FUNCTIONAL CHANGES OF THE VASCULATURE IN HIV/AIDS PATIENTS ON HAART AND HAART NAÏVE HIV PARTICIPANTS

by

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ABSTRACT

The present study sought to explore the functional changes that occur in the vasculature of HIV positive participants of African origin in Mthatha district of South africa which might lead to increased risk in their cardiovascular system. Available literature shows that arterial stiffness plays an important role in cardiovascular events such as stroke, vasculitis and myocardial infarction. Measurement of (aortic pulse wave velocity; PWV) provides some of the strongest evidence concerning the prognostic significance of large artery stiffening. This study was aimed at investigating the relationship between anthropometry, age, E-Selectin level, cytokine levels, haemodynamic variables, blood counts and blood lipid profile with pulse wave velocity. Some traditional cardiovascular risk factors such as alcohol, and smoking were also taken into account.

This was a cross-sectional study comprising of 169 participants (62 males and 107 females). 63 were HIV negative (group A), 54 HIV positive on treatment (group B), and 52 were HIV positive not on treatment (group C). Pulse wave velocity (PWV) was assessed using the Sphygmocor Vx. Statistically, ANOVA was used for variables with normal distribution and non parametric tests were used for variables with skewed distribution.

Notable significant differences were seen in the means of the following variables across all the 3 groups. The mean PWV value for group C (7.21±2.17) was greater

than that for group B (6.84± 1.17) which in turn was more than group A (6.38±1.67); P=0.037. In participants who are HIV negative, In univariate analysis PWV correlated significantly with the following: Augmentation index; AIx (75): (r=0.850,p=0.004): Systolic aortic blood pressure; Spa: (r=0.635, p<.000); diastolic blood pressure; dbp: (r=0.436, p<0.000); aortic pulse pressure; Ppa: (r=0.472, p=0.000); Mean arterial pressure; MP: (r=0.446 p=<0.00) and age (r=0.606, p<0.000).

In participants who are on HAART the following variables were positively correlated with PWV: Ppa: (r=0.338,p=0.012), MP: (r=0.400,p=0.400), monocytes (r=0.320,p=0.047). Neutrophils: (r=0.341,p=0.034), CD4: (r=-0.446,p=0.009). In participants who are HAART naïve and HIV positive the following correlated with PWV Spa: (r=0.369, p=0.012), MP: (r=0.400, r=0.003) Ppa: (r=0.338,p=0.012), waist to hip ratio: (r=0.319, p=0.037), platelets: (r=0.037, p=0.019), triglycerides: (r=0.490, p=0.002).

With multiple linear regression Spa, age and triglycerides as the only independent and significant determinants of PWV among HIV negatives R^2= 56.9% (adjusted R^2=54.7%), model adjusted for gender, anthropometric parameters, HDL-C, TC, LDL-C, haematologic data, haemodynamic data, cytokines, smoking and alcohol. Only MP and waist circumference were identified as the most important and significant independent determinants of PWV in HIV positive participants not on treatment.

Age, MP, HDL-C, and triglycerides were identified as the significant independent determinants of the variations of PWV in HIV positive participants on HAART. R^2 =57 %(adjusted R^2 =53.5%). Model adjusted for gender, anthropometric data, smoking, alcohol, cytokines, adhesion molecules, total cholesterol, LDL-C. Haematological data, CD4 count, and other haemodynamic parameters.

For Aix(75) In HIV negatives the multiple linear regression model identified age (positive correlation), height (negative correlation), CD4 (positive correlation) and MP (positive correlation) as the independent and significant determinants of AIx (75) among HIV negatives. Spa and Age were independently and significantly associated with the variations of Aix(75) among HIV positives not on HAART. On the other hand height was negatively and significantly associated with Aix(75) amongst HIV positives not on HAART. After excluding confounding factors, height (negative correlation) age (positive correlation), MP (positive correlation, HDL-C (negative correlation), platelets (positive correlation) alcohol intake (excessive consummation associated with positive correlation) and TNFα (negative correlation) were identified as the independent and significant variables associated with increase in AIx(75) among HIV positive participants on HAART.

Conclusion: This study showed that HIV infected patients with or without antiretroviral therapy have increase arterial stiffness which is associated with an increased cardiovascular risk. The sphygmocor is an accurate, non invassive and useful tool in the evaluation of arterial stiffness and its use in clinical practice should be encouraged. PWV and the augmentation index (AIx) are the two major non-

invasive methods of assessing arterial stiffness. Life style modification should be incorporated into the management of HIV patients so as the continuous monitoring of their haematological and lipid profile.

DECLARATION

I, Kofoworola Awotedu, Student number 209048085 declare that this thesis entitled: 'Functional changes of the vasculature leading to some cardiovascular risk factors in HIV/AIDS patients on HAART and HAART naïve HIV participants' is my original work. All sources used or quoted in the study that have been indicated and acknowledged by way of complete references.

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DEDICATION

This work is dedicated to the memory of my parents the late Hon. Mr Justice O. Akinkugbe (OFR) and Chief. Mrs M.M. Akinkugbe who imbibed in me the spirit of hardwork, endurance, diligence and discipline without which I will not be able to accomplish this work.

TABLE OF CONTENTS

Table of Contents

LIST OF FIGURES

LIST OF TABLES

ACRONYMS

ABC	Abacavir
AIDS	Acquired immunodeficiency syndrome
ACTG	Aids clinical trials group
AII	Angiotensin II
AIx(75)	Augmentation index
APV	Amprenavir
ATV	Atazanavir
ASVD	Arteriosclerotic vascular disease
ARV	Antiretroviral therapy
AZT	Zidovudine
APV	Amprenavir
ART	Antiretroviral treatment
ANG II	Angiotensin II
BMI	Body mass index
BH4	Tetrahydrobiopterin
cAMP	Cyclic AMP
CDC	Centres for disease control
CRFs	Circulating recombination forms
CRF A/B	Circulating recombination forms, a mixture of subtypes A and B
CD4+T	Cluster of differentiation 4 expressed on the surface of T helper cells
CD8+T	Cluster of differentiation 8 is a transmenmbrane glycoprotein
CHOD-PAP	Cholesterol oxidase Phenol Aminoantipyrine Peroxidase method
CAF	CD8 antiviral factor

CVD	Cardiovascular disease
CHD	Coronary heart disease
CCR5	Co-receptor molecule called chemokine receptor
CMS	Cardio metabolic syndrome
CNS	Central nervous system
CMV	Cytomegalovirus
CaM	Calmodulin
CAD	Coronary atherothrombotic disease
CRP	C-reactive protein
CT	Carotid intima Thickness
CV	Cardiovascular
CVD	Cardiovascular Disease
CRH	Corticotrophin releasing hormone
CSF	Colony stimulating factor
cGMP	Cyclic Guanosine Monophosphate
DNA	Deoxyribonucleic acid
D	Distance
d4T	Stavudine
ddI	Didanosine
DRV	Darunavir
DLV	Delavirdine
Dpa	Aortic diastolic blood pressure
Dbp	Brachial diastolic blood pressure
E	Young's modulus

EFV	Efavirenz
ETR	Etravirine
DbP	Diastolic blood pressure
eNO	Nitric oxide synthase
ET-1	Endothelin
ET-A	Endothelin-A
ET-B	Endothelin-B
ETR	Etravirine
EBV	Epstein-barr virus
ELAM-1	Endothelial leukocyte adhesion molecule 1
ELISA	Enzyme linked immunosorbent assay
FDCs	Follicular dendritic cells
EFV	Efavirenz
FMD	Flow mediated dilation
FTC	Emtricitabine
FOS-APV	Fosamprenavir
GTP	Guanosine triphosphate
GM-CSF	Granulocyte-macrophage
GM-CSF	Granulocyte-macrophage colony-stimulating factors
GTN	Glyceryl trinitrate
G-CSF	Granulocyte-colony stimulating factors
GDP	Guanosine diphosphate
GRO-alpha	Growth-related oncogene protein-alpha
HIV	Human immunodeficiency virus

HIV-1	Human immunodeficiency virus-type 1
HIV-2	Human immunodeficiency virus-type 2
HAART	Highly active antiretroviral therapy
HTLV-111	Human T lymphocyte virus-111
HDL-C	High density lipoprotein cholesterol
HOPS	HIV out patients
HR	Heart rate
IGF-I	Insulin-like growth factor-I
INH	Instititute of National Health
ICAM	Intercellular adhesion molecule
ICAM-1	Intercellular adhesion molecule-1
IDLs	Intermediate density lipoproteins
IDV	Indinavir
IFN	Interferon-alpha
IL-1	Interleukin-1
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-15	Interleukin-15
IgA	Immunoglobulin A

IMT	Intima-media thickness
IRS-1	Insulin receptor substrate
LECAM2	Leukocyte-endothelial cell adhesion molecule 2
LP(a)	Lipoprotein
LPL	Lipoprotein lipase
LdL-OX	Low density lipoproteins-oxidized
LAV	Lymphadenopathy associated virus
LDL	Low density lipoprotein
LPV	Lopinavir
LV	Left ventricle
RTV	Lopinavir
LDLc	Low density lipoprotein cholesterol
LECAM2	Leukocyte endothelial cell adhesion molecule 2
LPS	Lipopolysaccharide
MACS	Multicentre AIDS cohort study
M-CSF	Macrophage- colony stimulating factor
MIP-1beta	Macrophage inflammatory protein-1 beta
MDR	Multi drug resistant
MDC	Macrophage derived chemokine
MCP-1	Monocyte chemotactic protein-1
MI	Myocardial Infarction
M-CSF	Macrophage Colony Stimulating Factor
MVC	Maraviroc
MP	Mean arterial Pressure

NFV	Nelfinavir
NI	National institute of health
NRT	Nucleoside reverse transcription
NIAD -	National institute of allergy and diseases
NIAIDS	National institute of allergy and infectious diseases
NRTI	Nucleoside and nucleotide reverse transcriptase inhibitors
NNRT	None nucleotide reverse transcriptase inhibitors
NSP	National strategic plan
NO	Nitric Oxide
NOS	Nitric oxide synthase
NADPH	Nicotinamide adenine dinucleotide phosphate
N-CAMS	Neural cell adhesion molecules
NK	Natural killer
NVP	Nevirapine
PI	Protease inhibitor
PIs	Protease inhibitors
PrEP	Pre-exposure prophylaxis
Ppa	Aortic pulse pressure
Pbp	Brachial blood pressure
PAMPs	Pathogen associated molecular pattern
PCP	Pneumocystis pneumonia
PML	Progressive multifocal lekoencephalopathy
PMTCT	Prevention of mother to child transmission
PHC	Primary health care

PDGF	Platelet derived growth factor
PGF	Basic fibroblast growth factor
PP	Pulse Pressure
PAI	Plasminogen activator inhibitor-1
PWV	Pulse wave velocity system
RAL	Raltegravir
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RB	Reagent blank
RLPs	Remnant-like particles
RTV	Ritonavir
SA	Subclinical atherosclerosis
SAA	Serum amyloid A
Sbp	Systolic brachial Blood Pressure
Spa	Systolic aortic blood pressure
sVCAM	Soluble vascular cell adhesion molecule
sICAM-1	Soluble intercellular adhesion molecule
SANAC	South African national Aids council
SEVR	Subendocardial viability ratio
SQV	Squinavir
TNF-alpha	Tumor necrosis factor-alpha
TTT	Technical task team
TB	Tuberculosis
TDF	Tenofovir

TG	Triglycerides
TGF-B	Tissue growth factor
Th-1	Thymus cells 1
Th2	Thymus cells 2
TNF	Tumour necrosis factor
T	Time
T cells	Thymus dependent cells
UNAIDS	United nations programme on AIDS
UNICEF	United nations international children's emergency fund
USAID	United States Aid agency
VCAM	Vascular cell adhesion molecule
VCAM-1	Vascular cell adhesion molecule-1
VSMC	Vascular smooth muscle cell
vWF	Willebrand factor
VLDL	Very low density lipoproteins
WHO	World health organization
WC	Waist Circumference
WHR	Waist to Hip ratio

RESEARCH OUTLINE

The Thesis is divided into six chapters.

CHAPTER 1

This is the chapter that contains the introduction, background of the study and statement of the problem. Included in this section is the main research question, sub research questions, research objectives, and rationale of the study.

CHAPTER 2

This chapter contains the literature review. The body of the chapter is made up of health burden of the HIV/AIDS disease, deals with the structure, life cycle of HIV, and types of HIV. Types and classes of antiretroviral drugs are mentioned. The strucure of the major types of blood vessels are indicated and illustrated with pictures. Arterial stiffness and inflammation are reviewed. Arterial stiffness in relation to lipids, cytokines and adhesion molecules are also reviewed. Arterial stiffness was also related to immunity.

CHAPTER 3

This deals with the method and data analysis. It comprises the study design, study population, calculation of sample size, administration of questionnairres, methods for measuring total cholestrol (TC), low densiy lipoprotein cholesterol (LDL-C),High density lipoprotein cholesterol (HDL-C), and triglycerides (TG). Also included are methods for measuring cytokines, E-selectin and arterial stiffness using the Sphygmocor.

CHAPTER 4

This contains the results. These were illustrated with the aid of graphs, box plots, regression plots and tables where appropriate.

CHAPTER 5

This chapter deals with the discussion. It discusses the composition of the participants. The effect of HIV on arterial stiffness in the participants, role of HIV in atherosclerosis, influence of cardiovascular risk factors on arterial wall, arterial stiffness and anthropometry, arterial stiffness and aging, arterial stiffness and blood pressure. This chapter also included the limitations and perspectives.

CHAPTER 6

This chapter incorporates the conclusion.

CHAPTER 1.

INTRODUCTION

1.1.The Human immunodeficiency virus/AIDS

Human immunodeficiency virus (HIV)/AIDS is one of the greatest medical challenges facing the African continent today. The impact of the HIV epidemic on the cardiovascular system in Africans has received little attention in the world literature.

In 2010, 27 million people were infected with HIV, the number has now risen to an estimated 34 million people globally. This includes millions who have developed Acquired immunodeficiency sydrome (AIDS), (UNAIDS 2011).

HIV-infected patients have higher rates of atherosclerosis than HIV-seronegative persons (Hsue et al. 2004, Triant et al. 2007). Factors that may contribute to this risk, include direct antiretroviral drug toxicity (Nolan et al. 2007), virus-induced endothelial injury, and chronic inflammation (Ghandi et al. 2006). It had been deduced that both traditional and nontraditional risk factors contribute to atherosclerotic disease in HIV-infected patients (Lo et al. 2010).

Endothelial dysfunction, an early marker of atherosclerosis with prediction for cardiovascular events could be the link between HIV infection and atherosclerosis (Mundel 2007). The pathogenesis of endothelial dysfunction which is a surrogate of atherosclerosis in HIV-1 infection is also undergoing investigation. Several mechanisms have been postulated and include the following: HIV-induced endothelial cell injury, activation of endothelial cells by pro-inflammatory cytokines and mediators, and toxicity from ART which may itself have

direct and indirect actions. Hence the effect of these substances on the blood vessels of HIV participants will be studied.

In this environment where the first line drugs still being used in public hospitals are the nucleosides and non nucleosides, it is still unclear if metabolic complications consequent on the use of these drugs have significant effects on the vasculature. It is also not clear if these cardiovascular complications are due to the human immunodeficiency virus, effects of HAART, or the effects of some other factors like cytokines and lipaedemia. It is to answer these questions that this study seeks to determine the effect of HIV, its treatment and other cardiovascular risk factors on the vasculature.

1.2. STATEMENT OF THE PROBLEM

HIV infection is associated with increase in risks for cardiovascular disease (Wilkinson & Cockcroft 2007). Some of the risk factors are low levels of HDL cholesterol and elevated levels of triglycerides. Patients with hypercholesterolaemia have a higher central pulse pressure and stiffer blood vessels than matched controls, despite having similar peripheral blood pressures (Wilkinson & Cockcroft 2007). It has also been reported that the risk for myocardial nfarction is 70% to 80% higher among people with HIV compared with those who are HIV negative (Lo et al. 2010).

Some researchers (Carr et al. 2008, Obel et al. 2007) found that aortic stiffness is increased in HIV treatment-naive patients free from cardiovascular disease and without major atherosclerotic risk factors. These findings suggest HIV infection as a potentially relevant contributor to atherosclerosis and for the increased cardiovascular risk observed among HIV-infected individuals regardless of antiretroviral treatment (Carr et al. 2008, Obel et al. 2007).

1.3. AIM

The aim of the study was to evaluate the level of vascular dysfunction and the magnitude of its determinants as cardiovascular risks in antiretroviral therapy naïve HIV positive participants, HIV positive participants on antiretroviral therapy, compared with HIV seronegative participants in black Africans of Mthatha district of old Transkei in South Africa.

1.5. MAIN RESEARCH QUESTION

Does HIV infection increase cardiovascular risk in black Africans ?

1.4.1. Subresearch questions

They are raised as follows:

- does HIV infection affect the vasculature thereby giving rise to endothelial dysfunction with consequent arterial stiffness which may lead to increased cardiovascular risks?

- does HAART affect the vasculature giving rise to endothelial dysfunction with consequent arterial stiffness leading to increased cardiovascular risk in HIV positive participants on first line antiretroviral in the public clinics in Mthatha district of Eastern Cape Province?

- do cytokines, adhesion molecules, and dyslipidemia contribute to arterial stiffness in antiretroviral naïve HIV participants and participants on first line antiretroviral in the public clinics in Mthatha district of Eastern Cape Province?

1.5. RESEARCH OBJECTIVES

In order to answer the research questions the following objectives were defined:

- to assess cardiovascular risk factors such as smoking, body mass index, blood pressure, in antiretroviral therapy naive, HIV positive participants on treatment and HIV negative participants;

- to determine cardiovascular risk by assessing the functional changes of the vasculature (arterial stiffness) using SphygmoCor Vx (a non invasive method);

- to measure the lipid profiles among antiretroviral therapy naïve HIV positive participants, HIV positive participants on antiretroviral therapy, and HIV seronegative participants;

- to measure some cytokines such as, TNFα and IL-6 in antiretroviral therapy naïve HIV positive participants , HIV positive participants on antiretroviral therapy, and HIV seronegative participants;

- to measure adhesion molecules such as E- selectin in antiretroviral therapy naïve HIV positive participants, HIV positive participants and HIV seronegative participants.

1.6. Rationale and significance of study

The five countries with the highest prevalence rates of HIV infection in the world are situated in Southern Africa. South Africa in 2011 had an estimated 5.6 million people living with HIV and has more cases of HIV/AIDS than any other country (UNAIDS 2012). The impact of this on the country might worsen if more people are infected. Highly active antiretroviral therapy (HAART) has greatly reduced the risk of early death from opportunistic infections and extended the lifespan of people infected with HIV. Many complications and organ damage in the HIV infected population have thus emerged.

CHAPTER 2: LITERATURE REVIEW.

2.1.The Human Immunodeficiency virus and acquired immunodeficiency syndrome

This is a condition in humans in which the immune system begins to fail, leading to life threatening opportunistic infections (Quinn 2011). HIV primarily infects vital cells in the human immune system such as the helper T cells. These are cluster of differentiation 4 lymphocytes expressed on the surface of T helper cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells. HIV infection leads to low levels of $CD4^+$ T cells through three main mechanisms: firstly, direct killing of infected cells by the virus, secondly, by the virus increasing rates of apoptosis of infected cells ; and thirdly, killing of infected $CD4^+$ T cells by cluster of differentiation 8 (CD8) cytotoxic lymphocytes that recognize infected

cells. When $CD4^+$ T cell number declines below a critical level, cell mediated immunity is impaired.

