all-cause mortality among HIV patients on antiretroviral therapy in Malawi: A prospective cohort study. PloS one, 14(1), e0210629. doi: 10.1371/journal.pone.0210629
13. Julius H1, Basu D, Ricci E, et al. 2011. The burden of metabolic diseases amongst HIV positive patients on HAART attending The Johannesburg Hospital. Current HIV Research. June;9(4):247-52. doi: 10.2174/157016211796320360.
14. Brennan AT, Jamieson L, Crowther NJ, et al 2018. Prevalence, incidence, predictors, treatment, and control of hypertension among HIV-positive adults on antiretroviral treatment in public sector treatment programs in South Africa. PLoS One. 2018 Oct 3;13(10):e0204020. doi: 10.1371/journal.pone.0204020. eCollection 2018.
15. Bloomfield GS1, Hogan JW, Keter A, Sang E, Carter EJ, Velazquez EJ, Kimaiyo S. (2011). Hypertension and obesity as cardiovascular risk factors among HIV seropositive patients in Western Kenya. PLoS One. 2011;6(7):e22288. doi: 10.1371 14.

16. Lopez-Jaramillo, P., Velandia-Carrilo,C., Avarez-Camacho,J., Cohen D. D., Sanchez-solano,T., and Castilo Lopez, G., (2013). Inflammation and hypertension: are there regional differences? International Journal of Hypertension, 2013, 1-12, doi:10.1155/2013/492094.
17. El Chami, H. and P.M. Hassoun,(2012). Inflammatory mechanisms in the pathogenesis of pulmonary arterial hypertension. Progress in cardiovascular diseases journal. 1(4): p. 1929-41. doi. 10.1016/j.pcad.2012.07.006
18. Medina-Torne, S., Ganesan, A., Barahona, I. and Crum-Cianflone, N. F.,(2012). Hypertension is Common among HIV-Infected Persons, But Not Associated with HAART. Journal of the International Association of Physicians in AIDS Care (Chicago, Ill.: 2002). 11(1): p. 20-5.doi 10.1177/1545109711418361
19. Zakynthinos, E. and N. Pappa,(2009). Inflammatory biomarkers in coronary artery disease. Journal of Cardiology, 2009. 53(3): p. 317-33.doi: 10.1016/j.jjcc.2008.12.007.
20. Colas Tcherakian, Louis-Jean Couderc, Marc Humbert, Véronique Godot, Olivier Sitbon and Philippe Devillier., 2013. Inflammatory mechanisms in HIV-associated pulmonary arterial hypertension. Seminars in Respiratory and Critical Care Medicine, 2013. 34(5): p. 645-53. doi: 10.1055/s-0033-1356489
21. Chuang, S. Y., Hsu, P. F., Chang, H. Y., Bai, C. H. and Yeh, W. T.,(2013). C-reactive Protein Predicts Systolic Blood Pressure and Pulse Pressure but not Diastolic Blood Pressure: the Cardiovascular Disease Risk Factors Two-Township Study. American Journal of hypertension 2013. doi 10.1093/ajh/hps095.
22. Agarwal SK(1), Singh A, Anuradha S, Singh NP, Sokhi J, Baveja UK.,(2001).Cytokine profile in human immunodeficiency virus positive patients with and without tuberculosis. The Journal of the Association of Physicians of India, 2001. Aug;49:799-802.

23. Tasca KI1, Calvi SA, Souza Ldo R.,(2012). Immunovirological parameters and cytokines in HIV infection. Revista da Sociedade Brasileira de Medicina Tropical, 2012. 45(6): p. 663-9. doi.org/10.1590/S0037- 86822012000600002
24. Armah, K. A., McGinnis, K., Baker, J.,Gibert, C., Butt, A. A., Bryant, K.et al,(2012). HIV status, burden of comorbid disease, and biomarkers of inflammation, altered coagulation, and monocyte activation. Journal of Clinical Infectious Diseases. 55(1): p. 126-36. doi 10.1093/cid/cis406.
25. Kim DJ, Westfall AO, Chamot E, Willig AL, Mugavero MJ, Ritchie C, et al, (2012). Multimorbidity patterns in HIV-infected patients: the role of obesity in chronic disease clustering. Journal of acquired immune deficiency syndromes,2012. 61(5): p. 600-5. doi: 10.1097/QAI.0b013e31827303d5
26. Nou, E., Lo, J., & Grinspoon, S. K. (2016). Inflammation, immune activation, and cardiovascular disease in HIV. AIDS (London, England), 30(10), 1495–1509. doi:10.1097/QAD.0000000000001109
27. Lacerda HR1, Falcão Mda C, de Albuquerque VM, Zírpoli JC, Miranda-Filho Dde B, et al., (2011). Association of Inflammatory Cytokines and Endothelial Adhesion Molecules with Immunological, Virological, and Cardio-metabolic Disease in HIV-Infected Individuals. Journal of Interferon and Cytokine Research, 34(5):385-93. doi: 10.1089/jir.2013.0029.
28. Rossi G P(1), Seccia TM, Maniero C, Pessina AC.,(2011). Drug-related hypertension and resistance to antihypertensive treatment: a call for action. Journal of hypertension, 2011.29(12): p.2295-309. doi:10.1097/HJH.0b013e32834c465d
29. Hearps AC (1), Martin GE, Rajasuriar R and Crowe S.M., (2014). Inflammatory Co-morbidities in HIV+ Individuals: Learning Lessons from Healthy Ageing. Current HIV/AIDS reports, 2014. 11(1): p. 20-34.doi: 10.1007/s11904-013-0190-8