Viruses such as HIV cannot grow or reproduce on their own, they need to infect the cells of a living organism in order to replicate. The human immune system usually finds and kills viruses fairly quickly, but the HIV attacks the immune system itself. HIV infection is basically divided into four stages: incubation period, acute infection, latency stage and AIDS. Previous names for the virus include human T lymphocyte virus-111 (HTLV-111), lymphadenopathy associated virus (LAV) and AIDS associated retrovirus (Coffin et al. 1986, and Sowadsky et al. 1999).

HIV is a highly variable virus which mutates very readily. The major routes of transmission are: unprotected sexual intercourse, contaminated needles, blood products during transfusion, breast milk, and transmission from an infected mother to her baby at birth (Joint United Nations Programme on HIV/AIDS 2008). Within these body fluids, the virus is present as both free virus particles and virus within infected immune cells.

2.1.1. Types and subtypes of HIV

There are two types of HIV: HIV-1 and HIV-2. Both types are transmitted by sexual contact, through blood, and from mother to child, and they appear to cause clinically indistinguishable AIDS. However, it seems that HIV-2 is less easily transmitted, and the period between initial infection and illness is longer in the case of HIV-2 (Plantier et al. 2009). Worldwide, the predominant virus is HIV-1, and generally when people refer to HIV without specifying the type of virus they will be referring to HIV-1. The relatively uncommon HIV-2 type is concentrated in West Africa and is rarely found elsewhere (WHO 2011). It is known that there are many different strains of HIV, even within the body of a single infected person.

Based on genetic similarities, the numerous virus strains may be classified into types, groups and subtypes.

As shown in Figure 1, the strains of HIV-1 can be classified into four groups: the "major" group M, the "outlier" group O and two new groups, N and P. These four groups may represent four separate introductions of simian immunodeficiency virus into humans (Gao et al 1998).

Group O subgroup appears to be restricted to West-Central Africa and group N - a strain discovered in 1998 in Cameroon - is extremely rare.

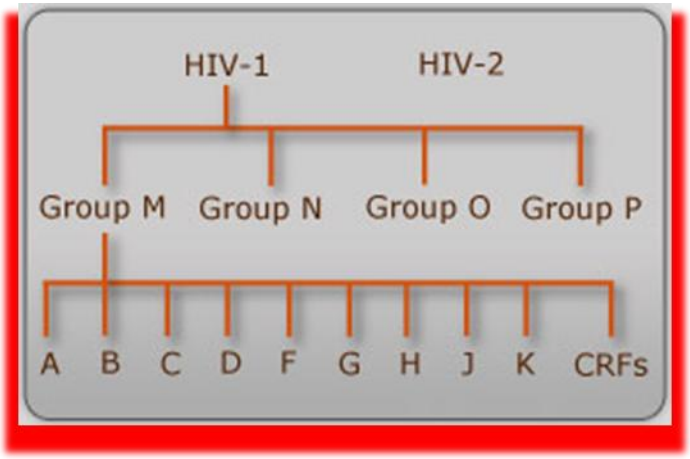

Figure 1. Types and subtypes of HIV.
Copied from Wainberg M A (2004, 3rd June) 'HIV-1 subtype distribution and the problem of drug resistance' AIDS 18(S3).

In 2009 a new strain closely relating to gorilla simian immunodeficiency virus was discovered in a Cameroonian woman (WHO 2011). It was designated HIV-1 group P. More than 90 per cent of HIV-1 infections belong to HIV-1 group M. Within group M there are known to be at least nine genetically distinct subtypes of HIV-1. These are subtypes A, B, C, D, F, G, H, J and K. (Gao et al. 1998). Occasionally, two viruses of different subtypes can

meet in the cell of an infected person and mix together their genetic material to create a new hybrid virus (a process similar to sexual reproduction, and sometimes called "viral sex").

Many of these new strains do not survive for long, but those that infect more than one person are known as "circulating recombinant forms" (CRFs). For example, the HIV Circulating Recombinant Forms (CRF A/B) is a mixture of subtypes A and B. The classification of HIV strains into subtypes and CRFs is a complex issue and the definitions are subject to change as new discoveries are made. Some scientists talk about subtypes A1, A2, A3, F1 and F2 instead of A and F, though others regard the former as sub-subtypes (Gao et al.1998).

2.1.2. Health burden of HIV/AIDS

HIV infection in humans is now a pandemic. The UNAIDS global report estimated that the number of people living with HIV/AIDS by the end of 2009 was 33 million. By 2008, the global funding for HIV/AIDS had climbed to $15.6 billion (Kates et al. 2009) and by 2009, WHO estimated that 5.2 million were on ART in low and middle income countries (WHO, 2010). As of January 2006, the Joint United Nations Programme on HIV/AIDS (UNAIDS 2006) and the World Health Organization (WHO) estimated that AIDS has killed more than 25 million people since it was first recognized in December 1, 1981.

It is estimated that 0.06% of the world's population was infected with HIV in 2008 (Joint United Programme on AIDS 2008). AIDS has decreased life expectancies by 20 years, and the high rates of HIV infection in adolescents and women of reproductive age have resulted in community destruction, family dissolution, and economic losses in many sub-Saharan African countries. AIDS prevention programme has been actively pursued, and there are

significant ongoing researches focused on the development of an HIV vaccine and effective antiretroviral drugs.

Africa disproportionately bears the burden of the HIV/AIDS pandemic. Although only 11% of the world's population lives in Africa, approximately 67% of those living with HIV/AIDS are in Africa. In Africa, there were 22.4 million people living with HIV and 1.9 million new HIV infections in 2008. An estimated 14 million children in Africa have been orphaned as a result of HIV/AIDS (WHO/UNAIDS/UNICEF 2011).

In 2008 prevalence rate in adults aged 15-49 years was 17.8%, average life expectancy was 48.3 years. It is 6.5 years less than it would have been without the disease. The number of adults aged 15 years and above living with HIV was 5,300,000. Women aged 15 years and above living with HIV was 3,300,000. Children aged 0 to 14 years living with HIV was 330,000. Almost 1,000 AIDS deaths occur every day in South Africa (UNAIDS 2008 report on the global AIDS epidemics). Hospitals are struggling to cope with the number of patients with HIV related diseases that they have to care for. In South Africa, parallel private and public systems exist. The public system serves the vast majority of the population, but is chronically underfunded and understaffed. The wealthiest 20% of the population uses the private system and are far better served. In 2005, South Africa spent 8.7% of GDP on health care, or US$437 per capita. Of that, approximately 42% was government expenditure (WHO 2008). About 79% of doctors work in the private sector (Atagbua 2010).

In 2006 a leading researcher estimated that HIV positive patients would soon account for 60-70% of medical expenditure in South African hospitals (Inter Press Service News Agency

May,2006). Schools have fewer teachers because of the AIDS epidemic. In 2006 it was estimated that 21% of teachers in South Africa were living with HIV (UNAIDS/WHO 2006).

The meagre healthcare budgets in most African countries are not sufficient to cope with the added burden imposed by the HIV pandemic. In an environment where most people are dependent on government for healthcare, the impact of AIDS has been crippling. In some countries up to 16% of healthcare workers are HIV positive, and it is estimated that between 19% and 53% of all government health employee deaths are AIDS related (Benatar 2004).

Clinic visits and hospital admissions have increased. The result has been the reversal of progress in socioeconomic development, healthcare, and improvement in life expectancy. It is difficult to overstate the suffering that HIV has caused in South Africa, with statistics showing that almost one in five adults are infected. For each person living with HIV, the impact is not only on their lives but also on their families, friends and wider communities.

New HIV infections were reduced by 21% since 1997, and deaths from AIDS-related illnesses also decreased by 21% since 2005. The favourable report that emerged from UNAIDS in November 2011 showed a downward trend in HIV/AIDS which gave some light and hope to the people of Africa. There have seen a massive scale up in access to HIV treatment which has had a dramatic effect on the lives of people everywhere. According to UNAIDS and WHO estimates, 47% (6.6 million) of the estimated 14.2 million people eligible for treatment in low and middle income countries were accessing lifesaving antiretroviral therapy in 2010 which is an increase of 1.35 million since 2009. The 2011 UNAIDS World AIDS Day report also highlighted that there are early signs that HIV treatment is having a significant impact on reducing the number of new HIV infections.

Decrease in AIDS-associated mortality coincided with the introduction of highly active antiretroviral therapy (HAART), including protease inhibitors. This suggests that survival following HIV infection increased from 10–12 years to more than 25 years (Greener 2002).

Figure 2 shows that an estimated 5.3 million people are infected in South Africa and 3.7 million in Nigeria in 2003. North Africa seem to have the lowest number of infections in the continent.

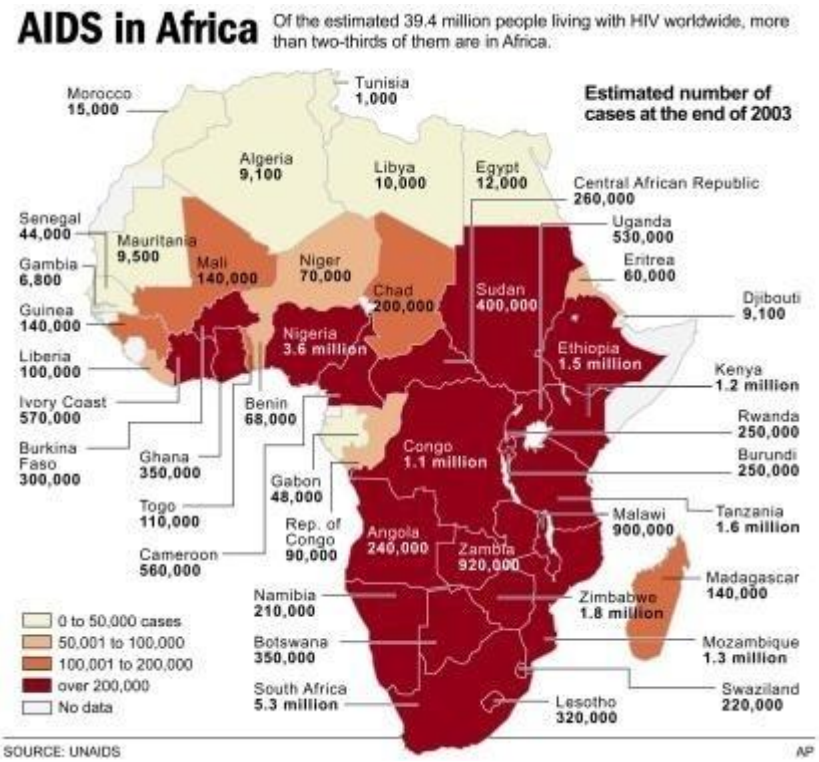

Figure 2. Map of HIV/AIDS in Africa. Source: (CIA World Factbook).
The darker portion of the map shows the area where HIV infection is highest in the continent.

2.1.3. The structure of the HIV

Figure 3. is that of the human immunodeficiency virus. It is roughly spherical with a diameter of about 120nm, which is 60 times smaller than a red blood cell, yet large for a virus. It is composed of two copies of positive single stranded RNA that code for the virus's nine genes enclosed by a conical capsid composed of 2000 copies of the viral protein. The single strand RNA is tightly bound to nucleocapsid proteins, p7 (Nielsen et al. 2005). .

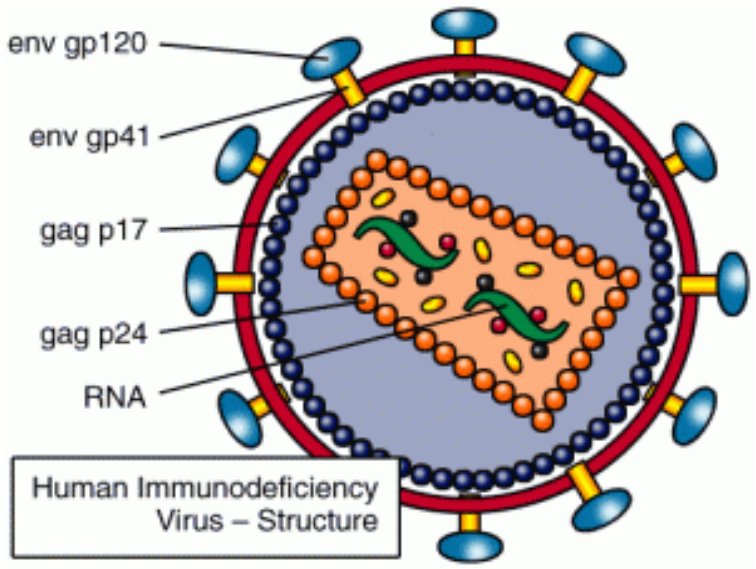

Figure 3. The structure of the Human immunodeficiency virus. Source: (National Institute of Allergy and Infectious Diseases, 2009).

HIV particles surround themselves with a coat of fatty material known as the viral envelope (or membrane). Projecting from the viral envelope are around 72 little spikes, which are formed from the proteins gp 120 and gp 41. Just below the viral envelope is a layer called the matrix, which is made from the protein p17 (Nielsen et al. 2005). The viral core (or capsid) is usually bullet-shaped and is made from the protein p24. Inside the core are three enzymes required for HIV replication called reverse transcriptase, integrase and protease. Also held within the core is HIV's genetic material, which consists of two identical strands of ribonucleic acid (RNA). Almost all organisms, including most viruses, store their genetic materials on long strands of DNA. Retroviruses are the exception because their genes are composed of only RNA.

RNA has a very similar structure to deoxyribose nucleic acid (DNA). However, small differences between the two molecules mean that HIV's replication process is a bit more complicated than that of most other viruses. HIV has just nine genes (compared to more than 500 genes in a bacterium, and around 20,000-25,000 in a human). Three of the HIV genes,

called gag, pol and env, contain information needed to make structural proteins for new virus particles. The other six genes, known as tat, rev, nef, vif, vpr and vpu, code for proteins that control the ability of HIV to infect a cell, produce new copies of virus, or cause disease. At either end of each strand of RNA is a sequence called the long terminal repeat, which helps to control HIV replication (Nielsen et al. 2005).

2.1.4. The life cycle of HIV

HIV can only replicate inside human cells. The process typically begins when a virus particle bumps into a cell that carries on its surface a special protein called CD4. The life cycle of HIV can be as short as about 1.5 days from viral entry into a cell, through replication, assembly and release of additional viruses, to infection of other cells. HIV enters

macrophages and $CD4^+$ T cells by the adsorption of glycoproteins on its surface to receptors on the target cell, followed by fusion of the viral envelope with the cell membrane and the release of the HIV capsid into the cell. The spikes on the surface of the virus particle sticks to the $CD4^+$ receptor on the cell and allows the viral envelope to fuse with the cell membrane (Figure 4). The contents of the HIV particle are then released into the cell, leaving the envelope behind. Once inside the cell, the HIV enzyme reverse transcriptase converts the viral RNA into DNA, which is compatible with human genetic material.

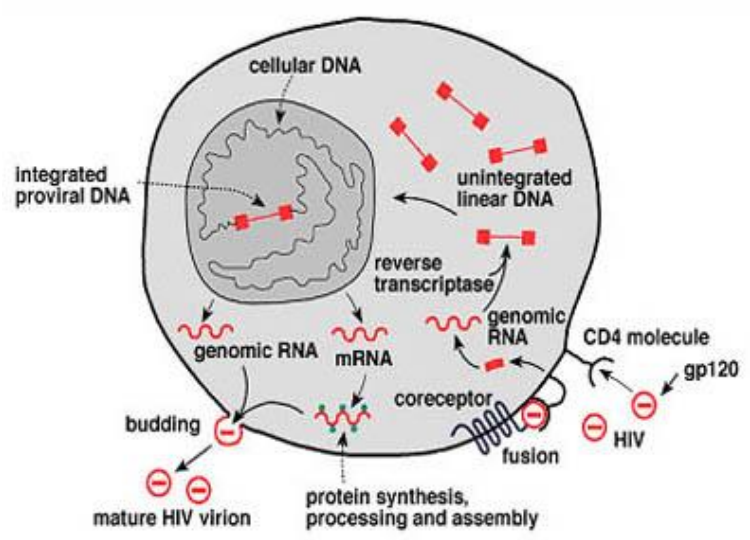

Figure 4. The life cycle of the HIV. Sources: NIH Health Topics and "Biology of HIV" (Nielsen et al. 2005).

2.1.4.1. Transportation of the DNA into the cell

This DNA is transported to the cell's nucleus, where it is spliced into the human DNA by the HIV enzyme integrase. Once integrated, the HIV DNA is known as provirus. HIV provirus may lie dormant within a cell for a long time. When the cell becomes activated, it treats HIV genes in much the same way as human genes. First it converts them into messenger RNA (using human enzymes). Then the messenger RNA is transported outside the nucleus, and is used as a blueprint for producing new HIV proteins and enzymes (Nielsen et al. 2005).

2.1.4.2. The release of new viral particles

Among the strands of messenger RNA produced by the cell are complete copies of HIV genetic material. These gather together with newly made HIV proteins and enzymes to form new viral particles. The HIV particles are then released or 'bud' from the cell (Figure 5).

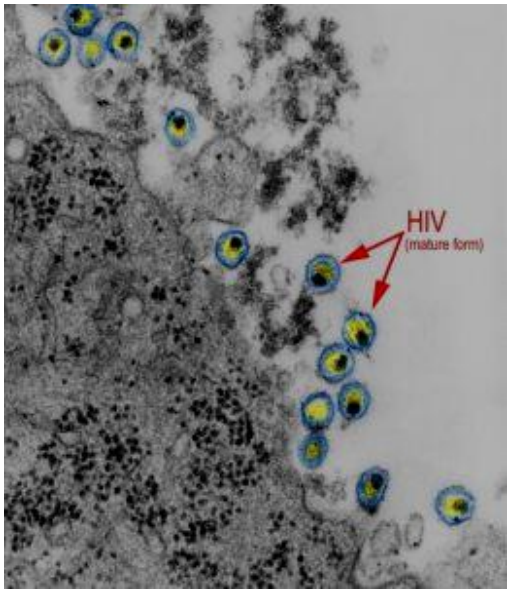

Figure 5. showing HIV budding from an infected cell. Sources: NIH Health Topics and "Biology of HIV"(Nielsen et al. 2005).

The enzyme protease plays a vital role at this stage of the HIV life cycle by chopping up long strands of protein into smaller pieces, which are used to construct mature viral cores. The newly matured HIV particles are ready to infect another cell and begin the replication process all over again.

In this way the virus quickly spreads through the human body. Once a person is infected, they can pass HIV on to others through their body fluids. HIV differs from many viruses in that it has very high genetic variability. This diversity is as a result of its fast replication cycle with the generation of 10^9 to 10^{10} virions every day coupled with a high mutation rate. This complex scenario, leads to the generation of many variants of HIV in a single cell infected patient in the course of one day (National Institute of Allergy and Infectious Diseases (NIAID) (2009).

2.1.5. Events in HIV infection

Untreated HIV disease is characterized by a gradual deterioration of immune function. Most notably, the T cells that contain CD4 proteins (represented as $CD4^+$ T cells) are disabled and killed during the typical course of infection. These cells, sometimes called 'T-helper cells', play a central role in the immune response, signaling other cells in the immune system to perform their special functions. A healthy, uninfected person usually has 800 to 1,200 $CD4^+$ T cells per cubic millimeter (mm^3) of blood. During HIV infection, the number of these cells in a person's blood progressively declines. When the $CD4^+$ T cell count falls below 200/mm^3, a person becomes particularly vulnerable to the opportunistic infections and cancers that typify AIDS, the end stage of HIV disease (CDC, 1994).

Most scientists think that HIV causes AIDS by inducing the death of $CD4^+$ T cells or interfering with their normal function, and by triggering other events that weaken a person's immune function. For example, the network of signaling molecules that normally regulates a person's immune response is disrupted during HIV disease, impairing a person's ability to fight other infections. The HIV-mediated destruction of the lymph nodes and related immunologic organs also plays a major role in causing the immunosuppression seen in people with AIDS.