30. Bastard J.P(1), Soulié C, Fellahi S, Haïm-Boukobza S, Simon A and Katlama C,(2012). Circulating interleukin-6 levels correlate with residual HIV viraemia and markers of immune dysfunction in treatment-controlled HIV-infected patients. Current HIV/AIDS reports, 2012. 17(5): p. 915-9. doi: 10.3851/IMP2093.
31. Kedzierska, K. and S.M. Crowe.,(2001). Cytokines and HIV-1: interactions and clinical implications. Antiviral chemistry & chemotherapy, 2001. 12(3): 133-50. doi: 10.1177/095632020101200301
32. Conley L.J(1), Bush TJ, Rupert AW, Sereti I, Patel P, Brooks JT and Baker JV. (2015). Obesity is associated with greater inflammation and monocyte activation among HIV-infected adults receiving antiretroviral therapy. Journal of infectious diseases. 2015. 29(16): p. 2201-7. doi: 10.1093/infdis/jiw096
33. Balt, C.A., (2013). Hypertension and HIV infection. The Journal of the Association of Nurses in AIDS Care: JANAC 2013. 24(1 Suppl): p. S127-34. Doi: 10.1016/j.jana.2012.06.012
34. Fisher SD, Bowles NE, Towbin JA and Lipshultz SE. (2003). Mediators in HIV-associated cardiovascular disease: a focus on cytokines and genes. AIDS, 2003. 17 Suppl 1: p. S29-35.
35. Kwiatkowska W1, Knysz B, Drelichowska-Durawa J, Czarnecki M, Gasiorowski J, Biłyk E., et al (2013).[Overweight, obesity and underweight in HIV infected patients]. Przeglad lekarski 2013. 70(3): p. 113-7.
36. Wandeler, G., Johnson L.F. and Egger M.,(2016). Trends in life expectancy of HIV-positive adults on antiretroviral therapy across the globe: comparisons with general population. Current opinion in HIV and AIDS, 2016. 11(5): p. 492-500.doi: 10.1097/coh.0000000000000298

37. Okpa HO, Bisong EM, Enang OE, Monjok E and Essien EJ., (2017). Predictors of hypertension in an urban HIV-infected population at the University of Calabar Teaching Hospital, Calabar, Nigeria. Journal HIV/AIDS - Research and Palliative Care, 2017. 9: p. 19-24.doi: doi.org/10.2147/HIV.S126374
38. Ogunmola OJ1, Oladosu OY1 and Olamoyegun A.M., (2014). Association of hypertension and obesity with HIV and antiretroviral therapy in a rural tertiary health center in Nigeria: a cross-sectional cohort study. Journal ofVascular health and risk management, 2014. 10: p. 129-37. doi: 10.2147/VHRM.S58449
39. Balt, C.A., (2013). Hypertension and HIV infection. The Journal of the Association of Nurses in AIDS Care: JANAC 2013. 24(1 Suppl): p. S127-34. Doi: 10.1016/j.jana.2012.06.012.
40. Flexor G, Zucman D, Berthé H, Meier F,Force G, Greder-Belanet A et al.,(2013). [Aging and HIV infection: 4years follow-up of 149 HIV infected patients older than 60years in West Paris agglomeration (COREVIH Ile-de-France Ouest).]. Presse Med, 2013. doi.org/10.1016/j.lpm.2012.11.005
41. Blanco F, San Román J, Vispo E, López M, Salto A.,et al (2010). Management of metabolic complications and cardiovascular risk in HIV-infected patients. AIDS reviews 2010. 12(4): p. 231-41.
42. Palella FJ Jr, Baker RK, Moorman AC, Chmiel JS, Wood KC., et al.,(2006). Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. Journal of acquired immune deficiencysyndromes(1999)2006.43(1):p.27-34.doi: 10.1097/01.qai.0000233310.90484.16

43. Safar, M.E., Blacher J. and Jankowski P.,(2011). Arterial stiffness, pulse pressure, and cardiovascular disease-is it possible to break the vicious circle? Atherosclerosis, 2011. 218(2): p. 263-71. doi: 10.1016/j.atherosclerosis.2011.04.039

44. Christian Akem Dimala , Julius Atashili, Josephine C. Mbuagbaw, Akam Wilfred et al.,(2016). Prevalence of Hypertension in HIV/AIDS Patients on Highly Active Antiretroviral Therapy (HAART) Compared with HAART-Naive Patients at the Limbe Regional Hospital, Cameroon. PLoS One, 2016. 11(2): p. e0148100. doi.org/10.1371/journal.pone.0148100

45. Myerson M, Poltavskiy E, Armstrong EJ, Kim S, Sharp V and Bang H.,(2014). Prevalence, treatment, and control of dyslipidemia and hypertension in 4278 HIV outpatients. Journal of Acquired Immune Deficiency Syndrome, 2014. 66(4):p.370-7. doi: 10.1097/QAI.0000000000000168.

46. Lang S1, Mary-Krause M, Cotte L, Gilquin J, Partisani M, Simon A., et al.,(2010). Impact of individual antiretroviral drugs on the risk of myocardial infarction in human immunodeficiency virus-infected patients: a case-control study nested within the French Hospital Database on HIV ANRS cohort CO4. Archives of internal medicine, 2010. 170(14): p. 1228-38. doi: 10.1001/archinternmed.2010.197

47. Palella FJ Jr and Phair JP.,(2011). Cardiovascular disease in HIV infection. Current opinion in HIV and AIDS, 2011. 6(4): p. 266-71. doi: 10.1097/COH.0b013e328347876c.

48. Carr RL and Traufler R.,(2011). Immune reconstitution inflammatory syndrome in HIV-infected patients: what a critical-care nurse needs to know. Dimensions of critical care nursing, 2011. 30(3): p. 139-43. doi: 10.1097/DCC.0b013e31820d213f.

49. Agita, A. and Alsagaff M.T.,(2017). Inflammation, Immunity, and Hypertension. Acta medica Indonesiana, 2017. 49(2): p. 158-165.

50. De Ciuceis C1, Rossini C, La Boria E, Porteri E, Petroboni B, Gavazzi A., et al.,(2014). Immune mechanisms in hypertension. High blood pressure & cardiovascular prevention, 2014. 21(4): p. 227-34. doi: 10.1007/s40292-014-0040-9
51. Virdis, A., Dell'Agnello U and Taddei S.,(2014). Impact of inflammation on vascular disease in hypertension. Maturitas, 2014. 78(3): p. 179-83. doi: 10.1016/j.maturitas.2014.04.012
52. Megan L. Salter, Bryan Lau, Shruti H. Mehta, Vivian F. Go,Sean Leng, and Gregory D. Kirk.,(2013). Correlates of elevated interleukin-6 and C-reactive protein in persons with or at high-risk for HCV and HIV infections.
53. Journal of Acquired Immune Deficiency Syndrome,2013. doi: 10.1097/QAI.0b013e3182a7ee2e
54. Barakat, M.G. and Arora R.R.,(2013). Coronary Artery Disease in the Human Immunodeficiency Virus Seropositive Population. American journal of therapeutics, 2013. doi: 10.1097/MJT.0b013e3182973924.
55. LaMarca BD, Ryan MJ, Gilbert JS, Murphy SR and Granger JP.,(2007). Inflammatory cytokines in the pathophysiology of hypertension during preeclampsia. Current hypertension reports 2007. 9(6): p. 480-5.
56. Post, W.S.,(2011). Predicting and preventing cardiovascular disease in HIV-infected patients. Topics in antiviral medicine, 2011. 19(5): p. 169-73.
57. Andreas Deckert ,Florian Neuhann ,Christina Klose, Thomas Bruckner, Claudia Beiersmann, John Haloka., et al.,(2017). Assessment of renal function in routine care of people living with HIV on ART in a resource-limited setting in urban Zambia. PLoS One, 2017. 12(9): p. e0184766. doi.org/10.1371/journal.pone.0184766