2.1.6. The acute phase of primary infection in HIV

Once the virus enters the body, it infects a large number of $CD4^+$ cells and replicates rapidly. During this acute phase of primary infection the blood contains many viral particles that spread throughout the body seeding various organs especially the lymphoid organs. Two to four weeks after exposure to the virus, some people may

suffer flu-like symptoms related to the acute infection. Their immune system fights back with killer T cells ($CD8^+$ T cells) and B-cell-produced antibodies, which dramatically reduce HIV levels (CDC, 1994).

A person's $CD4^+$ T cell count may rebound somewhat and even approach its original level. A person may then remain free of HIV-related symptoms for years despite continuous replication of HIV in the lymphoid organs that had been seeded during the acute phase of infection. One reason that HIV is unique is the fact that despite the body's aggressive immune responses, which are sufficient to clear most viral infections, some of the HIV invariably escapes. This is due in large part to the high rate of mutations that occur during the process of HIV replication. In addition, early in the course of HIV infection, people may lose HIV-specific $CD4^+$ T cell responses that normally slow the replication of viruses. Such responses include the secretion of interferons and other antiviral factors, and the orchestration of $CD8^+$ T cells. Finally, the virus may hide within the chromosomes of an infected cell and be shielded from surveillance by the immune system. Such cells can be considered as a latent reservoir of the virus. The antiviral agents currently in our therapeutic arsenal attack are not effective against hidden, inactive viral DNA (so-called provirus). New strategies to purge this latent reservoir of HIV have become one of the major goals for current research efforts (CDC 2009).

2.1.7. Variation in disease progression in HIV/AIDS

Among people enrolled in large epidemiologic studies in Western countries, the median time from infection with HIV to the development of AIDS-related symptoms

has been estimated to be 10 to 12 years without the use of antiretroviral therapy. Researchers, however, have observed a wide variation in disease progression. Approximately 10 per cent of HIV-infected people in some of these studies have progressed to AIDS within the first 2 to 3 years following infection, while up to 5 per cent of individuals in the studies have stable $CD4^+$ T cell counts and no symptoms even after 12 or more years. Factors such as age or genetic differences among individuals, the level of virulence of an individual strain of virus, and co-infection with other microbes may influence the rate and severity of disease progression. Drugs that fight the infections associated with AIDS have improved and prolonged the lives of HIV-infected people by preventing or treating conditions such as *Pneumocystis jirorecii* pneumonia, cytomegalovirus disease, and diseases caused by a number of fungi.

Recent research has shown that most infecting strains of HIV use a co-receptor molecule called chemokine receptor 5 (CCR5), in addition to the CD4 molecule, to enter certain of its target cells (Lederman et al 2006). HIV-infected people with a specific mutation in one of their two copies of the gene for this receptor may have a slower disease course than people with two normal copies of the gene. Rare individuals with two mutant copies of the CCR5 gene appear, in most cases, to be completely protected from HIV infection (Anderson & Sansom 2007). Mutations in the gene for other HIV co-receptors also may influence the rate of disease progression. Numerous studies show that people with high levels of HIV in their bloodstream are more likely to develop new AIDS-related symptoms or die than those with lower levels of virus (Quinn 2011). In the multicenter AIDS cohort study

(MACS), investigators showed that the level of HIV in an untreated person's plasma 6 months to a year after infection (the so-called viral "set point") is highly predictive of the rate of disease progression; that is, patients with high levels of virus are much more likely to get sick faster than those with low levels of virus. The MACS and other studies have provided the rationale for providing aggressive antiretroviral therapy to HIV-infected people, as well as for routinely using newly available blood tests to measure viral load when initiating, monitoring, and modifying anti-HIV therapy.

2.1.8. HAART and progression to AIDS

Potent combinations of three or more anti-HIV drugs known as highly active antiretroviral therapy, or HAART, can reduce a person's viral burden to very low levels and in many cases delay the progression of HIV disease for prolonged periods. Before the introduction of HAART therapy, 85 per cent of patients survived an average of less than 3 years following AIDS diagnosis. Antiretroviral regimens, however, have yet to completely and permanently suppress the virus in HIV-infected people.

Recent studies have shown that, in addition to the latent HIV reservoir, HIV persists in a replication-competent form in resting $CD4^+$ T cells even in people receiving aggressive antiretroviral therapy who have no readily detectable HIV in their blood. Investigators around the world are working to develop the next generation of anti-HIV drugs that can stop HIV, even in these biological scenarios. A treatment goal, along with reduction of viral burden, is the reconstitution of the person's immune system, which may have become sufficiently damaged that it cannot replenish itself.

2.1.9. HIV progresson and cytokines

Various strategies for assisting the immune system are being tested in clinical trials. Although HIV-infected people often show an extended period of clinical latency with little evidence of disease, the virus is never truly completely latent. Researchers have shown that even early in the disease, HIV actively replicates within the lymph nodes and related organs, the virus become trapped in networks of specialized cells. These cells are called follicular dendritic cells (FDCs). FDCs are located in hot spots of immune activity in lymphoid tissue called germinal centres. FDCs trap invading pathogens until B cells come along to start an immune response. Over a period of years significant amounts of virus accumulate in the lymphoid tissue, both within infected cells and bound to FDCs. Numerous $CD4^+$ T cells are probably activated by immune system cells within the lymphoid tissue in the germinal centres by increasing production of cytokines such as tumour necrosis factor (TNFα) and interleukin 6 (IL-6). Activating the CD4 cells allows uninfected cells to be more easily infected and increases replication of HIV in already infected cells (National Institute of Allergy and Disease, 2009). While greater quantities of certain cytokines such as TNFα and IL-6 are secreted during HIV infection, other cytokines with key roles in the regulation of normal immune function may be secreted in decreased amounts (National institute of Allergy and Disease, 2009). For example, $CD4^+$ T cells may lose their capacity to produce Interleukin 2 (IL-2), a cytokine that enhances the growth

of other thymus dependent cells (T cells) and helps to stimulate other cells' response to invaders (National institute of Allergy and Disease, 2009). Infected cells also have low levels of receptors for IL-2, which may reduce their ability to respond to signals from other cells. Ultimately, with chronic cell activation and secretion of inflammatory cytokines, the fine and complex inner structure of the lymph node breaks down and is replaced by scar tissue. With the breakdown in structure, cells in the lymph node cannot communicate and the immune system cannot function properly. Investigators also have reported recently that this scarring reduces the ability of the immune system to replenish itself following antiretroviral therapy that reduces the viral burden (National institute of Allergy and Disease, 2009).

2.1.10. CD8 + T Cells and HIV progression

$CD8^+$ T cells are critically important in the immune response to HIV. These cells attack and kill infected cells that are producing the virus. Thus, vaccine efforts are directed toward eliciting or enhancing these killer T cells, as well as eliciting antibodies that will neutralize the infectivity of HIV. $CD8^+$ T cells also appear to secrete soluble factors that suppress HIV replication (National institute of Allergy and Disease, 2009).

Several molecules, including "regulated upon activation normal T cell expressed (RANTES), and presumably secreted" molecules, macrophage Inflammatory Protein-1 alpha, (MIP-1alpha), macrophage Inflammatory Protein-1 beta, (MIP-1beta), and macrophage derived chemokine (MDC) appear to block HIV replication by occupying the co-receptors necessary for many strains of HIV to enter their target cells. There

may be other immune system molecules - including the so-called CD8 antiviral factor (CAF), the defensins (type of antimicrobials), and others yet undiscovered - that can suppress HIV replication to some degree (National Institute of Allergy and Infectious Disease, 2012).

2.1.11. Mutation, Cell death in HIV progression

HIV replicates rapidly and several billion new virus particles may be produced every day. In addition, the HIV reverse transcriptase enzyme makes many mistakes while making DNA copies from HIV RNA. As a consequence, many variants or strains of HIV develop in a person, some of which may escape destruction by antibodies or killer T cells. Additionally, different strains of HIV can recombine to produce a wide range of variants (Nielsen et al. 2005). During the course of HIV disease, viral strains emerge in an infected person that differs widely in their ability to infect and kill different cell types, as well as in their rate of replication. Scientists are investigating why strains of HIV from people with advanced disease appear to be more virulent and infect more cell types than strains obtained earlier from the same person.

Researchers around the world are studying how HIV destroys or disables $CD4^+$ T cells, and many think that a number of mechanisms may occur simultaneously in an HIV-infected person. Data suggest that billions of $CD4^+$ T cells may be destroyed every day, eventually overwhelming the immune system's capacity to regenerate. Infected CD4+ T cells may be killed directly when large amounts of virus are produced and bud out from the cell surface, disrupting the cell membrane, or when

viral proteins and nucleic acids collect inside the cell, interfering with cellular machinery. Infected $CD4^+$ T cells may be killed when the regulation of cell function is distorted by HIV proteins, probably leading to cell suicide by a process known as programmed cell death or apoptosis (National Institute of Allergy and Disease, 2009). Recent reports indicate that apoptosis occurs to a greater extent in HIV-infected people, both in their bloodstream and lymph nodes. Apoptosis is closely associated with the aberrant cellular activation seen in HIV disease. Uninfected cells also may undergo apoptosis.

Investigators have shown in cell cultures that the HIV envelope alone or bound to antibodies sends an inappropriate signal to $CD4^+$ T cells causing them to undergo apoptosis, even if not infected by HIV (NIAID 2009). Uninfected cells may die in an innocent bystander scenario: HIV particles may bind to the cell surface, giving them the appearance of an infected cell and marking them for destruction by killer T cells. This process is called antibody-dependent cellular cytotoxicity. Killer T cells also may mistakenly destroy uninfected cells that have consumed HIV particles and that display HIV fragments on their surfaces. HIV envelope proteins bear some resemblance to certain molecules that may appear on $CD4^+$ T cells, the body's immune responses may mistakenly damage such cells as well.

2.1.12. Cell activation and HIV progression

Researchers have shown in cell cultures that $CD4^+$ T cells can be turned off by activation signals from HIV that leaves them unable to respond to further immune stimulation (National Institute of Allergy and Disease, 2009). This inactivated state is

known as anergy. Studies suggest that HIV also destroys precursor cells that mature to have special immune functions, as well as the microenvironment of the bone marrow and the thymus needed for developing such cells (NIAID 2009). These organs probably lose the ability to regenerate, further compounding the suppression of the immune system. Although monocytes and macrophages can be infected by HIV, they appear to be relatively resistant to being killed by the virus (Kopperstaner et al. 2012). These cells, however, travel throughout the body and carry HIV to various organs, including the brain, which may serve as a hiding place or 'reservoir' for the virus that may be relatively resistant to most anti-HIV drugs.

2.1.13. The normal immune response

During a normal immune response, many parts of the immune system are mobilized to fight an invader. $CD4^+$ T cells, for instance, may quickly multiply and increase their cytokine secretion, thereby signalling other cells to perform their special functions. Scavenger cells called macrophages may double in size and develop numerous organelles, including lysosomes that contain digestive enzymes used to process ingested pathogens. Once the immune system clears the foreign antigen, it returns to a relative state of quiescence.

2.1.14. Chronic cell activation and disease progression

HIV disease for most of its course is characterized by immune system hyperactivation, which has negative consequences. HIV replication and spread are much more efficient in activated $CD4^+$ cells (NIAID 2009). Chronic immune system activation during HIV disease also may result in a massive stimulation of B cells, impairing the ability of these cells to make antibodies against other pathogens. Chronic immune activation also can result in apoptosis,

and an increased production of cytokines that not only may increase HIV replication but also have other deleterious effects.

Increased levels of TNF-alpha, for example, may be at least partly responsible for the severe weight loss or wasting syndrome seen in many HIV-infected people (National Institute of Health, 2001). The persistence of HIV and HIV replication plays an important role in the chronic state of immune activation seen in HIV-infected people. In addition, it has been shown that infections with other organisms activate immune cells and increase production of the virus in HIV-infected people (NIAID 2009). Chronic immune activation due to persistent infections or the cumulative effects of multiple episodes of immune activation and bursts of virus production, likely contribute to the progression of HIV disease.

2.1.15. Concept of cardiovascular risk and HIV

Ischaemic cardiovascular events increasingly occur in those patients infected for many years with HIV and are attributed either to the infection itself or to the use of HAART. In the context of declining rates of HIV-related death, proportions of HIV infected patients dying of other causes have increased. For example, a death certificate study in New York City showed that the proportion of deaths among HIV-infected patients due to non–HIV-related causes increased from 19.8% to 26.3% between 1999 and 2006, reflecting mortality resulting from cardiovascular disease (CVD), substance abuse, and non–AIDS-defining cancers (Sackoff et al. 2006). Among individuals aged 55 years or older, CVD was the leading cause of death. Numerous studies have indicated increased risk of myocardial infarction (MI) in HIV populations, with HIV infection considered at least a partial CVD risk factor in these studies (Triant et al. 2007). Triant et al (2007) found a 75% increase in risk of MI admissions in HIV-infected patients.

At the 9th Retrovirus conference in February 2002 different conflicting datas were presented about HAART and cardiovacular risk factors. In the research work presented by Holmberg et al (2002) they followed 5,676 HIV positive people at nine clinics, HIV Out Patient clinics (the HOPS cohort) from Centres for Disease Control and prevention (CDC) from 1993 to 2001. Slightly more than half the group used protease inhibitor (PI) -based regimens. Thirteen of 3,013 (0.43%) participants taking protease inhibitors (PIs) suffered myocardial infarctions during the study period, compared with two out of 2,663 (.075%) participants not using PIs (Holmberg et al. 2002). Bozzette et al (2002) claimed that there seems to be a slight decline in heart disease and strokes since the advent of HAART. They examined the medical records of 36,766 HIV positive patients at U.S. Veterans administration medical centers over eight-year period. During this time there were 1,800 hospital admissions and 500 deaths due to cardiovascular or cerebrovascular disease. They found out that since 1997, rates of hospital admissions and deaths due to heart attack or stroke have fallen compared to the rates prior to the availability of PIs. Over the course of the study period, heart attack and stroke admission and mortality rates declined by 10-20%. The researchers concluded that their findings did not support an association between PI, NRTI, or non-nucleoside reverse transcriptase inhibitor (NNRTI) use and excess cardiovascular or cerebrovascular problems. However, the results of the study by Bozzette et al (2002) may reflect the fact that by 1997 health-care providers were prescribing statins and other lipid-lowering therapies for HIV positive people with high blood lipid levels.

A smaller study by Klein et al (2002) also did not detect an increase in cardiovascular events among 4,159 HIV positive men in the Kaiser health-care system (Kaiser health-care system is an integrated managed care consortium, based in Oakland, California, United States, founded

in 1945 by industrialist Henry J. Kaiser and physician Sidney Garfield) since the introduction of PIs.

Arterial stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Atherosclerotic changes within the arterial wall are known to increase arterial stiffness and alter wave reflection (Laurent et al. 2006). Increased arterial wave reflection is associated with coronary artery disease and is an independent predictor of adverse coronary events (London et al. 2001). This has however not been well studied in the setting of HIV infection. This study will therefore involve looking into arterial stiffness which will be a surrogate of atherosclerosis in HIV positive participants on treatment and drug naïve HIV positive participants.

Some studies found HIV infection and HAART treatment are associated with increased arterial stiffness and increased heart rate. These vascular alterations are possible causes of the increased cardiovascular risk observed in HIV infected patients (Papita et al. 2011). Arterial stiffness and pulse pressure were found to be important determinants of cardiovascular risk. The two leading causes of death in the developed world, myocardial infarction and stroke, are both direct consequences of atherosclerosis.

In a study by Dube et al (2008), it was stated that HIV-related cardiovascular disease is an under recognized and unappreciated cause of symptomatic illness and a predictor of all cause mortality in late stage HIV infection (Dube at al. 2008). A high degree of suspicion and early screening may allow appropriate intervention and improved quality of life in those affected. It might be therefore imperative if HIV patients both on treatment and those who are treatment naïve be screened and evaluated for their vascular functions. It will also be worthy of note to know the effects of cytokines and adhesion molecules on arterial stiffness. The natural course

of the HIV virus itself will be looked into, including the mechanism by which the virus increases cardiovascular morbidity and mortality.

In 2008 the DAD (Data collection on adverse events on anti HIV Drugs) study group reported ways of decreasing cardiovascular risks in HIV. They reported that having HIV is associated with increases in two important risk factors for cardiac disease. These are; low levels of HDL- cholesterol and elevated levels of triglycerides. These scientists (DAD study group, 2008) noted that many HIV infected patients have abnormal levels of cholesterol, insulin resistance, diabetes, excess abdominal weight and other risk factors which may be side effects of antiretroviral medications. These disturbances in lipid and glucose metabolism may contribute to the excess cardiovascular disease morbidity and mortality observed in HIV infected individuals (Francisci et al. 2009).

Antiretroviral therapy has greatly reduced the risk of early death from opportunistic infections and extended the lifespan of people infected with the HIV. This has led to many complications and organ damage in the HIV infected population. Grunfeld et al (1992) mentioned that in the era before HAART the only metabolic abnormality noted in AIDS was the hypertriglyceridaemia associated with a wasting syndrome. This he attributed to high levels of cytokines accompanying chronic infection. Since the advent of effective antiretroviral therapy, infection with the human immunodeficiency virus has been transformed in the western world to a chronic disease associated with a variety of metabolic complications (Gkrania-Klotsas & Klotsas 2007). HAART has allowed for prolonged survival even in patients at an advanced stage of AIDS, with their life expectancy increased by more than 10 years. The association between the use of HAART and an increased risk in metabolic syndrome and cardiovascular disease remains unclear (Mondy et al. 2008). Large

international studies have also implicated the HIV itself and anti-retroviral therapy (ART) as potential mediators of this increased risk of CVD. There were whole lots of contradicting findings on cardiovascular risk factors in HIV positive patients on treatment and drug naïve participants (Monteiro et al. 2012, Saves et al. 2003).

The few studies evaluating the prevalence of traditional cardiovascular risk factors among HIV positive patients in sub-Saharan Africa suggest that rates are significantly lower than in developed countries but some of this difference may be attributed to under detection based on low clinical suspicion and small sample sizes.

Inflammation contributes to the pathogenesis of cardiovascular disease. TNFα in particular is a key mediator of inflammation and vascular dysfunction and progression of atherosclerotic disease (Haddy et al. 2003). Zhang (2008) showed that inflammatory cytokines like TNFα, IL-6, P selectin, Von Willebrand factor may all increase in chronic HIV infection. He cited inflammatory cytokines as important protagonists in formation of atherosclerotic plaque, eliciting their effects throughout the atherosclerotic vessel. These studies, however, have not included patients from sub-Saharan Africa.

2.1.16. Cardiovascular risk factors and HIV in Africa

Africa is home to the majority of the world's HIV infected population (UNAIDS 2004) and yet very few of recent reviews on the cardiovascular manifestation of HIV have examined the African experience (Barbaro 2002). Although the link between heart disease and HIV positive status has been proven in first world countries, there is difficulty in ascertaining the root cause because many people are already on HIV treatment.

Estimates from outside Africa are that the incidence of HIV-associated vasculitis is less than 1% (Johnson et al 2003). Ntsheke & Hakim (2005) reported that large-vessel vasculopathy involving the aorta or its major branches is increasingly being recognized in young African subjects with mean age of 31 years. It was reported that these young Africans had no evidence of atherosclerosis, syphilis or other causes of vasculitis. The large vessel vasculopathy occurred at a relatively early stage of HIV disease (median CD4 count $370x10^6$/L).