58. Ameh S, Klipstein-Grobusch M, Eustasius K. K, Tollman S and Gómez-Olivé F X.,(2017). Quality of integrated chronic disease care in rural South Africa: user and provider perspectives. Journal of Acquired Immunodeficiency Syndrome, 2017. 32(2): p. 257-266. doi: 10.1097/QAI.0000000000001437
59. Salter ML1, Lau B, Go VF, Mehta SH and Kirk GD,(2011). HIV infection, immune suppression, and uncontrolled viremia are associated with increased multimorbidity among aging injection drug users. Clinical infectious diseases, 2011. 53(12): p. 1256-64. doi: 10.1093/cid/cir673
60. Rasmussen LD, May MT, Kronborg G, Larsen CS, Pedersen C, Gerstoft J., et al.,(2015). Time trends for risk of severe age-related diseases in individuals with and without HIV infection in Denmark: a nationwide population-based cohort study. The Lancet HIV, 2015. **2**(7): p. e288-98. doi: 10.1016/S2352-3018(15)00077-6
61. Hart BB, Nordell AD, Okulicz JF, Palfreeman A, Horban A, Kedem E., et al.,(2018). Inflammation Related Morbidity and Mortality Among HIV-Positive Adults: How Extensive Is It? Journal of Acquired Immune Deficiency Syndromes(1999), 2018. Jan 1;77(1):1-7.doi: 10.1097/qai.0000000000001554
62. Peters B, Post F, Wierzbicki AS, Phillips A, Power L, Das S. et al.,(2013). Screening for chronic comorbid diseases in people with HIV: the need for a strategic approach. HIV Medicine, 2013. 14 Suppl 1: p. 1-11. doi: 10.1111/j.1468-1293.2012.01055.x.
63. Nancy A H, Sandra K S, Ling C H, Martha S, Edward L M, Sandra K S et al.,(2017). Gender differences in causes of death among persons with HIV/AIDS in San Francisco, California, 1996-2013. International Journal of STD and AIDS, 2017: 29(2). doi/abs/10.1177/0956462417720370

64. Schwarcz S K, Vu A, Hsu L C and Hessol N A.(2014). Changes in causes of death among persons with AIDS: San Francisco, California, 1996-2011. AIDS patient care and STDs, 2014. 28(10): p. 517-23. doi: 10.1089/apc.2014.0079.
65. Hernández-Aguilera A, Rull A, Rodríguez-Gallego E, Riera-Borrull M, Luciano-Mateo F, Camps J. et al., (2013). Mitochondrial dysfunction: a basic mechanism in inflammation-related non-communicable diseases and therapeutic opportunities. Mediators of Inflammation, 2013. 2013: p. 135698. doi.org/10.1155/2013/135698
66. Aberg J.A., (2012). Aging, inflammation, and HIV infection. Topics in antiviral medicine, 2012. 20(3): p. 101-5.
67. Hileman CO, Longenecker CT, Carman TL, Milne GL, Labbato DE, Storer NJ., et al(2012). Elevated D-dimer is independently associated with endothelial dysfunction: a cross-sectional study in HIV-infected adults on antiretroviral therapy. Antiviral Therapy, 2012. 17(7): p. 1345-9. doi: 10.3851/IMP2297
68. Longenecker CT, Funderburg NT, Jiang Y, Debanne S, Storer N, Labbato DE, et al., (2013). Markers of inflammation and CD8 T-cell activation, but not monocyte activation, are associated with subclinical carotid artery disease in HIV-infected individuals. HIV Medicine, 2013. 14(6): p. 385-90. doi: 10.1111/hiv.12013
69. Fourie CM, Schutte AE, Smith W, Kruger A and van Rooyen JM (2015). Endothelial activation and cardiometabolic profiles of treated and never-treated HIV infected Africans. Atherosclerosis, 2015. 240(1): p. 154-60. doi: 10.1016/j.atherosclerosis.2015.03.015
70. Harrison DG1, Guzik TJ, Goronzy J, Weyand C. (2008). Is hypertension an immunologic disease? Current cardiology reports, 2008. 10(6): p. 464-9.

71. Harrison D.G.(2014). The immune system in hypertension. Transactions of the American Clinical and Climatological Association, 2014. 125: p. 130-38; discussion 138-40.
72. Monteiro P, Miranda-Filho DB, Bandeira F, Lacerda HR, Chaves H, Albuquerque MF et al.,(2012). Is arterial stiffness in HIV-infected individuals associated with HIV-related factors? Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica .2012. 45(9): p. 818-26.
73. Aburawi E H , AlKaabi J, Zoubeidi T, Shehab A, Lessan N, Al Essa A et al.,(2016). Subclinical Inflammation and Endothelial Dysfunction in Young Patients with Diabetes: A Study from United Arab Emirates. PLoS One, 2016. 11(7): p. e0159808. doi.org/10.1371/journal.pone.0159808
74. Akdis, Mübeccel et al. (2016). Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: Receptors, functions, and roles in diseases. Journal of Allergy and Clinical Immunology, Volume 138, Issue 4, 984 - 1010
75. Spoulou V, Kanaka-Gantenbein C, Bathrellou I, Mora S, Mostrou G, Sidossis L et al.,(2011).Monitoring of lipodystrophic and metabolic abnormalities in HIV-1 infected children on antiretroviral therapy. Hormones (Athens), 2011. 10(2): p. 149-55.
76. Mutevedzi P.C, Rodger AJ, Kowal P, Nyirenda M and Marie-Louise N. (2013). Decreased Chronic Morbidity but Elevated HIV Associated Cytokine Levels in HIV-Infected Older Adults Receiving HIV Treatment: Benefit of Enhanced Access to Care? PLoS One, 2013. 8(10): p. e77379. doi.org/10.1371/journal.pone.0077379

77. Liebetrau, C, Hoffmann J, Dörr O, Gaede L, Blumenstein J, Biermannet H et al., (2015). Release kinetics of inflammatory biomarkers in a clinical model of acute myocardial infarction. Circulation Research, 2015. 116(5): p. 867-75. doi.org/10.1161/CIRCRESAHA.116.304653