Concurrently, the epidemiology and demography of sub-Saharan Africa is shifting towards older populations with a higher proportion of CVD due to chronic, non-communicable diseases. Despite these facts, the region is under-represented in studies examining the relationship between HIV and CVD risk factors. Africa is important due to the exceedingly high prevalence of HIV in this region. Furthermore, global efforts in sub-Saharan Africa aimed solely at HIV care may be missing a critical opportunity to improve overall cardiovascular health if chronic CVD risk factors is highly prevalent. The majority of the articles in Africa examined aspects of pericardial disease in HIV infected African subjects. The remainder described the prevalence of clinical spectrum and unusual features of cardiac disease in HIV infected subjects. Mutimura (2008) in Kigali, Rwanda suggested an increasing incidence of HIV-associated cardio metabolic syndrome (CMS), in developing countries especially in urban settings. Predictions indicate that the greatest increase in the prevalence of diabetes will occur in Africa over the next two decades due to lifestyle changes. This, coupled with increased access to HAART, may exponentially increase the prevalence of CMS in developing countries, where HIV infection is prevalent.

Appropriate evaluation and intervention programmes need to be implemented in the developing world, especially sub-Saharan Africa, to curtail HIV-related CMS. This should include routine cardiovascular risk assessments, management of HIV infection with more "metabolically friendly" HAART, and encouragement of lifestyle modifications, particularly smoking cessation, weight management, regular exercise, and adherence to a healthy diet.

2.1.17. Clinical staging of HIV/AIDS by WHO

The World Health Organization (WHO) has developed case definitions for HIV surveillance and clinical staging and immunological classification of HIV-related disease in adults and children. This system uses standardized clinical parameters to direct medical decision making for patients with HIV/AIDS and can be used based solely on patient clinical features, thus accommodating facilities with no or limited access to laboratory testing.

The WHO Clinical Staging system has been shown to be a practical and accurate way to manage HIV-infected patients, with international studies showing agreement between clinical manifestations included in the WHO staging system and laboratory markers including CD4 cell count and total lymphocyte count. With the progression of the HIV/AIDS epidemic, consideration of the entire spectrum of infection is necessary. Several discrete clinical phases can be recognized which correlate with the degree of immunodeficiency that arises with progression of HIV infection.

The revised WHO HIV/AIDS Clinical Staging System is intended for baseline assessment of patients and for use in provision of ongoing care (WHO 2005). It provides guidance, including when to start, switch, or stop prophylactic medications, antiretrovirals, and other interventions. It assists clinicians in the assessment of a patient's current clinical status;

encourages clinical providers to offer diagnostic HIV testing to patients who exhibit clinical symptoms and signs suggestive of HIV infection; classifies disease in a progressive sequence from least to most severe. It is designed to be used with reference to current and previous clinical events, making it useful for surveillance purposes.

2.1.18. The Clinical Stages of HIV/AIDS defining their illnesses

The WHO system for adults, sorts patients into one of four hierarchical clinical stages ranging from stage 1 (asymptomatic) to stage 4 (AIDS). Patients are assigned to a particular stage when they demonstrate at least one clinical condition in that stage's criteria. Patients remain at a higher stage after they recover from the clinical condition which placed them in that stage.

2.1.18.1. Stage 1

Patients who are asymptomatic or have persistent generalized lymphadenopathy (lymphadenopathy of at least two sites (not including inguinal) for longer than 6 months are categorized as being in stage 1, where they may remain for several years.

2.1.18.2. Stage 2

Clinical findings included in stage 2 (mildly symptomatic stage) are unexplained weight loss of less than 10 per cent of total body weight and recurrent respiratory infections (such as sinusitis, bronchitis, otitis media, and pharyngitis), as well as a range of dermatological conditions including herpes zoster flares, angular cheilitis, recurrent oral ulcerations, papular pruritic eruptions, seborrhoeic dermatitis, and fungal nail infections.

2.1.18.3. Stage 3

Those manifestations encompassed by the WHO clinical stage 3 (the moderately symptomatic stage) category are weight loss of greater than 10 per cent of total body weight, prolonged (more than 1 month), unexplained diarrhoea, pulmonary tuberculosis, and severe systemic bacterial infections including pneumonia, pyelonephritis, empyema, pyomyositis, meningitis, bone and joint infections, and bactereamia. Mucocutaneous conditions, including recurrent oral candidiasis, oral hairy leukoplakia, and acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis, may also occur at this stage.

2.1.18.4. **Stage 4**

The WHO clinical stage 4 (the severely symptomatic stage) designation includes all of the AIDS-defining illnesses. Clinical manifestations for stage 4 disease that allow presumptive diagnosis of AIDS to be made based on clinical findings alone are HIV wasting syndrome, pneumocystis pneumonia (PCP), recurrent severe or radiological bacterial pneumonia, extrapulmonary tuberculosis, HIV encephalopathy, central nervous system (CNS) toxoplasmosis, chronic (more than 1 month) or orolabial herpes simplex infection, esophageal candidiasis, and Kaposi's sarcoma.

A modified version of the WHO Clinical Staging System is available for infants and children under 15 years. According to the WHO, advanced HIV/AIDS disease is defined for surveillance purposes as any clinical stage-3 or stage-4 disease or any clinical stage with a CD4 count less than 350 per cubic mm, and this information can be used to calculate the burden of disease and the demand for antiretroviral therapy. WHO recommends definitive initiation of antiretroviral therapy in adults and adolescents in clinical stage 4, consideration of therapy initiation for those in clinical stage 3, and antiretroviral use for those in clinical stage 1 or 2 only if the CD4 count is less than 250 per cubic mm.

For patients taking antiretroviral therapy for more than 24 weeks, new or recurrent clinical staging events can be a guide to decision-making. Prior to 24 weeks of antiretroviral treatment, clinical events are largely influenced by immune reconstitution or treatment toxicity and may not accurately reflect immune deterioration.

2.2. Antiretrovirals

Antiretroviral drugs are medications for the treatment of infection by retroviruses, primarily HIV. This is the main type of treatment for HIV or AIDS. It is not a cure, but it can stop people from becoming ill for many years. The treatment consists of drugs that are taken every day for the rest of a person's life. The aim of antiretroviral treatment is to keep the amount of the virus in the body at a low level. This stops any weakening of the immune system and allows it to recover from any damage that HIV might have caused already.

The drugs are often referred to as:

- Antiretrovirals;
- ARVs;
- anti-HIV or anti-AIDS drugs.

Taking two or more antiretroviral drugs at a time is called combination therapy. Taking a combination of three or more anti-HIV drugs is sometimes referred to as Highly Active Antiretroviral Therapy (HAART). If only one drug was taken, HIV would quickly become resistant to it and the drug would stop being effective. Taking two or more antiretrovirals at the same time vastly reduces the rate at which resistance would develop, making treatment more effective in the long term. There are different classes of antiretroviral drugs that act at different stages of the HIV Cycle.

The key goal of HIV treatment is to slow HIV replication as much as possible and enable recovery of the immune system. Antiretroviral drugs are the mainstay of HIV treatment: each antiretroviral drug class targets a different step in the viral life-cycle. These include, but are not limited to:

- Nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs and NRTIs);
- Non-nucleoside reverse transcriptase inhibitors (NNRTIs);
- Protease inhibitors;
- Integrase inhibitors;
- Fusion inhibitors;
- CCR5 inhibitors.

There are a number of other candidate drugs in clinical trials, including one in a class called 'maturation inhibitors', as well as innovations in immune-based strategies (Thaczuk 2012).

The standard of care for anyone on antiretroviral treatment is highly active antiretroviral therapy (HAART), using drugs with at least two different mechanisms of action (for example, two NRTIs plus either an NNRTI or a protease inhibitor). Over time, HIV can develop mutations that make it resistant to drugs. By targeting multiple steps in the viral life-cycle simultaneously, the emergence of resistance can be slowed or prevented (Collin &Van Lint 2009).

Researchers have also explored other approaches for treating HIV, such as inhibiting cellular factors the virus needs for its replication, gene therapy that protects cells from infection, and removal of cells that are already infected (Allers et al. 2011). Many of these approaches are experimental and some remain purely theoretical. But there is evidence that complementary

therapies such as nutrient supplements (used in conjunction with antiretroviral therapy) can improve the overall health of people with HIV (Thaczuk 2012). New studies are showing that even at CD4 cell counts between 350 and 500 cells/mm^3, there is a greater risk of morbidity and mortality from non-AIDS illnesses (Zolopa et al. 2009).

While viral load suppression is considered an indicator of effective therapy, the ultimate goal of treatment is to preserve immune function, increase disease-free survival, and reduce mortality (Borrow et al. 2010).

2.2.1. Classes of drugs

Nucleoside and nucleotide reverse transcriptase inhibitors (NRTI), inhibit reverse transcription by being incorporated into the newly synthesized viral DNA and preventing its further elongation.

Non nucleoside reverse transcriptase inhibitors (NNRTI), inhibit reverse transcriptase directly by binding to the enzyme and interfering with its function.

Protease inhibitors (PIs), target viral assembly by inhibiting the activity of protease, an enzyme used by HIV to cleave nascent proteins for final assembly of new virons.

Integrase inhibitors, inhibit the enzyme integrase, which is responsible for integration of viral DNA into the DNA of the infected cell. There are several integrase inhibitors currently under clinical trial, and raltegravir became the first to receive FDA approval in October 2007.

Entry inhibitors (or fusion inhibitors), interfere with binding, fusion and entry of HIV -1 to the host cell by blocking one of several targets.

Maturation inhibitors, inhibit the last step in gag processing in which the viral capsid polyprotein is cleaved, thereby blocking the conversion of the polyprotein into the mature capsid protein. Because these viral particles have a defective core, the virions released consist mainly of non infectious particles.

CCR5 receptor antagonists are a class of small molecules that antagonize the CCR5 receptor. The C-C motif chemokine receptor CCR5 is involved in the process by which HIV, enters cells. Hence antagonists of this receptor are entry inhibitors and have potential therapeutic applications in the treatment of HIV infections (Ledermann et al. 2006).

NRTIs and NNRTIs are available in most countries. Fusion/entry inhibitors and integrase inhibitors are usually only available in resource-rich countries. Protease inhibitors are generally less suitable for starting treatment in resource-limited settings due to the cost, number of pills which need to be taken, and the particular side effects caused by protease drugs. The most common drug combination given to those beginning treatment consists of two NRTIs combined with either an NNRTI or a "boosted" protease inhibitor. An example of a common antiretroviral combination is the two NRTI's zidovudine and lamivudine, combined with the NNRTI efavirenz. Some antiretroviral drugs have been combined into one pill, which is known as a 'fixed dose combination'. This reduces the number of pills to be taken each day. Most people living with HIV in the developing world still have very limited access to antiretroviral treatment and often only receive treatment for the diseases that occur as a result of a weakened immune system. At the beginning of treatment, the combination of

drugs that a person is given is called first line therapy. If after a while HIV becomes resistant to this combination, or if side effects are particularly bad, then a change to second line therapy is usually recommended.

According to WHO and UNAIDS recommendations, the South African guidelines endorse the use of NRTI'S and NNRTI's as first line therapy. For the initiation of ARV therapy, 2 NRTIs and an NNRTI (one drug from category 1 and one from category 11 and one from category IV) may be prescribed. If the viral load is >55,000 a third NRTI (category 111) may be considered as part of a triple NRTI regimen.

Table 1. showing categories of antiretroviral agents

There are five categories of the antiretroviral drugs shown in this table.

Category 1	Category 11	Category 111	Category 1V	Category V
stavudine (dTT)	didanosine(ddl)	abacavir(abc)	nevirapine (Nvp)	nelfinavir(nfv
			favirenz	
	zalcitabine(ddc)		indinavir	
zidovudine			(idv)	
				ritonavir

	lamivudine		saquinavir
AZT(3TC)			softgel
			formulation)
			lopinavir/riton
			avir
			combination

2.2.2. Classes of antiretroviral agents used in public hospitals in South Africa

Antiretrovirals are deferred until patients are prepared to commit themselves to long term treatment and to maintaining good adherence to the therapy. Table 2. below shows the classification of antiretrovirals used in South Africa.

Table 2. The classification of antiretrovirals used in public hospitals in South Africa and their specific action

Classification of antiretroviral agent	Abbreviations	Enzyme inhibited	Specific action
nucleoside reverse transcriptase inhibitors	NRTI'S	reverse transcriptase	mimics the normal building blocks of HIV DNA.

non –nucleoside transcriptase inhibitors	NNRTI'S	reverse transcriptase	directly inhibits reverse transcriptase
protease inhibitors	PIs	protease	inhibits late stages of HIV replication

In July 2012 the WHO issued its first guidance to nations, considering providing ARVs (antiretrovirals) to HIV-negative, high risk people. WHO says its recommendations were based on human trials which showed that administering PrEP (pre-exposure prophylaxis) once daily to HIV-negative individuals who are at high risk of becoming infected is both safe and effective in helping prevent HIV infection.

The first antiretroviral drug to be licensed, zidovudine, became available in 1987. Until December 1995, the antiretroviral drugs available and approved for clinical use in the United States consisted of only five individual drugs belonging to a single class of antiretroviral agents, nucleoside analogue reverse transcriptase inhibitors. Since then, numerous other antiretroviral drugs and classes of antiretroviral drugs have been introduced.

There are currently more than 20 approved antiretroviral drugs in the US and Europe (including combined formulations) and many more in the expanded access programmes and trials. Most antiretroviral drugs have at least three names. Sometimes a drug is referred to by its research or chemical name, such as AZT. The second name is the generic name for all drugs with the same chemical structure; for

example AZT is also known as zidovudine. The third name is the brand name given by the pharmaceutical company; one of the brand names for zidovudine is Retrovir. Lastly, an abbreviation of the common name might sometimes also be used, such as ZDV, which is the fourth name given to zidovudine.

Brand name of drugs and date of approved drugs by the US Food and Drug Administration (FDA) are classified as follows:

Multi-class combinations:

Combination	Brand name	Date of FDA approval
EFV + TDF + FTC	atripla	12-Jul-06
d4T + 3TC + NVP	-	tentative only*
AZT + 3TC+ NVP	-	tentative only*

Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs):

Abbreviation	Generic name	Date of FDA approval
3TC	lamivudine	17-Nov-95
ABC	abacavir	17-Dec-98
AZT or ZDV	zidovudine	19-Mar-87
d4T	stavudine	24-Jun-94
ddI	didanosine	31-Oct-00
FTC	emtricitabine	02-Jul-03
TDF	tenofovir	26-Oct-01

Combined NRTIs:

Combination	Brand name	Date of FDA approval
ABC + 3TC	epzicom (US) kivexa (Europe)	02-Aug-04

ABC + AZT + 3TC	trizivir	14-Nov-00
AZT + 3TC	combivir	27-Sep-97
TDF + FTC	truvada	02-Aug-04
d4T + 3TC	-	Tentative only*

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs):

Abbreviation	Generic name	Date of FDA approval
DLV	delavirdine	04-Apr-97
EFV	efavirenz	17-Sep-98
ETR	etravirine	18-Jan-08
NVP	nevirapine	21-Jun-96
	rilpivirine	20-May-11

Protease Inhibitors (PIs):

Abbreviation	Generic name	Date of FDA approval
APV	amprenavir	15-Apr-99
FOS-APV	fosamprenavir	20-Oct-03
ATV	atazanavir[8]	20-Jun-03
DRV	darunavir	23-Jun-06
IDV	indinavir	13-Mar-96
LPV/RTV	lopinavir+ritonavir	15-Sep-00
NFV	nelfinavir	14-Mar-97
RTV	ritonavir	01-Mar-96
SQV	saquinavir	06-Dec-95

Fusion or Entry Inhibitors:

Abbreviation	Generic name	Date of FDA approval
T-20	enfuvirtide	13-Mar-03
MVC	maraviroc	18-Sep-07

Integrase Inhibitors:

Abbreviation	Generic name	Date of FDA approval
RAL	raltegravir	12-Oct-07

The South Afica Department of Health has endorsed the replacement of d4T with TDF for first-line regimens (South African DOH 2012). Table 3 presents the antiretroviral drugs currently avalable in South Africa.

Table 3. Antiretroviral agents currently available in South Africa

GENERIC NAME	TRADE NAME	CLASS OF DRUG
zidovudine (AZT)	retrovir®*	NRTI
didanosine (ddI)	videx®*	NRTI
zalcitabine (ddC)	hivid®	NRTI
lamivudine (3TC)	3TC®*	NRTI
stavudine (d4T)	zerit®*	NRTI
abacavir	ziagen®*	NRTI
nevirapine	viramune®*	NNRTI
efavirenz	stocrin®	NNRTI
nelfinavir	vira-cept®*	PI
indinavir	crixivan®	PI
ritonavir	norvir®*	PI
saquinavir(hard gel formulation)	invi-rase®	PI
saquinavir (soft gel formulation)	forto-vase®	PI
amprenavir Preclir®*		PI
lopinavir/ritonavir Kaletra®		PI

* Available in paediatric formulations

2.2.3. South African HIV Clinicians Guidelines for Antiretrovirals

The primary goals of antiretroviral therapy are:

- maximal and durable suppression of viral load;
- restoration and/or preservation of immunological function;
- improvement of quality of life and;
- reduction of HIV related morbidity and mortality.

In accordance with WHO and UNAIDS recommendations, the guidelines for the treatment of HIV endorse the use of NRTIs and NNRTIs as first-line therapy.

For initiation of HAART therapy 2 NRTIs and an NNRTI (one drug from Category 1, one from Category 11, and one from Category 1V) is prescribed. If the viral load is <55 000 a third NRTI (Category 111) may be considered as part of a triple NRTI regimen.

Below are indications for starting antiretrovirals in South Africa (Table 4).

Source:(South Africa Clinicians Society Clinical Guideline).

Table 4. Indications for starting antiretrovirals in South Africa

Symptomatic patient	**Treatment**
Presence of HIV related symptoms, current or previous HIV associated disease*	Treatment recommended
Primary infection**	Treatment recommended
Asymptomatic patient with	**Treatment**
$CD4^+$ count<200	Treatment recommended
$CD4^+$ count 200 -350	Monitor CD4+ count Commence treatment if the CD4 annual decline is in excess of the expected 20-80 cells/year or if the CD4 count approaches 200.
$CD4^+$ count >350	Defer treatment

2.2.4. Recommendations for changes in treatment pattern

In 2009 the South Africa National Aids Council (SANAC) Treatment Technical Task Team (TTT) finalized recommendations for changes to the national standard treatment guidelines for adult, paediatric management and treatment, as well as the changes in the prevention of mother

to child transmission (PMTCT) of HIV guidelines. At the same time, the Department of Health convened a working group on costing the antiretroviral treatment (ART) programme, consisting of the Department of Health, the US Government, the Clinton Foundation Health Access Initiative, and the Boston University/Health Economics and Epidemiology Research Office. These two initiatives laid the groundwork for the announcement by the South African President, Mr Zuma on World AIDS Day (1 December 2009), for changes in the national HAART programme. Subsequently, additional changes were made to the treatment guidelines to be in line with these new Presidential mandates, which are effective as of the new government financial year, 1 April 2010. Due to the high cost associated with ART, and the high burden of people in need of ART in South Africa, eligibility criteria have been adapted only for priority groups. These are:

HIV-infected pregnant women;

HIV-infected infants;

people with both TB and HIV infection; and

people with multi-drug resistant (MDR) or extensively drug resistant (XDR) TB.

The other major change to the treatment guidelines is the decentralisation of HIV care and treatment to a primary health care (PHC) level, as contained in the 2007-2011 National Strategic Plan (NSP).

2.2.5. Eligibility to Start ART

The followings are the eligibility criteria for starting ART.

- CD4 count <200cells/mm^3 irrespective of clinical stage ;
- CD4 count <350cells/mm^3 in patients with TB/HIV co-infection,
- or pregnant women ;
- WHO stage 4 disease, irrespective of CD4 count ;

- MDR/XDR TB, irrespective of CD4 count.