78. Cecchi E, D'Alfonso MG, Chiostri M, Parigi E, Landi D, Valente S,et al.(2013). Impact of Hypertension History on Short and Long-Term Prognosis in Patients with Acute Myocardial Infarction Treated with Percutaneous Angioplasty: Comparison Between STEMI and NSTEMI. High blood pressure & cardiovascular prevention, 2013. doi: 10.1007/s40292-013-0032-1

79. Nüesch R, Wang Q, Elzi L, Bernasconi E, Weber R, Cavassini M, Vernazza P et al.,(2013). Risk of Cardiovascular Events and Blood Pressure Control in Hypertensive HIV-Infected Patients: Swiss HIV Cohort Study (SHCS). Journal of Acquired Immune Deficiency Syndrome, 2013.doi: 10.1097/QAI.0b013e3182847cd0

80. Armah, K. A.,Chang, C. C.,Baker, J. V.,Ramachandran, V. S.,Budoff, M. J.Crane, H. M. et al., (2013). Prehypertension, Hypertension, and the Risk of Acute Myocardial Infarction in HIV-Infected and -Uninfected Veterans. Clinical infectious diseases, 2013. doi: 10.1093/cid/cit652

81. Sauce, D., Elbim C., and Appay V. (2013). Monitoring cellular immune markers in HIV infection: from activation to exhaustion. Current opinion in HIV and AIDS, 2013. 8(2): p. 125-31. doi: 10.1097/COH.0b013e32835d08a9

82. Reinsch N1, Neuhaus K, Esser S, Potthoff A, Hower M, Mostardt S,et al.,(2012). Are HIV patients undertreated? Cardiovascular risk factors in HIV: results of the HIV-HEART study. European journal of preventive cardiology, 2012. 19(2): p. 267-74. doi: 10.1177/1741826711398431.

83. Yanni X, Xiaodan S, Sanyi T, Yicang Z, Zhihang P, Jianhong W, et al.,(2017). Personalized life expectancy and treatment benefit index of antiretroviral therapy. Theoretical Biology & Medical Modelling, 2017. 14(1): p.1. doi: 10.1186/s12976-016-0047-0

84. Joseph L. G, Shawn L. F, David R, Adeel B, Cynthia G, Rodriguez-Barradaset M et al.,(2007). Aging and infectious diseases: do patterns of comorbidity vary by HIV status, age, and HIV severity? Clinical Infectious Diseases, 2007. 45(12): p. 1593-601. doi: 10.1086/523577

85. Chu C, Umanski G, Blank A, Meissner P, Grossberg R, Selwyn PA (2011). Comorbidity-related treatment outcomes among HIV-infected adults in the Bronx, NY. Journal of urban health, 2011. 88(3): p. 507-16. doi: 10.1007/s11524-010-9540-7

86. Monteiro, V.S., Lacerda H.R, Uellendah M, Chang T M, Maria de Albuquerque V, Zirpoli J.C et al.,(2011). Calcium score in the evaluation of atherosclerosis in patients with HIV/AIDS. Arq Bras Cardiol, 2011. 97(5): p. 427-33. doi.org/10.1590/S0066-782X2011005000094

87. Tripathi A, Liese AD, Winniford MD, Jerrell JM, Albrecht H, Rizvi AA et al.,(2014). Impact of clinical and therapeutic factors on incident cardiovascular and cerebrovascular events in a population-based cohort of HIV-infected and non-HIV-infected adults. Clinical Cardiology, 2014. 37(9): p. 517-22. doi: 10.1002/clc.22311.

88. Amorosa V, Synnestvedt M, Gross R, Friedman H, MacGregor RR, Gudonis D, et al.,(2005). A tale of 2 epidemics: the intersection between obesity and HIV infection in Philadelphia. Journal of Acquired Immune Deficiency Syndrome (1999), 2005. 39(5): p. 557-61.

89. Krauskopf K, Mark L.V.N, Ronald P.D.,Ackatz G S, Adrienne L ,Federman A, et al.,(2013). Correlates of hypertension in patients with AIDS in the era of highly active antiretroviral therapy. Journal of the International Association of Providers of AIDS Care. 2013. 12(5): p. 325-33. doi: 10.1177/2325957413491432
90. Simmons R D, Ciancio BC, Kall M M, Rice BD, Delpech VC (2013). Ten-year mortality trends among persons diagnosed with HIV infection in England and Wales in the era of antiretroviral therapy: AIDS remains a silent killer. HIV Medicine, 2013.14(10):p.596-604. doi: 10.1111/hiv.12045.
91. Calò, L A., Caielli P, Maiolino G, Rossi G (2013). Arterial hypertension and Cardiovascular risk in HIV-infected patients. Journal of Cardiovascular Medicine, 2013. 14(8): p. 553-8. doi: 10.2459/JCM.0b013e3283621f01
92. Dentali, F., E. Nicolini, and W. Ageno,(2012). Venous and arterial thrombosis associated with HIV infection. Seminars in Thrombosis and hemostasis, 2012. 38(5): p. 524-9. doi: 10.1055/s-0032-1306434. Epub 2012 Mar 7.
93. Ridha E, Devitt E, Boffito M and Boag F (2011). Antiretroviral therapy and cardiovascular risk. BMJ Case Reports, 2011. 2011. doi: 10.1136/bcr.10.2010.3429
94. Desvarieux M, Boccara F, Meynard JL, Bastard JP, Mallat Z, Charbit B et al.,(2013). Infection duration and inflammatory imbalance are associated with atherosclerotic risk in HIV-infected never-smokers independent of antiretroviral therapy. AIDS, 2013. **27**(16): p. 2603-14. doi: 10.1097/QAD.0b013e3283634819.
95. Fisher et al.1998.Sample size calculation for cross sectional studies.
96. Arruda Junior ER, Lacerda HR, Moura LC, Albuquerque Mde F, Miranda Filho Dde B, Diniz GT et al.,(2010). Risk factors related to hypertension among patients in a cohort living with HIV/AIDS. Brazilian Journal of Infectious Diseases, 2010. 14(3): p. 281-7.

97. Neto M.G., Zwirtes R., and Brites C.(2013). A literature review on cardiovascular risk in human immunodeficiency virus-infected patients: implications for clinical management. Brazilian Journal of Infectious Diseases, 2013. 17(6): p. 691-700. doi: 10.1016/j.bjid.2013.05.004

98. Gutierrez, J., Elkind M.S. and Marshall R.S.(2013). Cardiovascular profile and events of US adults 20-49 years with HIV: Results from the NHANES 1999-2008. AIDS Care, 2013. 25(11):1385-91.doi: 10.1080/09540121.2013.769493

99. Shin S, Song H, Oh SK, Choi KE, Kim H and Jang S(2013). Effect of antihypertensive medication adherence on hospitalization for cardiovascular disease and mortality in hypertensive patients. Hypertension Research, 2013. 36(11):1000-5. doi: 10.1038/hr.2013.85.

100. Enriquez, M., & McKinsey, D. S. (2011). Strategies to improve HIV treatment adherence in developed countries: clinical management at the individual level. *HIV/AIDS (Auckland, N.Z.)*, *3*, 45–51. doi:10.2147/HIV.S8993

101. Juega-Mariño J1, Bonjoch A, Pérez-Alvarez N, Negredo E, Bayes B, Bonet J,et al.,(2017). Prevalence, evolution, and related risk factors of kidney disease among Spanish HIV-infected individuals. Medicine (Baltimore), 2017. 96(37): p. e7421. doi: 10.1097/MD.0000000000007421

102. Bener A1, Zirie M, Musallam M, Khader YS, Al-Hamaq AO (2009). Prevalence of metabolic syndrome according to Adult Treatment Panel III and International Diabetes Federation criteria: a population-based study. Metabolic Syndrome and Related Disorders, 2009. **7**(3): p. 221-9. doi: 10.1089/met.2008.0077

103. Okeke, N.L., et al., Hypertension Among HIV-infected Patients in Clinical Care, 1996-2013. Clin Infect Dis, 2016. 63(2): p. 242-8. doi: 10.1093/cid/ciw223

104. Emamian M, Hasanian SM, Tayefi M, Bijari M, Movahedian Far F, Shafiee M et al.,(2017). Association of hematocrit with blood pressure and hypertension. Journal of Clinical Laboratory Analysis, 2017. doi: 10.1002/jcla.22124

105. Chen Y., Liu C., Lu W., Li M., Hadadi C., Wang E. W et al.,(2016). Clinical characteristics and risk factors of pulmonary hypertension associated with chronic respiratory diseases: a retrospective study. Journal of Thoracic Disease, 2016. 8(3): p. 350-8. doi: 10.21037/jtd.2016.02.58

106. Peck RN, Shedafa R, Kalluvya S, Downs JA, Todd J, Suthanthiran M et al., (2014). Hypertension, kidney disease, HIV and antiretroviral therapy among Tanzanian adults: a cross-sectional study. BMC Medicine, 2014. 12: p. 125. doi: 10.1186/s12916-014-0125-2.

107. Marina N, Alfa M, Akum A,Donna S, Claudia H, Armstrong C et al.,(2016). Prevalence of Hypertension and Its Associated Risk Factors among 34,111 HAART Naïve HIV-Infected Adults in Dar es Salaam, Tanzania. International Journal of Hypertension, 2016. doi: 10.1155/2016/5958382

108. John R. K, Cathy A. J, Bryan E. S, Samuel E. S, and Timothy R. S. (2011). An optimal body mass index range associated with improved immune reconstitution among HIV-infected adults initiating antiretroviral therapy. Clinical Infectious Diseases, 2011. 53(9): p. 952-60. doi: 10.1093/cid/cir606

109. Koethe J R, Jenkins C A, Lau B, Shepherd B E, Wester W, Rebeiro P F, et al., (2016). Higher Time-Updated Body Mass Index: Association with Improved CD4+ Cell Recovery on HIV Treatment. Journal of Acquired Immune Deficiency Syndrome, 2016. 73(2): p. 197-204. doi: 10.1097/QAI.0000000000001035.

110. McKay H S, Bream J H, Margolick J B, Martínez-Maza O, Phair J P, Rinaldo C R et al.,(2016) Host factors associated with serologic inflammatory markers assessed using multiplex assays. Cytokine, 2016. 85: p. 71-9. doi: 10.1016/j.cyto.2016.05.016.

111. Abraham A G, Althoff K N, Jing Y, Estrella M M, Kitahata M M, Wester C W., et al.,(2015). End-stage renal disease among HIV-infected adults in North America. Clinical Infectious Diseases, 2015. 60(6): p. 941-9. doi: 10.1093/cid/ciu919.

112. Jotwani V, Li Y, Grunfeld C, Choi AI and Shlipak MG(2012). Risk factors for ESRD in HIV-infected individuals: traditional and HIV-related factors. American Journal of Kidney diseases, 2012. 59(5): p. 628-35. doi: 10.1053/j.ajkd.2011.10.050.

113. Khang Y.H (2012). Burden of noncommunicable diseases and national strategies to control them in Korea. Journal of Preventive Medicine and Public Health, 2013. 46(4): p. 155-64. doi: 10.3961/jpmph.2013.46.4.155.

114. Harrison, D.G., Marvar P.J. and Titze J.M. (2012). Vascular inflammatory cells in hypertension. Frontiers in Physiology, 2012. 3: p. 128. doi: 10.3389/fphys.2012.00128

115. Gorenec L, Zidovec Lepej S, Grgic I, Planinic A, Iscic Bes J, Vince A., et al.,(2016). The comparison of Th1, Th2, Th9, Th17 and Th22 cytokine profiles in acute and chronic HIV-1 infection. Microb Pathog, 2016. 97: p. 125-30. doi: 10.1016/j.micpath.2016.06.008.

116. de Medeiros RM, Valverde-Villegas JM, Junqueira DM, Gräf T, Lindenau JD, de Mello MG, et al. (2016). Rapid and Slow Progressors Show Increased IL-6 and IL-10 Levels in the Pre-AIDS Stage of HIV Infection. PLoS One, 2016. 11(5): p. e0156163. doi.org/10.1371/journal.pone.0156163

117. McKay H S, Bream J H, Margolick J B, Martínez-Maza O, Phair J P, Rinaldo C R et al., (2016) Host factors associated with serologic inflammatory markers assessed using multiplex assays. Cytokine, 2016. 85: p. 71-9. doi: 10.1016/j.cyto.2016.05.016.