In addition, certain patients are fast-tracked to be initiated on ART, which means they should be started within two weeks of receiving their CD4 result, and choosing to start lifelong ART:

- pregnant women;
- patients with a CD4 level below 100;
- any patient with WHO Stage 4 disease;
- any patient with MDR or XDR TB;
- any patient that tests HIV positive, but do not yet meet eligibility criteria, should be enrolled in a wellness programme, with repeat CD4 tests every 6 months.

To assist with this, the Department of Health is introducing a national standard pre-ART register to allow for follow-up. If the TB screening rules out active TB, HIV-infected patients should be started on pimary prophylaxis against tuberculosis.

2.2.6. National regimens for adults and adolescents

National regimens for adults and adolescents are prescribed using first and second line drugs.

2.2.6.1. First Line

All new patients, including pregnant women: tenofovir + lamivudine/emtracitibine + efavirenz/nevirapine.

Currently on a stavudine-based regimen, with no side-effects: stavudine + lamivudine + efavirenz.

Patients with renal problems (for which tenofovir is contraindicated): zidovudine + lamivudine + efavirenz/nevirapine.

2.2.6.2. Second Line

Failing on a stavudine or zidovudine-based first line regimen: tenofovir + lamivudine/emtricitabine + lopinavir/ritonavir. Failing on a tenofovir-based first line regimen: zidovudine + lamivudine + lopinavir/ritonavir.

2.2.7. Clinical and Laboratory Monitoring

There have been only minor changes to the guidelines in terms of monitoring tests, with the major change being the reduction in the periodicity of some tests (e.g. CD4 and Viral Load only done at month 6, month 12, and then every 12 months only), and the addition of creatinine clearance to identify any toxicity to tenofovir.

2.2.8. Children and HAART

For children, eligibility criteria to start HAART are:

- All children under 1 year of age, irrespective of CD4 level;
- Children between 1 and 5 years with clinical stage 3 or 4, or a CD4 percentage of 25 or below, or with an absolute CD4 count under 750 cells/mm^3;
- Children over 5 years and up to 15 years with clinical stage 3 or 4, or CD4 350 cells/mm^3 and below.

The first line regimens for children are:

- Infants and children under 3: abacavir + lamivudine + lopinavir/ritonavir;
- Children 3 years and older: abacavir + lamivudine + efavirenz.

If a child is currently on a stavudine-based regimen, and is not experiencing any side effects, the regimen should be maintained. Substitutions are only made once lipodystrophy is

suspected.

2.3. The Vasculature

The blood vessels are the part of the circulatory system that transports blood throughout the body. There are three major types of blood vessels: the arteries, which carry blood away from the heart; the capillaries, which enable the actual exchange of water and chemicals between the blood and the tissues; and the veins, which carry blood from the capillaries back toward the heart. The arteries and veins both have three layers, but the middle layer is thicker in the arteries than it is in the veins.

Figure 6. is a cross section of a blood vessel showing the different layers.

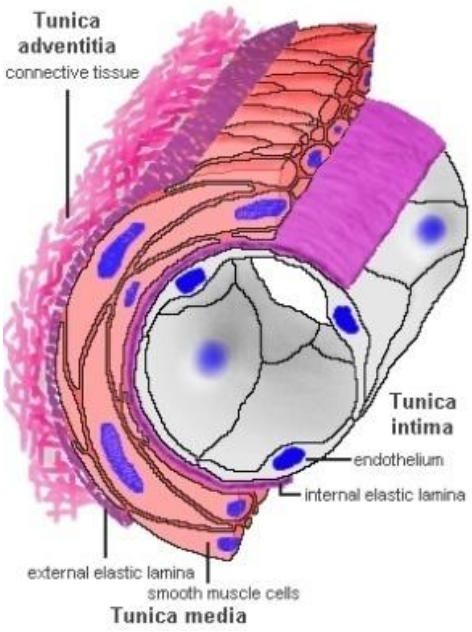

Figure 6. A section of a blood vessel.
Source:http://www.courseweb.uoottawa.ca/medicine.

2.3.1. Arteries

Arteries carry blood away from the heart. They are classified into three types according to their sizes: large or elastic arteries; medium (or muscular or distributive) arteries; and small arteries or arterioles, which are less than 0.5 mm in diameter. These types are all continuous with one another. A characteristic feature of arteries, regardless of size, is a well-defined lumen, rounded or oval, maintained by the muscularity of the vessel wall.

Figure 7. shows the wall of a large artery at 400x magnification. The blue arrow indicates the tunica intima. The black bracket represents the tunica media and the green bracket is the tunica adventitia.

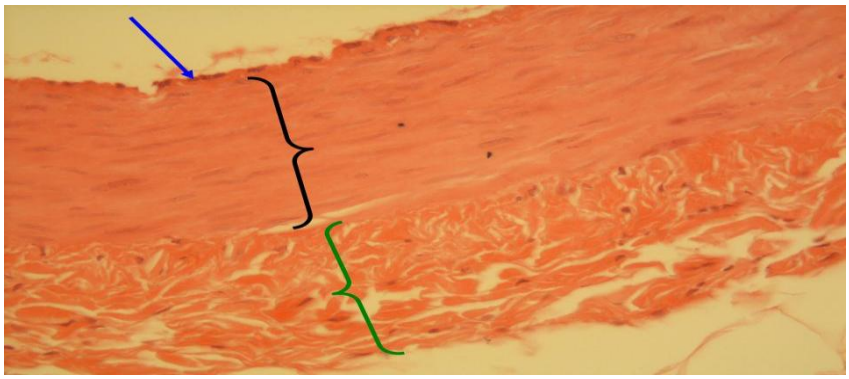

Figure 7. A large artery. Source**:**(http://www.courseweb.uoottawa.ca/medicine.

The aorta and its branches (brachiocephalic, subclavian, pulmonary, beginning of common carotid and iliac) are distinguished by their great elasticity. This helps them smoothen out the large fluctuations in blood pressure created by the heartbeat. During systole, their elastic laminae are stretched and reduce blood pressure. During diastole, the elastic rebound helps maintain arterial pressure.

2.3.1.1. **Tunica intima**

Large arteries often have large subendothelial layers, which grow with age or disease conditions (arteriosclerosis). Both connective tissue and smooth muscle are present in the intima. The border of the intima is delineated by the internal elastic membrane. The internal elastic membrane may not be conspicuous because of the abundance of elastic material in the tunica media.

2.3.1.2.Tunica media

This is the thickest of the three layers. The smooth muscle cells are arranged in a spiral around the long axis of the vessel. They secrete elastin in the form of sheets, or lamellae, which are fenestrated to facilitate diffusion. The number of lamellae increase with age (few at birth, 40-70 in adult) and with hypertension. These lamellae, and the large sizes of the media,

are the most striking histological features of elastic arteries. In addition to elastin, the smooth muscle cells of the media secrete reticular and fine collagen fibres and proteoglycans.

2.3.1.3.Tunica adventitia

This is a relatively thin connective tissue layer. Fibroblasts are the predominant cell type, and many macrophages are also present. Collagen fibres predominate and elastic fibres (not lamellae) are also present. The collagen in the adventitia prevents elastic arteries from stretching beyond their physiological limits during systole. Blood vessels supplying the adventitia and outer media are present and are called vasa vasorum ("vessels of the vessels"). The inner part of the media is supplied from the lumen via pinocytic transport.

2.3.1.4. Medium arteries

The majority of named arteries are medium (muscular or distributive) arteries (Figure 8). There is no sharp dividing line between elastic (large) and muscular (medium) arteries; Medium arteries have less elastic tissue than large arteries, the predominant constituent of the tunica media is smooth muscle.

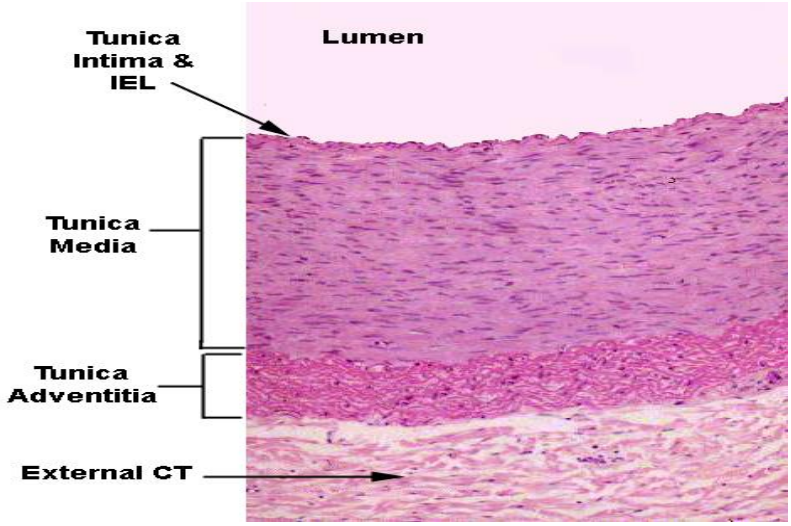

Figure 8. Muscular or medium artery.
Source:http://www.courseweb.uoottawa.ca/medicine.

2.3.1.4.1. Tunica intima of medium arteries

The tunica intima is thinner in medium arteries than in large arteries, there are fewer smooth muscle cells and less elastic tissues. The outermost part of the intima is defined by a very prominent internal elastic membrane (not obscured by elastic lamellae as in large arteries). The basement membrane of the endothelium may rest directly on the internal elastic membrane, or be separated by a subendothelial layer. The tunica intima increases in thickness with age, and may also become expanded by lipid deposits.

2.3.1.4.2.Tunica media

Smooth muscle cells predominate in the tunica media, and little elastic material is present. As in large arteries, no fibroblasts are present. Elastic fibres (few), collagen, and ground substance are produced by the smooth muscle cells. These are arranged in a spiral fashion and their contraction help maintain blood pressure. In tissue preparation, the internal elastic membrane of the intima appears wavy due to the contraction of the smooth muscle of the media.

2.3.1.4.3. Tunica adventitia

The main constituent of the adventitia is collagen fibres, secreted by fibroblasts. Elastic fibres are also present, a concentration of such fibres at the inner boundary of the adventitia is called the external elastic membrane. The external elastic membrane is not as prominent as the internal, and as arteries get smaller it disappears much earlier. The tunica adventitia is relatively larger than in elastic arteries, it can be up to the same size as the media. Adipose cells may be present.

2.3.1.5. Small arteries

The general features of small arteries are very similar to that of muscular arteries (figure 9). The media is still muscular and has up to 8-10 layers of smooth muscle cells. This number is reduced as the arteries get smaller, the smallest arterioles have 1-2 layers of smooth muscle cells. The adventitia becomes thinner and the external elastic membrane disappears. The intima becomes smaller and the internal elastic membrane also eventually disappears. However, it persists much longer than the external, and it is not uncommon to see very small arteries which still have an internal elastic membrane. Small arteries also maintain their shape, and tend to be round or oval.

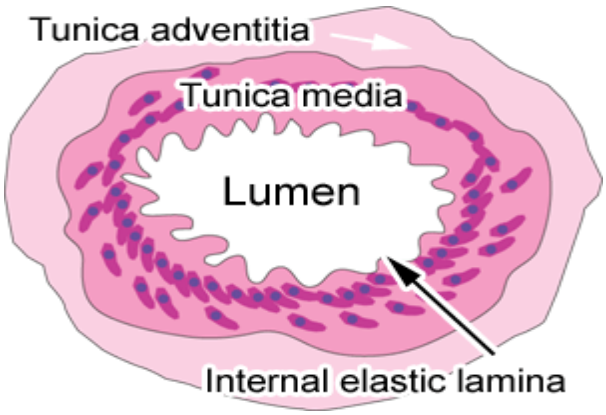

Figure 9. A small artery or arteriole.
Source: (http://www.courseweb.uoottawa.ca/medicine).

2.3.2. Veins

Veins are the vessels that return blood to the heart. Like arteries, they are classified as large, medium and small, and the sizes blend into one another with no sharp demarcations. Although the same layers (intima, media and adventitia) are present, they are often not as well defined as in arteries. A big difference between arteries and veins is the thickness of their walls and the relative amount of muscle tissue (media). In comparably sized vessels, arteries have thicker walls and a much larger media. In veins, the adventitia is larger than the media. Because of these features, veins do not retain their shape. They often appear floppy in

sections, and the lumen may not be patent. Veins are frequently of an irregular shape. Veins also have less elastic tissue than do arteries. Even in larger veins, the internal elastic membrane may be poorly developed or absent. Valves, which function to prevent the backflow of blood especially in the lower part of the body, are also seen quite frequently in veins. Veins often travel in close proximity to their arterial counterparts, which is convenient for histological comparison. The endothelium appears to have been stripped away as no endothelial cell nuclei are identifiable. Bundles of smooth muscle sit in the wavy collagen fibres of the adventitia. Blood vessels (vasa vasorum) are also present.

2.3.2.1. Medium veins

The tunica consists of the endothelium and a thin subendothelial layer with smooth muscle cells among the connective tissue elements. A thin internal elastic membrane may or may not be present. The tunica media is much thinner relative to that of an artery, and consists mostly of circularly arranged smooth muscle but also contains collagen fibres. The tunicas intima and media therefore tend to be less distinct from one another than is the case in arteries. The tunica adventitia is usually thicker than the media and is made up mostly of collagen fibres. It may contain longitudinally oriented smooth muscle bundles

2.3.2.2. Small artery and vein

The media of the small artery is much larger than that of the small veins. A few bulgy endothelial cells can be seen in the artery, but otherwise an intima is not distinguishable. Elastic tissue is also not identifiable. A few flattened endothelial cells appear to be present in the vein. The adventitia of the vessels blends in with the surrounding connective tissue.

2.3.3. Capillaries

Capillaries are the smallest diameter vessels and the site of exchange of metabolites between blood and tissues. Capillaries are just wide enough to allow the passage of red blood cells, only one cell at a time. Capillaries consist of a single layer of endothelial cells and their basement membrane. The endothelial cells are joined together by tight junctions. At intervals, these tight junctions are interrupted, leaving small spaces allowing the passage of fluid between blood and extracellular fluid. These interruptions do not occur in the brain, and the lack thereof is responsible for the blood-brain barrier present in most of the brain. Endothelial cells also have pinocytotic vesicles which are involved in transporting macromolecules.

2.3.4. The Endothelium

The endothelium as shown in Figure 10, lines the blood vessels throughout the body, from the larger arteries and veins down to the small capillaries that branch through organs. The endothelium is the thin layer of cells that lines the interior surface of blood vessels and lymphatic vessels, forming an interface between circulating blood and lymph in the lumen and the rest of the vessel wall.

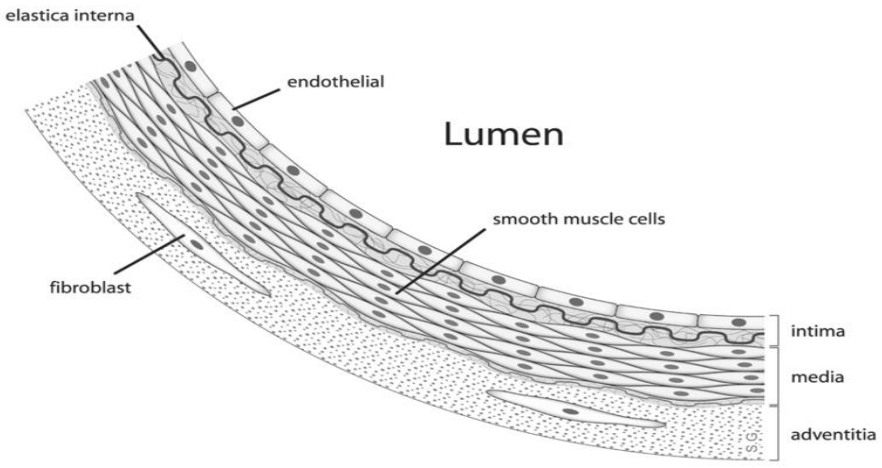

Figure 10. Cross section showing the endothelium of a blood vessel. Source:http://www.gettyimages.com/creative/endothelium.

2.3.4.1. Structure of the Endothelium

Endothelial cells in direct contact with blood are called vascular endothelial cells whereas those in direct contact with lymph are known as lymphatic endothelial cells (figure 11). The cells are arranged, edge to edge, in a single layer. The wavy or denticulate edges of neighbouring cells fit into each other, being separated by a mere line of the intercellular substance which in this tissue has received the name of cement substance.

The endothelium, the largest organ in the body, is strategically located between the wall of blood vessels and the blood stream. Endothelial cells possess thin membranous bodies, except at the site of the nucleus. The intercellular substance is minimal in amount; clear and homogeneous in character. The cells of endothelium vary somewhat in size and shape. They may be polygonal, rhomboid, or stellate in form, and during life are soft and extensible so that their sizes may be modified by stretching or tension in one or more directions. The cell-bodies, or cytoplasm, are usually clear and apparently structureless or only slightly granular, but occasionally some of the cells are smaller and more granular than the majority. The endothelial cell layer represents a mechanical and biological barrier between the blood and blood and the vascular wall and is likely to serve as the 'missing link' between any given risk factor and its detrimental vascular effects**.**

The location of the endothelium as seen in the wall of a blood vessel in Figure 11. It is such that it is often the first structure to be exposed to any changes in blood and is therefore the first to be able to respond to such changes.

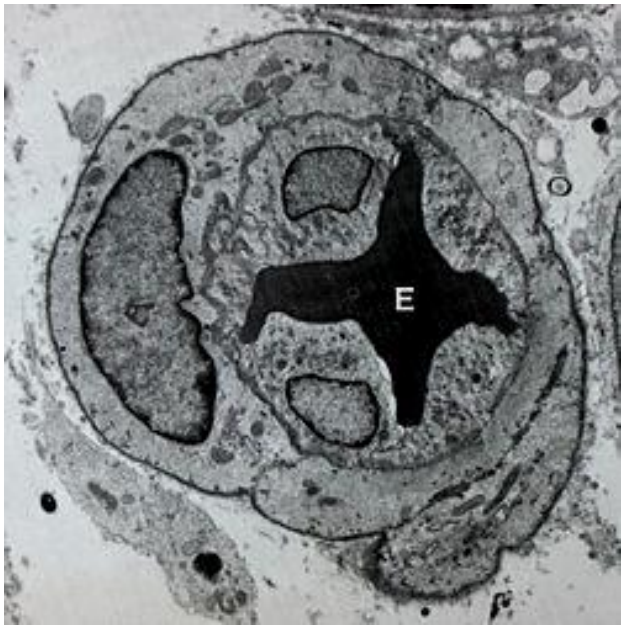

Figure 11. The endothelium of a blood vessel.
Source: http://www.gettyimages.com/creative/endothelium.

2.3.4.2. Some substances released from the endothelium

Among the vasodilatory, vasoconstrictor and other substances, produced by the endothelium are nitric oxide (NO), prostacyclin, different endothelium-derived hyperpolarizing factors, and C-type natriuretic peptide (Table 4). The pioneering experiments of Furchgott and Zawadiski in (1980) first demonstrated an endothelium- derived relaxing factor that was subsequently shown to be nitric oxide (NO).

Table 5. Substances released by endothelium

Substances	Examples
Vasodilators :	Nitric oxide; bradykinin; prostacyclin; endothelium-derived hyperpolarizing factor; serotonin; histamine; substance P.
Vasoconstrictors:	Angiotensin II (AII); endothelin (ET-1); thromboxane A2; serotonin; arachidonic acid; prostaglandin H2; thrombin.
Promoters:	Platelet derived growth factor (PDGF); basic fibroblast growth factor (PGF); insulin-like growth factor – I (IGF-I); endothelin (ET1); Angiotensin.
Inhibitors:	Nitric oxide; prostacyclin; bradykinin; heparin sulfate; transforming growth factor I (TGFB).
Adhesion molecules:	Endothelial leukocyte adhesion molecule; intercellular adhesion molecule (ICAM); vascular cell adhesion molecule (VCAM).
Thrombolytic factors:	Tissue-type plasminogen activator; plasminogen activator inhibitor-1 (PAI-I); Thrombomodulin.

Source: http://geocities.com/agnihotrimed. and Chhobra, N., (2009) Internet Journal of Medical update vol 4. No1.

2.3.4.3. Functions of the endothelium

The endothelium performs multiple functions that are often modified by regional location. Endothelial cells which line the internal lumen of the vasculature are part of a complex system that regulates vasodilatation, vasoconstriction, and growth of vascular smooth muscle, inflammation and haemostasis. They also maintain proper blood supply to tissues and regulate inflammation (Zhang 2008).

2.3.4.4. The endothelium and NO

There is increased intercellular Ca^{++} in response to vasodilator agonists or shear stress which displaces the inhibitor caveolin from calmodulin (CaM), activating nitric oxide Synthase (eNOS). eNOS can be activated, upon stimulation of the endothelium by a variety of stimuli including shear stress, substance P and acetyl choline. The most critical of the substances released by the endothelium is nitric oxide (NO), which is produced by the conversion of the substrate L-arginine to L-citrulline by the enzyme, nitric oxide synthase (eNOS). This reaction requires a number of cofactors, including tetrahydrobiopterin (BH4) and nicotinamide adenine dinucleotide phosphate (NADPH) (Forstermann and Munzel 2006). NO thus formed diffuses into the underlying vascular smooth muscles to stimulate the activation of guanylate cyclase, which in turn converts guanosine triphosphate (GTP) to the smooth muscle relaxant compound cyclic guanosine monophosphate (c-GMP) in the vascular smooth muscle cells**.** The increase in c-GMP leads to activation of cyclic GMP- dependent protein kinase, which in turn leads to phosphorylation of potassium dependent channels with consecutive hyperpolarization and extrusion of calcium ions resulting in VSMC (vascular smooth muscle cell) relaxation**.**

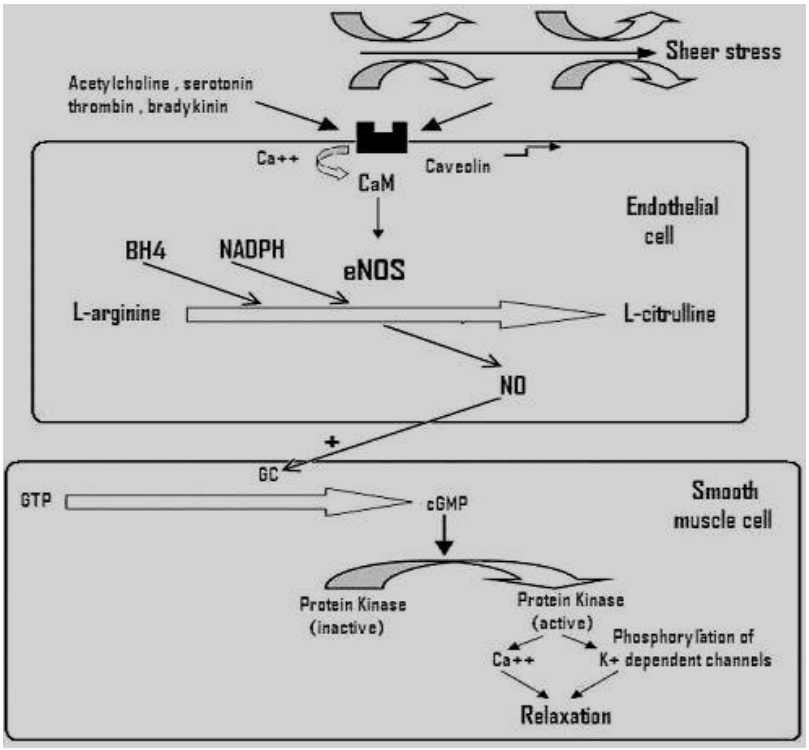

Figure 12: Production of nitric oxide (NO) by eNOS from endothelial cells and its action on smooth vascular cell to produce relaxation. Source: Chhobra, N., (2009) Internet Journal of Medical update vol 4. No1.

Nitric oxide not only produces vasodilatation, but it also participates in various processes that are beneficial to the vasculature such as reduction of vascular smooth muscle cell migration and growth, platelet aggregation and thrombosis, monocyte and macrophage adhesion and inflammation. In general the substances released by the endothelium have important autocrine as well as paracrine functions and help to maintain not only the normal health of vascular wall but also control the haemostatic functions and the haemodynamic balance of the entire body (Libby et al. 2002).

Factors such as smoking, dyslipidemia, diabetes, aging and sedentary lifestyle have all been shown to reduce NO synthesis and therefore impair endothelial function.

2.3.4.5. The endothelium,vasoconstrictors and other modulators

Vasoconstrictors include endothelin-1 (ET-1), angiotensin II (Ang II), thromboxane A_2, and reactive oxygen species (ROS). Inflammatory modulators include NO, intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), E-selectin, and NF-κB. Modulation of haemostasis includes release of plasminogen activator, tissue factor inhibitor, von Willebrand factor, NO, prostacyclin, thromboxane A_2, plasminogen-activator inhibitor-1, and fibrinogen.

Endothelin-1 acts via 2 receptor subtypes, endothelin-A and endothelin-B (ET-A and ETB), which are expressed in varying quantities. Endogenous levels of ET-1 act via ET-A to induce coronary vasoconstriction but also serve to increase smooth muscle proliferation and induce cytokine production in vitro (Davignon & Ganz, 2004). Selective antagonism of ET-A receptors has been shown to improve endothelial function (Verhaar et al. 1998). The endothelium also contributes to mitogenesis, angiogenesis, vascular permeability, and fluid balance. Vasoactive mediators on the endothelium include bradykinin and thrombin.

In addition to providing a balance between vasoconstriction, vasodilatation and pro-coagulation, the endothelium is the source of growth factors and cytokines (Krishnaswamy et al. 2004). It is also involved in the remodeling of vascular structure and long term organ perfusion.

2.3.4.6. Endothelial dysfunction

Endothelial dysfunction is broadly used to describe any defect in the endothelium. However it often refers to an impaired NO system either by reduced NO production or increased breakdown due to oxidative stress.

Endothelial dysfunction is a systemic pathological state of the inner lining of the blood vessels. It can be broadly defined as an imbalance between vasodilating and vasoconstriction substances produced by (or acting on) the endothelium (Deanfield et al. 2007). What is generally referred to as endothelial dysfunction should be more appropriately considered as endothelial activation, which may eventually contribute to arterial disease when certain conditions are fulfilled.

Endothelial activation represents a switch from a quiescent state to one that involves the host defence response (Deanfield et al. 2007). Endothelial dysfunction is now regarded as an early pivotal event in atherogenesis and has been shown to precede development of clinically detectable atherosclerotic plaques in the coronary arteries. It has also been considered an important event in the development of microvascular complications in diabetes. Endothelial dysfunction, aside from denoting impaired endothelium dependent vasodilatation, also comprises a specific state of 'Endothelial activation' which is characterized by a pro-inflammatory, proliferative and procoagulatory milieu that favours all stages of atherogenesis.

2.3.4.7. Endothelial dysfunction and NO

A key and quantifiable feature of endothelial dysfunction is the inability of arteries and arterioles to dilate fully in response to an appropriate stimulus that stimulates release of

vasodilators from the endothelium like NO. Endothelial dysfunction is commonly associated with decreased NO bioavailability which is due to impaired NO production by the endothelium and/or increased inactivation of NO by reactive oxygen species (Lopez et al. 2005).This can be tested by direct administration of various vasoactive agents to segments of blood vessels. It is thought that people with endothelial dysfunction have low NO bioavailability in their blood vessels. They also have a decreased capacity to dilate in response to certain stimuli compared to those with normal endothelial function (Deanfield et al. 2005).

2.3.4.8. Endothelial dysfunction and artherosclerosis

Endothelial dysfunction is a chronic inflammatory response in the walls of arteries, which cal also be caused largely by the accumulation of macrophage white blood cells and promoted by low-density lipoproteins. Endothelial dysfunction is thought to be a key event in the development of atherosclerosis and predates clinically obvious vascular pathology by many years (Mondy et al. 2008). Decreased NO and increased local mediators, increased adhesion molecules, cytokines and growth factors leads to vasoconstriction, plaque rupture and inflammation (Figure 13).

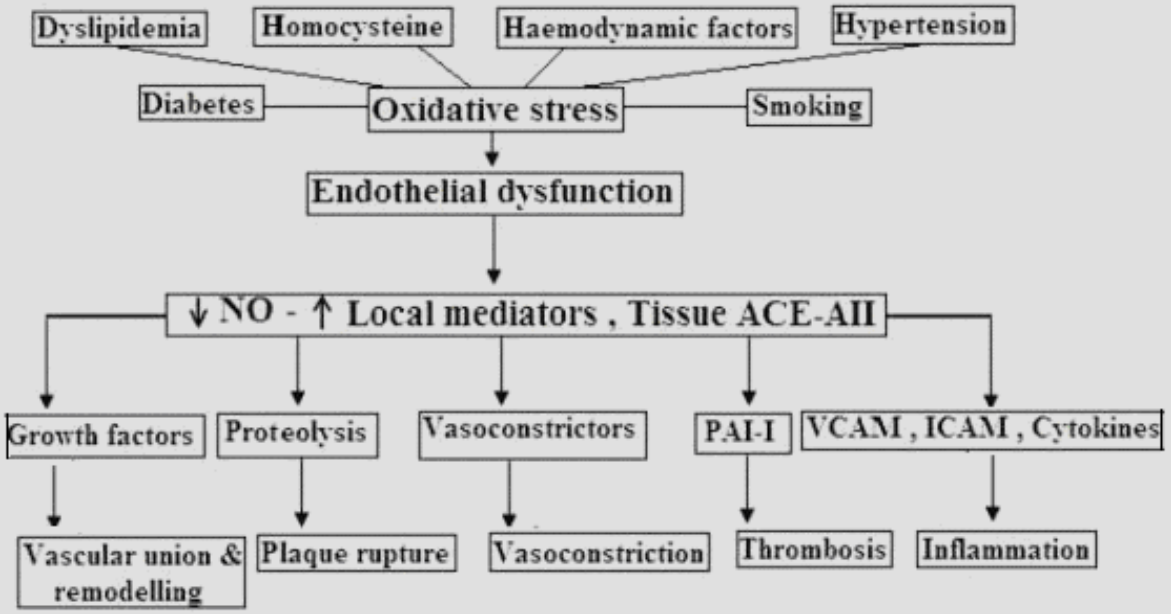

Figure 13. Role of endothelial dysfunction in the causation and progression of atherosclerosis.

Source: http://geocities.com/agnihotrimed. Chhobra, N., (2009) Internet Journal of Medical update vol 4. No1

Given this relationship between endothelial dysfunction and atherosclerosis, it is likely that the status of endothelial function may reflect the propensity of an individual to develop atherosclerotic disease, and thus the presence of endothelial dysfunction may serve as a marker of an unfavorable cardiovascular prognosis (Kinlay & Ghanz, 1997). Although the association between cardiovascular risk factors and atherosclerotic disease is well documented, the mechanism by which these risk factors induce lesion formation leading to the events is not entirely defined (Kinlay & Ghanz, 1997).

By reacting with NO, ROS may reduce vascular NO bioavailability and promote cell damage. Hence increased oxidative stress is considered a major mechanism involved in the pathogenesis of endothelial dysfunction and may serve as a common pathogenic mechanism of the effect of risk factors on the endothelium (Chhabra, 2009).

Risk to develop endothelial dysfunction increases with the number of risk factors present in an individual, but the potential to alter endothelial function may vary between risk factors (Chhabra 2009). Taken together, the status of endothelial function represent an integrated index of both the overall cardiovascular risk factor burden and the sum of all vasculoprotective factors in any given individual (Chhabra 2009). Given its pivotal role in the atherogenic process, endothelial dysfunction may be regarded as the "ultimate risk of the risk factors" indicating the existence of a specific atherogenic vascular milieu which is associated with perfusion abnormalities and cardiovascular events (Bonneti et al 2003).

Endothelial dysfunction is characterized by a reduced ability of vessels to vasodilate. Subsequently, there is an accumulation of platelets, fibrin and monocytes over the injured endothelium; these then release substances such as platelet derived growth factor (PDGF), and tissue growth factor B, (TGF-B) which stimulate smooth muscle proliferation and connective tissue production. Most cardiovascular risk factors activate molecular machinery in the endothelium that results in expression of chemokines, cytokines and adhesion molecules. These are designed to interact with leucocytes, platelets and target inflammation to specific tissues (Hanson 2006).

HIV associated activated macrophages may predispose subjects to endothelial dysfunction and enhance atheroma formation. Consistent association between HIV infection and elevated risk of atherosclerosis suggested a connection between virus-induced changes in cholesterol metabolism and atherogenesis, but the mechanisms of such connection have not been identified (Bukrinsky et al 2006, Cui et al 2009). Both endothelial dysfunction and arterial stiffness are considered surrogate markers of cardiovascular disease and future risk. Several studies have assessed endothelial function in HIV infected patients with associated cardiovascular risk factors. Jose et al (2006) reported a link between endothelial function and

cardiovascular risk in HIV infected patients under treatment, when he compared these patients to treatment naïve and control patients.

Atherosclerosis is a hardening of an artery specifically due to an atheromatous plaque. Atherosclerosis (also known as arteriosclerotic vascular disease or ASVD) is a condition in which an artery wall thickens as a result of the accumulation of fatty materials such as cholesterol. It is a syndrome affecting arterial blood vessels. Atherosclerosis is a chronic disease that remains asymptomatic for decades (Finn et al 2010). The progression of atherosclerosis is due to endothelial dysfuncyion and starts ealy in life (Okumura et al 2005) (Figure14).

Atherosclerosis is a complex disease involving the arterial part of the vasculature. It is a fibroproliferative inflammatory process that proceeds through a series of pathological events involving the cardiovascular system, the inflammatory and immune systems, lipid and cholesterol handling mechanisms and blood clotting mechanisms. The disease is modulated by genetic and environmental factors with respect to the end point presentation of the atherosclerotic plaque.

The term atherogenic is used for substances or processes that cause atherosclerosis. The following terms are similar, yet distinct, in both spelling and meaning, and can be easily confused: arteriosclerosis, arteriolosclerosis, and atherosclerosis. Arteriosclerosis is a general term describing any hardening (and loss of elasticity) of medium or large arteries. Arteriolosclerosis is any hardening (and loss of elasticity) of arterioles.

Atherosclerosis develops from low-density lipoprotein molecules becoming oxidized (LdL-ox) by free radicals, particularly reactive oxygen species (ROS). When oxidized LDL comes in contact with an artery wall.

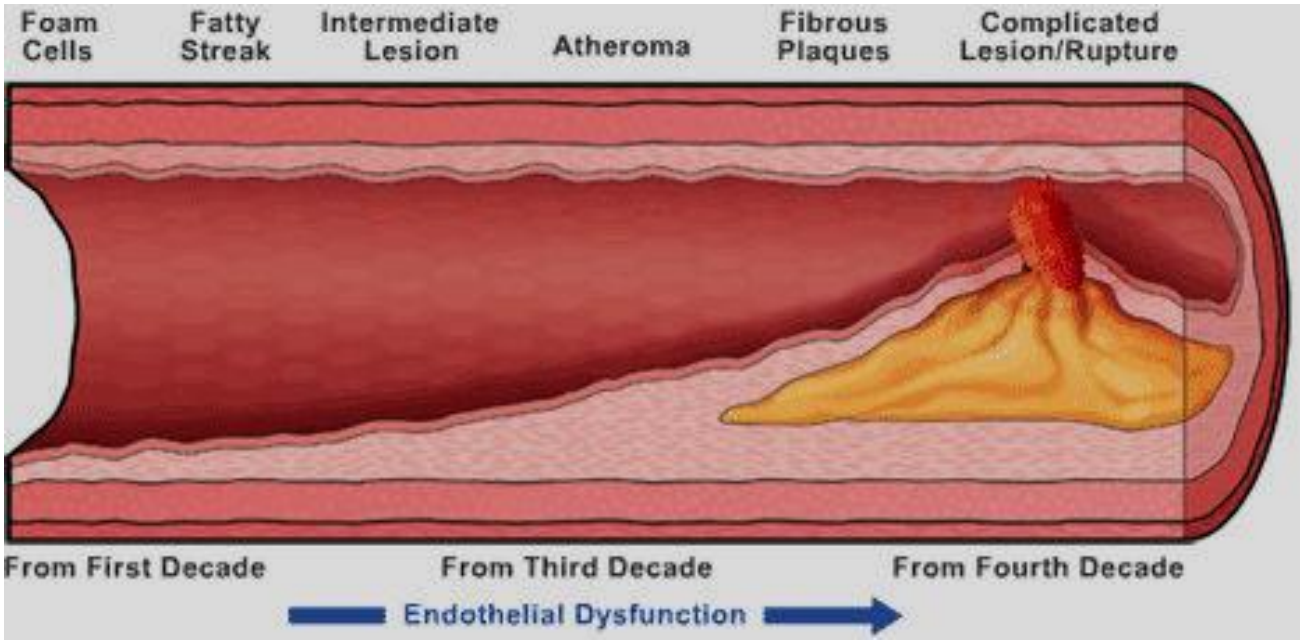

Figure 14. Section through a blood vessel showing endothelial dysfunction leading to atherosclerosis. Source: The American Heritage @medical Dictionary Copyright @2010 by Houghton Mifflin Harcourt Publishing Company.

The LDL molecule is globular shaped with a hollow core to carry cholesterol throughout the body. Once inside the vessel wall, LDL molecules become susceptible to oxidation by free radicals (Sparrow et al 1993), and become toxic to the cells. Oxidatively modified LDL induces the vascular wall cells to synthesize a chemoattractant cytokine, MCP-1 (Romano et al 2000, Munro et al. 1996). Cytokines (protein mediators of inflammation) such as IL-1 and TNF-α induce or augment the expression of the adhesion molecules (VCAM-1 and ICAM-1) on the endothelial surface (Libby et al. 2002). VCAM-1 is an immunoglobulin-like adhesion

molecule expressed on activated endothelial cells (Osborn et al. 1989). It is is expressed principally on endothelial cells, where it is cytokine-inducible and facilitates leukocyte adhesion. VCAM-1 up-regulation has been shown to be important in inflammatory diseases including atherosclerosis, where induction precedes leukocyte adhesion and transmigration across the vascular endothelium (Ley & Huo 2001). VCAM-1 binds to $\alpha_4\beta_1$ integrin, which is constitutively expressed on T lymphocytes, monocytes, and eosinophils, the first step of invasion of the vessel wall by inflammatory cells. The expression of VCAM-1, ICAM-1, and E-selectin plays a role in the initiation of the inflammatory process. Monocytes are transformed to lipid-loaded foam cells. (Libby 2002).

LDL is formed in the early stages of atherosclerosis, while ox-LDL is formed in the later stages (Kuhn & Chan 1997). Although LDL binds to LDL receptor and ox-LDL binds to scavenger receptor, the vascular effects of minimally modified LDL and ox-LDL are similar. Both of these derivatives activate endothelial cells, smooth muscle cells and monocytes. They also facilitate vasoconstriction, thrombosis and platelet aggregation associated with the activation of intracellular protein kinases and transcription factors such as NFκB or activator protein-1. Ox-LDL also induces a vasoconstrictor state by reducing the formation of the endothelium-derived vasodilators NO and prostaglandin while enhancing the production of the vasoconstrictor endothelin-1 (ET-1).

The damage caused by the oxidized LDL molecules triggers a cascade of immune responses which over time can produce an atheroma. A series of reactions occur to repair the damage to the artery wall caused by oxidized LDL. Platelets also play an important role in the pathophysiology of atherosclerosis in that they induce the formation of monocyte

chemotactic protein 1 (MCP-1) and the expression of Intercellular adhesion molecule (ICAM-1).