118.de Larrañaga GF, Petroni A, Deluchi G, Alonso BS, Benetucci JA(2003). Viral load and disease progression as responsible for endothelial activation and/or injury in human immunodeficiency virus-1-infected patients. Blood Coagulation and Fibrinolysis, 2003. 14(1): p. 15-8. doi: 10.1097/01.mbc.0000046173.06450.40

119.Chastain D B, King T S, Stover K R, (2016). Infectious and Non-infectious Etiologies of Cardiovascular Disease in Human Immunodeficiency Virus Infection. Open AIDS Journal, 2016. 10: p. 113-26. doi: 10.2174/1874613601610010113

120.Chastain DB, Henderson H, Stover K R(2015). Epidemiology and management of antiretroviral-associated cardiovascular disease. Open AIDS Journal, 2015. 9: p. 23-37. doi: 10.2174/1874613601509010023

121.Stonbraker S, Montina B, Lerebours N L, Halpern M, and Elaine L E(2016). Evaluating the utility of provider-recorded clinical status in the medical records of HIV-positive adults in a limited-resource setting. International Journal of STD and AIDS, 2016. 28(7): 685–692 doi: 10.1177/0956462416663990

122.Pirrone V, Libon D J, Sell C, Lerner C A, Nonnemacher M R and Wigdah B (2013). Impact of age on markers of HIV-1 disease. Future virology, 2013. 8(1): p. 81-101. doi: 10.2217/fvl.12.127

123. Munyazesa E, Emile I, Mutimura E, Hoover DR, Shi Q, McGinn AP, et al.,(2012). Assessment of haematological parameters in HIV-infected and uninfected Rwandan women: a cross-sectional study. BMJ Open, 2012. **2**(6). doi: 10.1136/bmjopen-2012-001600

124. Hasse B, Ledergerber B, Furrer H, Battegay M, Hirschel B, Cavassini M, et al.,(2011). Morbidity and aging in HIV-infected persons: the Swiss HIV cohort study. Clinical Infectious Diseases, 2011. 53(11): p. 1130-9. doi: 10.1093/cid/cir626.

125. Hasse B, Bernasconi E, Furrer H, Eyer MM, Kovari H(2014). [HIV-associated non-AIDS conditions]. Therapeutische Umschau. Revue therapeutique, 2014. 71(8): p. 483-9. doi: 10.1024/0040-5930/a000541.

126. Cerrato E, Calcagno A, D'Ascenzo F, Biondi-Zoccai G, Mancone M, Grosso Marra W et al.,(2015). Cardiovascular disease in HIV patients: from bench to bedside and backwards. Open Heart, 2015. **2**(1). doi: 10.1136/openhrt-2014-000174

127. Bolduc, P., et al., Care of Patients with HIV Infection: Primary Care. FP Essent, 2016. 443: p. 31-42.

128. Clarke R, Valdes-Marquez E, Hill M on behalf of the PROCARDIS Consortium, et al Plasma cytokines and risk of coronary heart disease in the PROCARDIS study Open Heart 2018;5:e000807. doi: 10.1136/openhrt-2018-000807

Appendix 1: QUESTIONNAIRE: SERIAL NO._____

RESEARCH TITLE: INFLAMMATORY CYTOKINE PROFILES IN HIV/AIDS PATIENTS WITH HYPERTENSION COMORBIDITY

a). Demographic information

1. Gender (**a)** Male ☐ (**b**) Female ☐
2. Occupation (**a)** Formal employment (**b**) Self- employment (**c)** Not employed
3. Home residence (**a**) Within Nairobi (**b**) Outside of Nairobi
4. Level of Education (**a**) Primary (**b**) Secondary (**c**) Tertiary
5. Approximate age in years a) 31-39 yrs. b) 40-49 yrs. c) 50 yrs & above

b). Clinical data

1. Past medical history: a) illnesses b) treatments c) Not applicable
2. Past medical interventions: i) Surgery ii) hospitalization c) N/A

c). Hypertension care parameters

1. Hypertension status: **a)** Hypertensive ☐ **b)** Non- Hypertensive ☐
2. Blood pressure range: **a)** Prehypertension ☐ **b**) Moderate hypertension ☐ **c)** Severe hypertension ☐
3. Antihypertensive treatment: **a)** Yes **b)** No
4. Length of treatment: **a)** less than 2 years **b)** between 2-4 years **c)** more than 4 years
5. Type of treatment: **a)** 1st line treatment **b)** 2nd line treatment
6. Side effects of antihypertensive treatment: **a)** dizziness **b)** Fatigue **c)** Diuresis/excessive thirst **d)** None

d). HIV care parameters

1. Is the client on ART treatment: **a**) Yes **b**) No, If Yes above, for how long?
2. Duration of ART treatment: **a)** less than 6 months **b)** between 6 months & 2 years **c**) More than 2 years.
3. State the ART combination; **a)** First line **b)** Second line
4. Reported side effects **a)** Gastrointestinal **b)** Integumentary **c)** Neurological **d**) Others(indicate)_____
5. Is the participant a member of a social support group; **a)** Yes **b)** No

e). Participants' Clinical reviews after diagnosis

1. Frequency of Clinical review:

   a) monthly **b)** two monthly **c)** three monthly **d)** four monthly

2. Any Opportunistic infections ever while on care:

   a) TB **b)** Meningitis **c)** Skin/oral cavity infection **d)** Diarrhea **e)** Typhoid

3. Any manifestations of wasting; **a**) Present **b)** absent
4. Analysis of stress levels **a)** Mild **b**) Moderate **c**) Severe
5. Description of social behavior/habits **a)** Smokes tobacco **b)** Uses alcohol c) None
6. Physical exercises **a)** Yes **b)** No

f). Laboratory analysis for cytokine levels obtained from participants plasma samples

Group	Individual cytokines
Pro-inflammatory cytokines	IL 8, IL- 17A, TNFα, IFN- γ, IL6.
Anti-inflammatory cytokines	IL2, IL4, IL-10, $IL_{6.}$

APPENDIX 2: PARTICIPANT RECRUITMENT PROCEDURE

1) Patients were identified using random identification using the existing clinical data base at KNH Comprehensive Care Centre.

2) Screening was done to identify eligible patients (both hypertensive and non-hypertensive on or without HAART and free of any other chronic /metabolic disorder.

3) The participants were taken through an informed consenting process. They will be informed about the study, its objectives and the benefits that are projected to be obtained as well as what are expected of them as participants.

APPENDIX 3: PARTICIPANT CONSENT EXPLANATION FORM

a). **Explanation form in English**

Dear Participant,

My name is Angeline Chepchirchir, a PhD Research Fellow at the Institute of Tropical and Infectious Diseases, University of Nairobi. This is to request you to take part in the proposed study. The participation sought for is entirely voluntary.

You have been randomly identified as a potential participant in the research study entitled, "**Inflammatory Cytokine Profiles in HIV/AIDs patients with Hypertension comorbidity**".

This research study is part of the requirements for the degree of Doctor of Philosophy of the University of Nairobi. The objective is to determine the prevalence, role of inflammation and effects of hypertension on HIV/AIDS progression in patients on care.