Once resident in the intima, the monocytes differentiate into macrophages and cause the enhanced expression of scavenger receptors, which bind lipoproteins for endocytosis. In an effort to clear the lipids, these macrophages first attach the lipoproteins to the scavenger receptors, and they result in the formation of foam cells. The formation of foam cells is the hallmark of atherosclerotic lesions. Ox-LDL is chemotactic for monocytes and T-cells. Lack of the LDL receptors responsible for endocytosis is seen in patients with familial hypercholesterolemia, where they have an abundance of arterial lesions and multiple xanthomata containing foam cell-rich lesions. Macrophages engulfing the modified lipoproteins produce cytokines and growth factors, which in turn cause further recruitment of the macrophages and vascular smooth muscle cell to the site of the lesion.

The atheromatous plaque is divided into three distinct components:

- The atheroma which is the nodular accumulation of a soft, flaky, yellowish material at the center of large plaques, composed of macrophages nearest the lumen of the artery;
    - underlying areas of cholesterol crystals;
    - calcification at the outer base of older/more advanced lesions.

The pathophysiology of atherosclerotic lesions is very complicated but generally, stable atherosclerotic plaques, which tend to be asymptomatic, are rich in extracellular matrix and smooth muscle cells, while unstable plaques are rich in macrophages and foam cells and the extracellular matrix separating the lesion from the arterial lumen (also known as the fibrous cap) is usually weak and prone to rupture. Ruptures of the fibrous cap expose thrombogenic material, such as collagen to the circulation and eventually induce thrombus formation in the

lumen (Didangelos et al. 2009). Upon formation, intraluminal thrombi can occlude arteries outright (i.e. coronary occlusion), but more often they detach, move into the circulation and eventually occlude smaller downstream branches causing thromboembolism.

These complications of advanced atherosclerosis are chronic, slowly progressive and cumulative. Most commonly, soft plaque suddenly ruptures, causing the formation of a thrombus that will rapidly slow or stop blood flow, leading to death of the tissues fed by the artery in approximately 5 minutes . One of the most common recognized scenarios is called coronary thrombosis of a coronary artery, causing myocardial infarction. The same process in an artery to the brain is commonly called stroke. Another common scenario in very advanced disease is claudication from insufficient blood supply to the legs, typically caused by a combination of both stenosis and aneurysmal segments narrowed with clots (Williams & Tabas 1995).

Atherosclerosis affects the entire artery tree, but mostly larger, high-pressure vessels such as the coronary, renal, femoral, cerebral, and carotid arteries. These are termed "clinically silent" because the person having the infarction does not notice the problem and does not seek medical help, or when they do, physicians do not recognize what has happened. The main cause of atherosclerosis is yet unknown, but is hypothesized to fundamentally be initiated by inflammatory processes in the vessel wall in response to retained low-density lipoprotein (LDL) molecules (Williams & Tabas 1995).

2.3.4.9. Endothelial markers and HIV

HIV may influence endothelial function via activated monocytes and resultant cytokine secretion, and via a direct effect of the secreted HIV proteins tat and gp120. A simple

measure of the chronic inflammation in HIV-infected patients is the higher levels of high sensitivity C-reactive protein (CRP) found in HIV-infected patients compared to control subjects, indicating a higher risk for cardiovascular events (Hsue et al. 2004).

Other inflammatory markers such as interleukins are said to be elevated in HIV infection. Endothelial markers are increased as well, including soluble vascular cell adhesion molecule (sVCAM-1), soluble intercellular adhesion molecule (sICAM-1), and von Willebrand factor (vWF) (Wolf et al. 2002). These markers indicate chronic endothelial activation and subsequent endothelial dysfunction, which may trigger inflammation and a hypercoagulable state. Endothelial activation also triggers platelet activation, which can upregulate adhesion molecules.

2. 4. Lipids and lipoproteins

A lipoprotein is a biochemical assembly that contains both proteins and lipids.

Lipoproteins are small spherules that transport fats in the body and consist of protein, cholesterol, triglycerides, and phospholipids. The terms "good" and "bad" cholesterol refer to High Density Lipoproteins (HDL-C) and Low Density Lipoproteins (LDL-C), respectively. High levels of LDL are associated with coronary atherosclerosis, whereas high levels of HDL appear to protect against cardiovascular diseases.

The lipoproteins are linked to cholesterol (Figure 15).

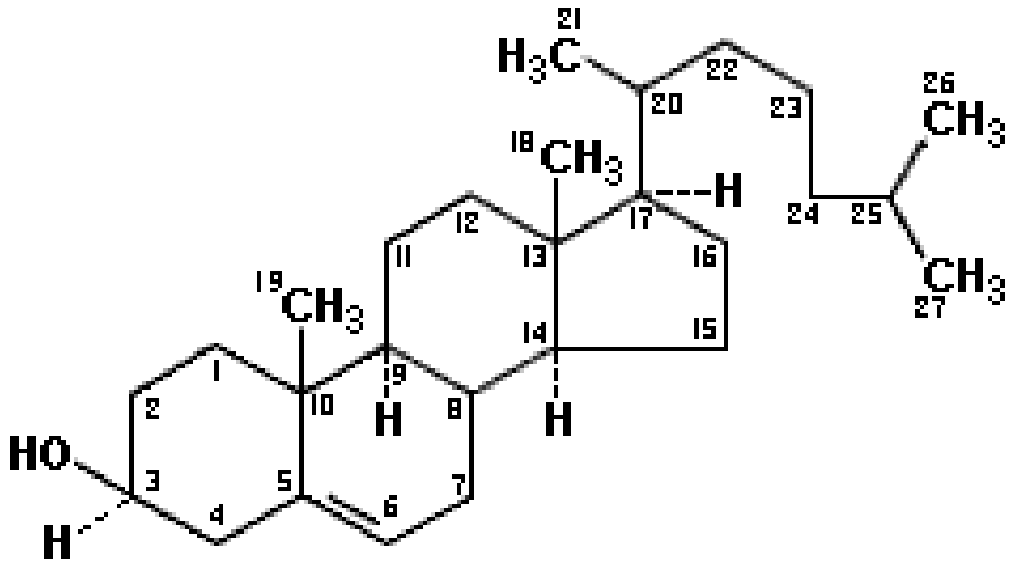

Figure 15. Cholesterol molecule. Source: USDA National Nutrient Database for Standard Reference, Release 18 (2005).

The outer layer of a lipoprotein consists of a water-soluble (hydrophilic) layer of apolipoproteins, phospholipids and cholesterol. The center of a lipoprotein is composed of cholesteryl esters, triglycerides, fatty acids and fat-soluble vitamins (Figure 16).

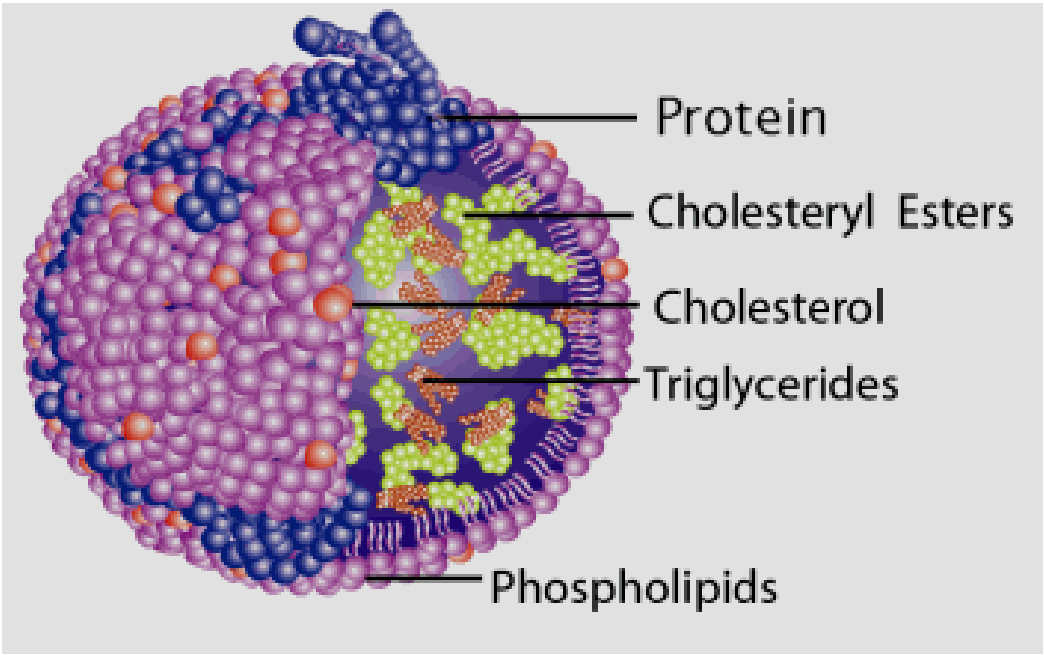

Figure 16. The structure of a lipoprotein. Source: USDA National Nutrient Database for Standard Reference, Release 18 (2005).

The proteins serve to emulsify the lipid (otherwise called fat) molecules. Many enzymes, transporters, structural proteins, antigens, adhesins, and toxins are lipoproteins. Examples include the high-density (HDL) and low-density (LDL) lipoproteins, which enable fats to be carried in the blood stream.

Listed from larger and less dense to smaller and denser. Lipoproteins are larger and less dense when the fat to protein ratio is increased. Lipoprotein particles range in size from 10 to 1000 nanometers. The largest lipoproteins are about one tenth the size of a red blood cell. The density of lipoproteins increases in proportion to their ratio of proteins to lipids. In general, as the density of a lipoproteins increases, the size of the particles decreases. Lipoproteins are characterized by density ,class, diameter, concentration of proteins, cholesterol, phospholipids, triacylglycerol and choleterol esters (Table 6). They are classified on the basis of electrophoresis and ultracentrifugation.

Table 6. Characteristics of diferent classes of lipoproteins. Source: Wilkipedia the free encyclopaedia

Density (g/ml)	Class	Diameter (nm)	Protein (%)	Cholesterol (%)	Phospholipids (%)	Triglycerol& cholesterol ester%
>1.063-1.019	HDL	5-1	33	30	29	4
1.063	LDL	18-28	25	50	21	8
1.006-1.019	IDL	25-50	18	29	22	31
0.95-1.006	VLDL	30-80	10	22	18	50
<0.95	Chylomicrons	100-1000	<2	8	7	84

Chylomicrons carry triglycerides (fat) from the intestines to the liver, to skeletal muscle, and to adipose tissue.

- Very-low-density lipoproteins (VLDL) carry (newly synthesised) triglycerides from the liver to adipose tissue.

- Intermediate-density lipoproteins (IDL) are intermediate between VLDL and LDL. They are not usually detectable in the blood.

- Low-density lipoproteins (LDL) carry cholesterol from the liver to cells of the body. LDLs are sometimes referred to as the "bad cholesterol" lipoprotein.

- High-density lipoproteins (HDL) collect cholesterol from the body's tissues, and bring it back to the liver. HDLs are sometimes referred to as the "good cholesterol" lipoprotein.

2.4.1. Metabolism of lipoproteins

The handling of lipoproteins in the body is referred to as lipoprotein metabolism. It is divided into two pathways, exogenous and endogenous, depending in large part, on whether the lipoproteins in question are composed chiefly of dietary (exogenous) lipids or whether they originated in the liver (endogenous).

2.4.1.1. Exogenous pathway

Epithelial cells lining the small intestine readily absorb lipids from nutritive substances. These lipids, including triglycerides, phospholipids, and cholesterol, are assembled with apolipoprotein B-48 into chylomicrons. These nascent chylomicrons are secreted from the intestinal epithelial cells into the lymphatic circulation in a process that depends heavily on

apolipoprotein B-48. As they circulate through the lymphatic vessels, nascent chylomicrons bypass the liver circulation and are drained via the thoracic duct into the bloodstream.

In the bloodstream, HDL particles donate apolipoprotein C-II and apolipoprotein E to the nascent chylomicron; the chylomicron is now considered mature. Apolipoprotein C-II, mature chylomicrons activate lipoprotein lipase (LPL), an enzyme on endothelial cells lining the blood vessels. LPL catalyzes the hydrolysis of triacylglycerol (i.e., glycerol covalently joined to three fatty acids) that ultimately releases glycerol and fatty acids from the chylomicrons. Glycerol and fatty acids can then be absorbed in peripheral tissues, especially adipose and muscle, for energy and storage. The hydrolyzed chylomicrons are now considered chylomicron remnants. The chylomicron remnants continue circulating until they interact via apolipoprotein E with chylomicron remnant receptors, found chiefly in the liver. This interaction causes the endocytosis of the chylomicron remnants, which are subsequently hydrolyzed within lysosomes. Lysosomal hydrolysis releases glycerol and fatty acids into the cell, which can be used for energy or stored for later use.

2.4.1.2. Endogenous pathway

The liver is another important source of lipoproteins, principally VLDL. Triacylglycerol and cholesterol are assembled with apolipoprotein B-100 to form VLDL particles. Nascent VLDL particles are released into the bloodstream via a process that depends upon apolipoprotein B-100. As in chylomicron metabolism, the apolipoprotein C-II and apolipoprotein E of VLDL particles are acquired from HDL particles. Once loaded with apolipoprotein C-II and E, the nascent VLDL particle is considered mature. Again like chylomicrons, VLDL particles circulate and encounter LPL expressed on endothelial cells. Apolipoprotein C-II activates LPL, causing hydrolysis of the VLDL particle and the release of glycerol and fatty acids.

These products can be absorbed from the blood by peripheral tissues, principally adipose and muscle. The hydrolyzed VLDL particles are now called VLDL remnants or intermediate-density lipoproteins (IDLs). VLDL remnants can circulate and, via an interaction between apolipoprotein E and the remnant receptor, be absorbed by the liver, or they can be further hydrolyzed by hepatic lipase. Hydrolysis of VLDL by hepatic lipase releases glycerol and fatty acids, leaving behind IDL remnants, called low-density lipoproteins (LDL), which contain a relatively high cholesterol content. LDL circulates in the blood and is absorbed by the liver and peripheral cells. Binding of LDL to its target tissue occurs through an interaction between the LDL receptor and apolipoprotein B-100 or E on the LDL particle. Absorption occurs through endocytosis, and the internalized LDL particles are hydrolyzed within lysosomes, releasing lipids, chiefly cholesterol (Kumar 2011).

2.4.2. Lipoproteins and atherosclerosis

Epidemiological studies have shown a positive relationship between total cholesterol concentrations and mortality from coronary heart disease (CHD). Total cholesterol does not accurately predict the risk of CHD in many patients, however, because it is the sum of all cholesterol carried not only by atherogenic lipoproteins (eg, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL) but also by antiatherogenic lipoproteins (ie, high-density lipoprotein (HDL).

The decision to treat is based on LDL-cholesterol values. Yet, treatment decisions may also need to take into account LDL heterogeneity, which has been recognized for many years. Small, dense LDL particles are more atherogenic than large, buoyant LDL particles, and oxidation of LDL also increases its atherogenicity. In addition, LDL belongs to the group of

lipoproteins that contain apolipoprotein (apo) B-100. Some of the particles in this highly heterogeneous group contain other apolipoproteins, such as apo C-II, apo C-III, and apo E.

Some particles are larger and rich in triglycerides (large VLDL), whereas others are smaller and rich in cholesteryl esters (small VLDL, IDL). It is now known that remnant lipoproteins containing apo C-III are highly atherogenic, as is lipoprotein(a) [Lp(a)] another member of the apo B-100 group. The small, dense LDL phenotype rarely occurs as an isolated disorder. It is most frequently accompanied by hypertriglyceridemia, reduced HDL-cholesterol levels, abdominal obesity, and insulin resistance (all of which are components of the metabolic syndrome) and by a series of other metabolic alterations predictive of an impaired endothelial function and increased susceptibility to thrombosis.

Results from the Québec Cardiovascular Study have indicated that persons displaying elevated plasma concentrations of insulin and apo B together with small, dense LDL particles showed a remarkable increase in CHD risk (Lamarche et al 1999).

2.4.3. Oxidized LDL and atherosclerosis

It has been suggested for 20 years that oxidative stress, and particularly LDL oxidation, could induce atherosclerosis (Toshima et al 2000), and that markers of LDL oxidation in plasma (circulating oxidized LDL, autoantibodies against oxidized LDL) could be used to assess the development of atherosclerosis in patients. Circulating oxidized LDL is additive to the global risk score based on age, sex, total and HDL cholesterol, diabetes mellitus, hypertension, and smoking as useful markers for identifying persons at risk for CAD.

Circulating oxidized LDL was associated with both subclinical atherosclerosis (clinically silent ultrasound-assessed atherosclerotic changes in the carotid and femoral arteries) and

inflammatory variables (C-reactive protein and the inflammatory cytokines interleukin-6 and tumor necrosis factor-α (Sherer et al 2001). Autoantibodies against oxidized LDL have been reported to be associated with atherosclerosis. However, the data are not consistent: some studies have reported a positive relationship between autoantibodies against oxidized LDL and CHD (Erkkila et al. 2000), whereas another did not (Leinonen et al 1998). There is a strong cross-reactivity between autoantibodies against oxidized LDL and anticardiolipin antibodies, which have been positively associated with CHD. Many studies have found elevated LDL-cholesterol levels are associated with a high risk of CHD, and LDL cholesterol continues to be the primary target of therapy for the prevention of CHD (Grundy et al. 2004).

Triglyceride-rich lipoproteins comprise a great variety of nascent and metabolically modified lipoprotein particles differing in size, density, lipid and apolipoprotein composition. Studies have shown an inverse relationship between the size of lipoproteins and their ability to cross the endothelial barrier to enter the arterial intima. Chylomicrons and large VLDLs are probably not capable of entering the arterial wall. On the other hand, small VLDLs can enter the arterial intima. Therefore, certain triglyceride-rich lipoproteins are atherogenic, whereas others are not. A large body of evidence suggests that small VLDLs and IDLs are independently associated with atherosclerosis. A study (Sniderman 2001) in 174 patients with type 2 diabetes mellitus who were not receiving lipid-lowering therapy concluded that the severity of CHD (as examined by angiography) was related to the number of circulating fasting triglyceride-rich lipoprotein particles. This relationship was stronger in women than in men and was independent of HDL and LDL.

Remnant-like particles (RLPs) can be isolated from the plasma or serum by an antibody-based assay. In the Framingham Offspring Study (Carmena et al 2004) mean RLP

cholesterol and RLP triglycerides were highly significantly (P<0.0001) increased in diabetic women and significantly (P<0.001) increased in diabetic men compared with respective controls. Apo C-III in VLDL is associated with denser, smaller VLDL subclasses and is believed to be particularly atherogenic. Remnants associated with apo C-III are more related to the development of atherosclerosis than are triglycerides. Fasting remnant lipoproteins could predict future clinical coronary events independently of other risk factors. The concentration of apo C-III in VLDL and IDL particles has been significantly correlated with development of coronary stenosis.

2.4.4. HIV and dyslipaedimia

It has been demonstrated that patients on highly active antiretroviral therapy are at increased risk for developing metabolic abnormalities that include elevated levels of serum triglycerides and low-density lipoprotein cholesterol and reduced levels of high-density lipoprotein cholesterol (Ridler et al. 2003). This dyslipidemia is similar to that seen in the metabolic syndrome, raising the concern that highly active antiretroviral therapy also potentially increases the risk for cardiovascular complications. The natural history of HIV infection changed abruptly in the developing world in 1995, subsequent to the introduction of highly active antiretroviral therapy (HAART), resulting in markedly lower mortality rates. However, treatment-associated toxicities were soon recognized, including a constellation of metabolic and body composition alterations, often referred to as HIV-associated lipodystrophy. Their development initially was ascribed solely to protease inhibitor (PI) therapy but later found to also be related to certain nucleoside reverse transcriptase inhibitors (NRTIs). The similarities of HIV-associated lipodystrophy to the metabolic syndrome

suggested that cardiovascular (CV) risk may also be elevated, a concern that has been corroborated in several, but not all analyses.