The study will involve the use of questionnaires to collect participant information as well as obtain a one-time blood sample for laboratory analysis of selected cytokine levels.

As a participant, you are expected to respond to questions outlined in a semi structured questionnaire and provide a sample of blood for laboratory analysis. The questionnaire will be administered by the principal researcher and/or the research assistants.

The study findings will indirectly benefit you and the HIV infected population because additional knowledge sought for on the interaction between inflammation and hypertension development may be used to design appropriate prevention and control interventions. As a participant, you will not receive any money nor material gifts.

All the information you will provide, and laboratory findings will be held confidentially and used only for the intended purpose. The questionnaire and clinical data forms will be serialized. No names shall be included in every documentation. Administration of questionnaires shall be done in a quiet and confidential location within the Comprehensive care Centre.

Your participation will be entirely voluntary, and you can at any time withdraw from the study without interference on the quality or quantity of care you are currently receiving. If at any time you do not feel like answering a question or discuss, you are free not to do so. If you choose not to participate, you shall not be penalized. There are no direct benefits attached to the study.

As a participant, you can ask any questions that would help you further to understand the nature of this research study. The principal investigator or assistant will attempt to answer them appropriately to your satisfaction where possible

If you change your mind regarding participation in this study or suffer any unforeseen inconvenience due to participation in the study, inform the principal investigator or the ERC committee using contacts provided below. Should you be dissatisfied with the way the study is being contacted or want to raise any pertinent issues about it; please forward your complaint to;

I. **The Ethics and research Committee.**

The Chairman

Ethics and Research Committee

KNH/University of Nairobi

P.O Box 20723, Nairobi.

Landline 0202 276300 ext.44102

II. Principal Investigator's Contacts.

Angeline Chepchirchir

UNITID/University of Nairobi

P.O Box 2558, 00202, Nairobi.

Cell 0720 440 665

III. Supervisors' Contacts

1. Dr. Joshua Nyagol (PhD),

Dept. of Pathology,

University of Nairobi.

P.O Box 19676, 00202, NBI.

2. Prof. Walter Jaoko

KAVI – Institute of Clinical Research

University of Nairobi.

P.O Box 19676, 00202, NBI.

APPENDIX 4: PARTICIPANT'S CONSENT FORM

Participant's endorsement for voluntary participation

I_____(Participants name/Initials) have understood the objectives and purpose of the study. The explanations that I have received are satisfactory to the best of my understanding. I voluntarily agree/disagree to participate in the said study.

Signature_____ Date _____

Researcher (Name/Initials)

_____Signature_____Date_____

Witness (Name/initials)_____Signature_____Date_____

For any enquiries or concerns about the study contact Principal investigator;

Angeline chepchirchir,

UNITID,

University of Nairobi,

Phone no.0720440665

APPENDIX 5: LABORATORY PROCEDURE: CYTOKINE ANALYSIS .

Use of the BD Cytometric Bead Array (CBD) Human Th1/Th2/Th17 cytokine kit to assess inflammatory cytokines in plasma.

Procedure	**Incubation time**
1. Preparing standards	15 minutes
2. Preparing mixed capture beads (when analyzing serum or plasma samples only)	30 minutes
3. Preparing Cytometer Setup Beads	30 minutes
4.Performing the assay	3 hours

Additional requirements necessary;

1) A dual-laser flow cytometer equipped with a 488- nm or 532-nm and a 633-nm or 635-nm laser capable of distinguishing 576-nm, 660-nm, and >680-nm fluorescence.

2) BD Falcon™12×75-mm sample acquisition tubes (Catalog No. 352008), or equivalent

3) 15-mL conical, polypropylene tubes (BD Falcon, Catalog No. 352097), or equivalent.

4) FCAP Array software (Catalog No. 641488 [PC] or 645447 [Mac])

5) Millipore Multiscreen-BV 1.2 μm clear nonsterile filter plates [Catalog No. MSBVN1210 (10 pack) or MSBVN1250 (50 pack)]

6) Millipore Multiscreen Vacuum Manifold, (Catalog No. MSVMHTS00)

7) MTS 2/4 Digital Stirrer, IKA Works, VWR (Catalog No. 82006-096)

8) Vacuum source

9) Vacuum gauge and regulator (if not using the recommended manifold)

PROCEDURE

A. PREPARATION OF STANDARDS AND CONTROLS

1. Open one vial of lyophilized Human Th1/Th2/Th17 Standards. Transfer the standard spheres to a 15-mL conical, polypropylene tube. Label the tube "Top Standard."

2. Reconstitute the standards with 2 mL of Assay Diluent.

a. Allow the reconstituted standard to equilibrate for at least 15 minutes at room temperature.

b. Gently mix the reconstituted protein by pipette only. Do not vortex or mix vigorously.

3. Label 12 × 75-mm tubes and arrange them in the following order: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256.

4. Pipette 300 µL of Assay Diluent in each of the 12 ×75-mm tubes.

5. Perform serial dilutions:

a. Transfer 300 µL from the Top Standard to the 1:2 dilution tube and mix thoroughly by pipette only.

b. Continue making serial dilutions by transferring 300 µL from the 1:2 tube to the 1:4 tube and so on to the 1:256 tube.

c. Mix thoroughly by pipette only. Do not vortex.

6. Prepare one 12 × 75-mm tube containing only Assay. Diluent will serve as the 0 pg/mL negative control.

B. MIXING HUMAN TH1/TH2/TH17 CYTOKINE CAPTURE BEADS

1. Determine the number of assay tubes (including standards and controls) required for the experiment (e.g, 8 unknowns, 9 cytokine standard dilutions, and

1 negative control = 18 assay tubes).

2. Vigorously vortex each Capture Bead suspension for 3 to 5 seconds before mixing.

Note: The antibody-conjugated beads will settle out of suspension over time. It is necessary to vortex the vial before taking a bead-suspension aliquot.

3. Add a 10-µL aliquot of each Capture Bead, for each assay tube to be analyzed, into a single tube labeled "mixed Capture Beads" (eg, 10 µL of IL-2 Capture Beads × 18 assay tubes = 180 µL of IL-2 Capture Beads required).

4. Vortex the bead mixture thoroughly.

C. SUSPENSION OF BEADS AS APPLICABLE FOR PLASMA AND SERUM SAMPLES.

1) Use serum enhancement buffer

2) Centrifuge the mixed Capture Beads at 200g for 5 minutes.

3) Carefully aspirate and discard the supernatant**4.3.3l**

4) Suspend the mixed Capture Beads pellet in Serum Enhancement Buffer (equal to the volume removed in step 2) and vortex thoroughly.

5) Incubate the mixed Capture Beads for 30 minutes at room temperature, protected from light.

6) After 30 minutes, transfer Capture Beads to the assay tubes. Discard excess.

7) Do not store after mixing.

D. DILUTION OF SAMPLES

To dilute samples with a known high cytokine concentration:

1. Dilute the sample by the desired dilution factor (i.e., 1:2, 1:10, or 1:100) using the appropriate volume of Assay Diluent.

2. Mix sample dilutions thoroughly. Note: Optimal recovery from serum samples

Typically requires a 1:4 dilution.

E. STAINING OF SAMPLES

To perform the assay:

1. Vortex the mixed Capture Beads and add 50 μL to all assay tubes.

2. Add 50 μL of the Human Th1/Th2/Th17 Cytokine Standard dilutions to the control tubes as listed in the following table:

Tube label	**Concentration (pg/ml)**	**Cytokine standard dilution**
1	0 (negative control)	No standard dilution (Assay diluent only)

2	20	1:256
3	40	1:128
4	80	1:64
5	156	1:32
6	312.5	1:16
7	625	1:8
8	1250	1:4
9	2500	1:2
10	5000	Top standard

3. Add 50 µL of each unknown sample to the appropriately labeled sample tubes.

4. Add 50 µL of the Human Th1/Th2/Th17 PE Detection Reagent to all assay tubes.

5. Incubate the assay tubes for 3 hours at room temperature, protected from light.

Note: If you have not yet performed cytometer setup, you may wish to do so during this incubation.

6. Add 1 mL of Wash Buffer to each assay tube and centrifuge at 200g for 5 minutes.

7. Carefully aspirate and discard the supernatant from each assay tube.

8. Add 300 µL of Wash Buffer to each assay tube to suspend the bead pellet.

F. PERFORM ASSAYS ON FILTER PLATES:

1. Wet the plate by adding 100 µL of wash buffer to each well.

2. Place the plate on the vacuum manifold.

3. Aspirate for 2 to 10 seconds until the wells are drained.

4. Remove the plate from the manifold, and then blot the bottom of the plate on paper towels.

5. Add 50 µL of each of the following to the wells in the filter plate:

• Capture Beads (vortex before adding)

• Standard or sample (add standards from the lowest concentration to the highest, followed by samples)

• Human Th1/Th2/Th17 PE Detection Reagent

6. Cover the plate and shake it for 5 minutes at 1,100 rpm on a plate shaker.

7. Incubate the plate for 3 hours at room temperature on a non-absorbent, dry surface.

Note: Place the plate on a non-absorbent, dry surface during incubation. Absorbent or wet surfaces can cause the contents of the wells to leak.

8. Remove the cover from the plate and apply the plate to the vacuum manifold.

9. Vacuum aspirate for 2 to 10 seconds until the wells are drained.

10. Remove the plate from the manifold, and then blot the bottom of the plate on paper towels after aspiration.

11. Add 120 µL of wash buffer to each well to suspend the beads.

12. Cover the plate and shake it for 2 minutes at 1,100 rpm before you begin sample acquisition.

G. ACQUIRE THE SAMPLES ON THE FLOW CYTOMETER

1) Acquire standards from lowest (0 pg/mL) to highest (Top Standard) concentration, followed by the test samples.

2) If running sample dilutions, acquire sequentially starting with the most concentrated sample.

3) Store all FCS files (standards and samples) in a single folder

H. PERFORM SAMPLE READINGS.

Analyze Human Th1/Th2/Th17 Cytokine data using FCAP Array software.

APPENDIX 6: DATA ANALYSIS GUIDE

1. Obtain descriptive and inferential statistics where applicable.

    a. Determine proportion of participants who are known to be hypertensive and non-hypertensive.

    b. Compare the proportions to establish any associations with selected sociodemographic, clinical and laboratory parameters.

Socio-demographic variables.	**Clinical parameters**	**Cytokines levels**
• Age	• CD4+ cell count • WHO HIV/AIDS stage • Creatinine levels • Hemoglobin levels • BMI • Hematocrit	• IL 6
• Gender		• IL8
• Alcohol use		• IL17A
• Smoking		

3. Establish proportions of participants in respect to selected parameters

- Establish proportions of participants at the different stages of HIV infection as per the WHO HIV/AIDS classification
- Determine the observations /variations in clinical parameters associated with chronic inflammation in hypertensive and non- hypertensive participants.

WHO stage	Clinical parameters
Stage I	● CD4 count ● Creatinine ● Hematocrit levels(HCT) ● BMI changes
Stage II	
Stage III	
Stage IV	

5. Establish participants 'proportions and associated social behavior characteristics

- Calculate proportion of participants who smoke and /or drink alcohol.
- Establish any correlation(s) associated with social habits and clinical parameters

6. Establish the burden of hypertension in HIV disease.

PART B. OBJECTIVE 2.

1. Generate descriptive statistics for individual cytokine levels.
2. Establish any correlation between participants 'hypertension status and levels of cytokines measured.
3. Compare the expression of IL_6 & IL_8 in hypertensive and non-hypertensive participants. The two are associated with cardiovascular damage and risk of atherosclerosis.
4. Cytokines were grouped as follows:

Group	Individual cytokines
Pro-inflammatory cytokines	IL 8, IL- 17A, TNFα, IFN- γ, IL6.
Anti-inflammatory cytokines	IL2, IL4, IL-10, IL_6.

Analytic tests performed

Dependent variable	Independent/ Explanatory variable	Tests applied
HIV/AIDS progression	**CD4+ cell counts** Comparison of means between the hypertensive and non-hypertensive groups on different forms of HIV care and at various stages of disease progression	Independent Sample t-test was used, applicable for 2 means ANOVA was used where categories of means were 2 or more than 2. Levene's test of homogeneity of variances was used. Spearman's rank correlation analysis was used
	Hematocrit levels Compare means between the hypertensive and non-hypertensives at different stages of HIV disease progression.	
	Creatinine levels Compare means between the hypertensive and non-hypertensives at different stages of HIV disease progression.	

	<u>Body Mass Index(BMI)</u> Comparison of means between the hypertensive and non-hypertensive groups on different forms of HIV care and at various stages of disease progression **<u>Inflammatory Cytokines' levels</u>** Comparison of means for Individual and group of Inflammatory cytokines (IL-17A, IL-2, IL-4, IL-6, IL-8, IL-10, TNF α, IFN γ).	chi square tests Simple linear regression Logistic regression