An association between dyslipidemia and myocardial infarction (MI) has long been recognized, as has the great overlap in serum cholesterol concentrations seen between those who do and do not develop symptomatic CVD. The latter observation implies the presence of other risk factors. Two general patterns of dyslipidemia are related to CVD. The first is an increase in the concentration of low-density lipoprotein cholesterol (LDL-C), usually with a genetic predisposition. A different defect leads to elevated serum triglyceride and low high-density lipoprotein cholesterol (HDL-C) concentrations and often is seen in obese patients with other components of the metabolic syndrome such as central obesity, diabetes mellitus, and hypertension (Kotler 2008). Some authors have considered untreated HIV infection to be an example of the type of inflammation that might promote atherosclerosis (Kotler 2008).

2.4.5. Atherosclerosis

Atherosclerosis, which is the buildup of fat and cholesterol-laden plaque in the walls of the arteries, can result from lipid disorders (Ginsberg 1997). When the coronary arteries are affected by arteriosclerosis, the person can develop angina, heart attack, congestive heart failure, or abnormal cardiac rhythms. When the arteries of the brain are affected by arteriosclerosis, the person can develop transient ischemic attack, or stroke.

Hardening of the arteries is a progressive condition that may begin in childhood. Fatty streaks can develop in the aorta shortly after birth. In those people with familial history of high cholesterol, the condition may worsen rapidly in the early 20s and progressively become more severe in the 40s and 50s.

The body's immune system responds to the damage to the artery wall caused by oxidized LDL by sending specialized white blood cells (macrophages and T-lymphocytes) to absorb the oxidized-LDL forming specialized foam cells. Unfortunately, these white blood cells are not able to process the oxidized-LDL, and ultimately grow, then rupture, depositing a greater amount of oxidized cholesterol into the artery wall. This triggers more white blood cells, continuing the cycle (Ross et al, 1999). Eventually, the artery becomes inflamed. The cholesterol plaque causes the muscle cells to enlarge and form a hard cover over the affected area. This hard cover is what causes a narrowing of the artery as in Figure 16. The plaque reduces the blood flow and increases blood pressure. This eventually leads to atherosclerosis.

Atherosclerosis is a complex disease involving the arterial part of the vasculature. The disease is modulated by genetic and environmental factors with respect to the end point presentation of the atherosclerotic plaque (Figure 16).

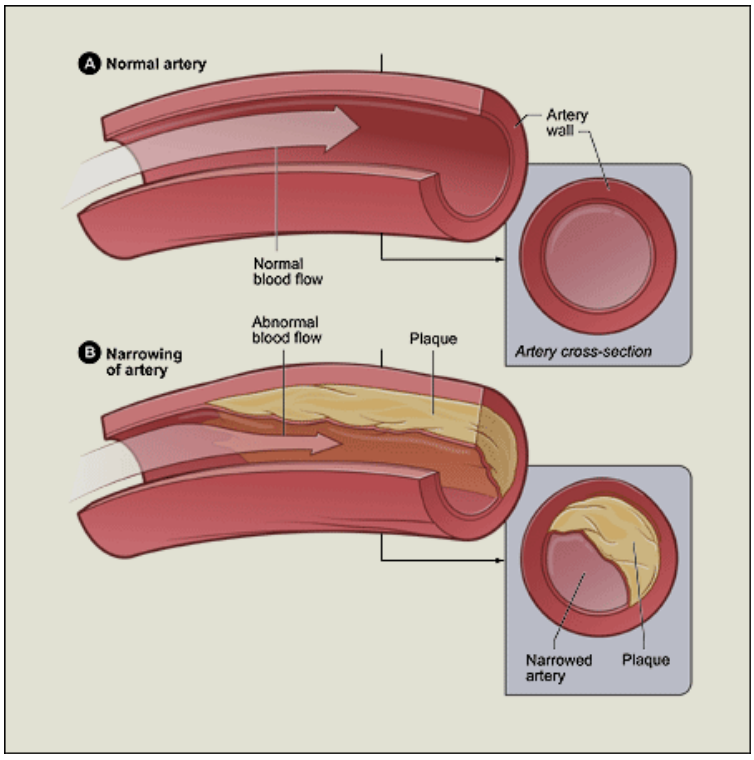

Figure 16. Formation of plaques in the blood vessel. Source: The American Heritage @medical Dictionary Copyright @2010.

The main cause of atherosclerosis is yet unknown, but is hypothesized to fundamentally be initiated by inflammatory processes in the vessel wall in response to retained low-density lipoprotein (LDL) molecules.

The development of atherosclerosis probably begins by damage in the endothelium. This damage causes cholesterol and fat to penetrate into the vessel walls and deposit there. This also induces cells to proliferate. Later, calcium salts are also deposited (Holt 2010).

Factors that may cause endothelial damage and thus atherosclerosis are:

- high content of cholesterol in the blood ;
- high content of blood fat and especially saturated fat;
- Inflammation in the blood vessels. A sign of such inflammation is the presence of C-reactive protein;
- high amount of oxidizing agents in the blood ;
- high blood pressure ;
- high content of low density lipoprotein (LDL) in the blood serum, and low content of high density lipoprotein (HDL) in the blood;
- diabetes ;
- advancing age;
- smoking;
- men have a somewhat higher chance of getting this condition than women;
- high content of the amino acid homocystein in the blood serum. (Holt, 2010).

Atherosclerosis can affect any artery in the body, including arteries in the heart, brain, arms, legs, pelvis, and kidneys. As a result, different diseases may develop based on which arteries are affected.

2.4.5.1. Atherosclerosis and inflammation

Inflammation appears to be an important pathogenic event in the progression of atherosclerosis and in the rupture of the plaque's fibrous cap, which is usually the key event leading to acute ischemic accidents. Infection-induced chronic inflammation may thus contribute to the increased incidence of cardiovascular events in HIV-infected individuals.

Endothelial cells have been shown to express interleukin-1 (IL-1), IL-5, IL-6, IL-8, IL-11, IL-15, several colony-stimulating factors (CSF), granulocyte-CSF (G-CSF), macrophage CSF (M-CSF) and granulocyte-macrophage CSF (GM-CSF), and the chemokines which are monocyte chemotactic protein-1 (MCP-1), RANTES, and growth-related oncogene protein-alpha (GRO-alpha), IL-1 and tumor necrosis factor-alpha (TNF-alpha). Endothelial cells can be induced to express several of these cytokines as well as adhesion molecules. Induction of these cytokines in endothelial cells has been demonstrated by such diverse processes as hypoxia and bacterial infection.

Recent studies have demonstrated that adhesive interactions between endothelial cells and recruited inflammatory cells can also signal the secretion of inflammatory cytokines. This cross-talk between inflammatory cells and the endothelium may be critical to the development of chronic inflammatory states (Krishnaswamy et al 2004).

2.4.5.2. Antiretroviral therapy and atherosclerosis

The role of antiretroviral therapy and HIV infection itself in the development of atherosclerosis is not well known, however it is known that they induce changes in lipid profile and insulin resistance.

HIV infection may lead to changes in the vascular endothelium by depressed immunity, sustained inflammation, and viral replication. At the present we do not know if HIV infection itself is an independent risk factor for the onset and progression of atherosclerosis. Some studies have shown an increased arterial stiffness in HIV infected patients who were not receiving ARV therapy (Charakida et al 2009 a).

2.4.5.3. Arterial stiffness and pulse wave velocity

Arterial stiffening occurs mainly because of structural alterations in connective tissue proteins in the intima media, endothelial and smooth muscle cells. This alterations may influence the visco- elastic properties of the arterial wall thereby affecting the arterial stiffness (Malik et al. 2008). The arterial pressure waveform is a composite of the forward pressure wave created by ventricular contraction and a reflected wave. Waves are reflected from the periphery, mainly at branch points or sites of impedance mismatch. In elastic vessels, because PWV is low, reflected wave tends to arrive back at the aortic root during diastole.

In the human body, wave reflections originate in various locations, including peripheral bifurcations of conducting arteries (Taylor 1964) and smaller muscular arteries. The geometry, number of arterioles, and the architecture of the microvascular network play an important role in wave reflection. With increased arterial stiffness, as observed, for example,

in older subjects or hypertensive patients, the reflected wave travels more rapidly along the arterial tree.

Both small and large arteries contribute to early reflected waves because of pulse pressure amplification between central and peripheral arteries.

The arterial tree does not have separate conduit and cushioning functions: both functions are features of the aorta and its major branches, which are distensible tubes. In addition, there is a progressive loss of the cushioning function, from the ascending aorta (the most elastic artery) to the more muscular and less elastic peripheral arteries, and an increasingly predominant conduit function of large arteries from the heart to the periphery. The respective amounts of cushioning and conduit functions in adjacent arterial segments determine this heterogeneity (Boutouyrie et al 1994). Peripheral arteries are stiffer than central arteries in healthy subjects, and this phenomenon leads to an increase in the amplitude of the pressure wave in the vessels, from the heart to the periphery, known as pressure amplification. In addition, the stiffness of medium-sized peripheral arteries is modulated by the vasomotor tone, either depending on the endothelial function or the sympathetic nervous system (Boutouyrie et al 1994) or the renin–angiotensin system (Giannattasio al 1995). When modeling the arterial tree, O'Rourke et al (1982, 1986) have also suggested that because the tube's end has a high level of resistance, waves are reflected and retrograde waves are generated. This would account for the secondary fluctuations of the pressure waveform in diastole and differences in the amplitude of the pressure wave between central and peripheral arteries. This fits well with pathophysiological observations.

2.4.5.3.1. Arterial stiffness and augmentation index

Carotid pressure waveform is recorded by applanation tonometry. The arterial waveform has two components; the forward moving wave when the left ventricle contracts and the reflected wave returning from the periphery. When the reflected wave arrives back at the central arteries earlier this leads to an augmentation of central aortic pressure. The arrival time and degree of augmentation depends on the stiffness of the arteries. This can be quantified through the augmentation pressure which is the difference between the forward and reflected systolic pulse wave peaks. When expressed as a percentage of the pulse pressure it is called augmentation index (Figure 18).

The augmentation index has been also identified as an independent risk marker of the severity of premature coronary artery disease and as a predictor of cardiovascular disease among healthy individuals (Weber et al 2004). The height of the late systolic peak (P1) above the inflection (P2) defines the augmentation pressure, and the ratio of augmentation pressure to pulse pressure (PP) defines the AIx (75) (in percent) (Figure 18). Apart from a high PWV, also changes in reflection sites can influence the AIx (75). In clinical investigation, not only diastolic blood pressure (dbp) and height, which are related to reflection sites, but also age and aortic PWV, are the main determinants of the augmentation index. In the case of stiff arteries, PWV rises, and the reflected wave arrives back at the central arteries earlier, adding to the forward wave and augmenting the systolic pressure. This phenomenon can be quantified through the augmentation index AIx (75) which is the augmentation index at a normal heart rate of 75 beats per minute for standardization, (Figure 18).

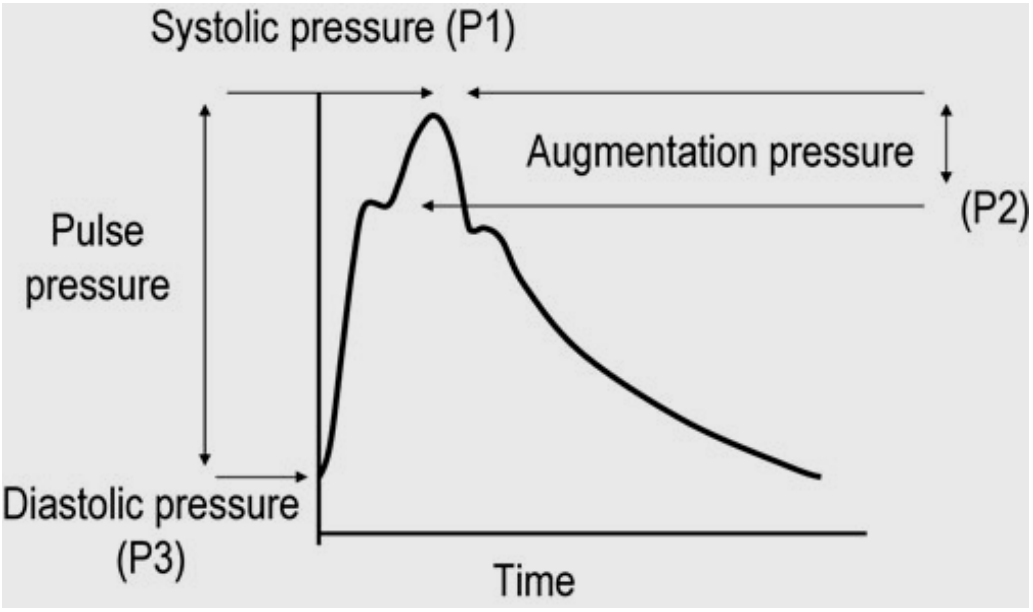

Figure 18. Carotid pressure waveform recorded by applanation tonometry. Source: O'Rourke MF, Pauca A, Kon N, et al. 1999. Am J Hypertens. 1999;12.

2.4.5.3.2. Arterial stiffness and the endothelium derived factors.

Endothelium derived factors including nitric oxide affect several biological processes in the arterial wall which includes smooth muscle reactivity. They may have both short and long term effects on the degree of arterial stiffness (Wilkinson et al 2004). An association between regional endothelial function and arterial stiffness in different vascular beds has been demonstrated using invasive methods in experimental animals as well as in human volunteers (Wilkinson et al 2002).

2.4.5.3.3. Measurement of arterial stiffness

In recent years, great emphasis has been placed on the role of arterial stiffness in the development of cardiovascular (CV) diseases. Indeed, the assessment of arterial stiffness is increasingly used in the clinical assessment of patients. Several papers have previously addressed the methodological issues concerning the various indices of arterial stiffness

currently available, and their clinical applications, (Laurent et al 2003). Clinicians and researchers still report difficulties in selecting the most appropriate methodology for their specific use. The measurement of aortic stiffness, which integrates the alterations of the arterial wall, may also reflect parallel lesions present at the site of cerebral vasculature.

The measurement of PWV is generally accepted as the most simple, non-invasive, robust, and reproducible method to determine arterial stiffness. Carotid-femoral PWV is a direct measurement, and it corresponds to the widely accepted propagative model of the arterial system. PWV is usually measured using the foot-to-foot velocity method from various waveforms. These are usually obtained, transcutaneously at the right common carotid artery and the right femoral artery (i.e. 'carotid-femoral' PWV), and the time delay (Dt or transit time) measured between the feet of the two waveforms. Carotid-femoral PWV has been used in the epidemiological studies demonstrating the predictive value of aortic stiffness for CV events. PWV is measured in various segments of the arterial circulation. When two pressure waves are recorded at two different sites of the vascular tree such as the carotid and femoral arteries, it is possible due to the propagation of the waves, to measure the time delay (t) and the distance (D) between these two waves.

Arterial PWV can be determined by the foot –to foot flow wave velocity method. (Avolio et al 1983). The foot of the flow wave will be identified between the two recording sites as the beginning of the sharp systolic up-stroke. The time delay is measured between the feet of the flow waves recorded at these different points and designated as pulse transmit time.

Carotid-femoral PWV is considered as the 'gold-standard' measurement of arterial stiffness. The higher the arterial stiffness, the higher the speed of travel of forward and retrograde

waves. Local arterial stiffness can be measured directly, and noninvasively, at various sites along the arterial tree. A major advantage of the regional and local evaluations of arterial stiffness is that they are based on direct measurements of parameters strongly linked to wall stiffness.

The aorta is a major vessel of interest when determining regional arterial stiffness for at least two reasons: the thoracic and abdominal aorta makes the largest contribution to the arterial buffering function. During each heart beat a pulse wave travels from the heart down the arterial wall in advance of blood flow (Lakatta 1999). The more rigid the wall of the artery, the faster the wave moves. When the wave hits the major branching points, such as the renal and femoral arteries, these waves are reflected back so that they reverse direction and travel back to their point of origin. Normally the reflected waves get back to the starting point after the aortic valve is closed. This amplifies diastolic pressure and facilitates blood flow to the coronary arteries. However the increased wave velocity of the initial wave and the subsequent reflected wave that occurs with age means the wave can get back to the point of origin before the aortic valve closes which increases systolic rather than diastolic pressure. Increased wave velocity also decreases the contribution of the reflected wave to the filling of the coronary arteries. These changes can have significant clinical implications in terms of coronary artery blood flow and can contribute to an increase in systolic blood pressure, pulse pressure and thus aortic stiffness.

Pulse wave velocity is a well-accepted index of arterial stiffness. In humans, pulse wave velocity is usually in the range 500-2,000 cm/sec. Pulse wave velocity (PWV) is related to Young's modulus (E), by the Moens Kortewig equation. PWV= square root of E.h/2r.P where h is arterial wall thickness, r is internal radius, and p is density of blood. Another commonly

used measure of arterial stiffness is the aortic characteristic impedance (Zc), which can be determined from pressure and flow waves recorded simultaneously in the ascending aorta (O'Rourke et al 1983). When expressed in terms of linear flow velocity (dyne-sec-cm"3, not volume flow) this is numerically very similar to pulse wave velocity according to the Waterhammer formula Zc=PWV x blood density (Fitchett et al. 1988).

Carotid-femoral PWV is a direct measurement, and it corresponds to the widely accepted propagative model of the arterial system. It is measured along the aortic and aorto-iliac pathway, it is the most clinically relevant, since the aorta and its first branches are what the left ventricle (LV) 'sees' and are thus responsible for most of the pathophysiological effects of arterial stiffness (Pannier 2005).

2.4.5.3.4. Terminology and Physical Principles

Young's modulus, pulse wave velocity, and characteristic impedance all increase with rise in stiffness and decrease with its fall (Fitchet et al. 1988). A higher value denotes a stiffer artery. The media of an artery is responsible for its physical properties. This is regarded as a "two phase "structure with tension at low distending pressure borne by the elastin fibers and at high pressure predominantly by less extensible collagen fibers (Roach et al. 1957).

A number of alternative non invasive approaches have been developed to study vascular biology. These rely on the ability of the β2 agonist salbutamol to reduce arterial stiffness in a nitric oxide dependent manner without significant reduction in blood pressure when given by inhaler at standard clinical doses (Hayward 2002).

Celermajer et al (1994) reported a non invasive ultrasound based test to assess conduit artery vascular function in the systemic circulation. In this method, brachial artery diameter was measured before and after an increase in shear stress that is induced by reactive hyperemia (FMD). Using a sphygmanometer, the cuff placed on the forearm distal to the brachial artery is inflated to 200mmHg and subsequently released four to five minutes late. FMD (Flow mediated dilation) occurs predominantly as a result of local endothelial release of NO.

There are modern software development of this method. There are practical challenges that need to be overcome such as environmental, and physiological like exercise, eating and caffeine ingestion. Variations in temperature have to be overcome and some of these equipments are expensive (Corretti et al. 2002).

2.4.5.4. HIV, arterial stiffness and increased cardiovascular morbidity

In the last two decades the introduction of efficient antiviral therapy transformed HIV infection into a chronic disease but, at the same time, a positive correlation between this therapy and an increased cardiovascular risk was also reported. Patients with HIV infection have the same classical cardiovascular risk factors as the general population in addition to HIV infection itself (Papita et al. 2011). Malik et al (2008) demonstrated that impaired vascular reactivity and arterial stiffening are manifestations of vascular dysfunction in the presence of cardiovascular risk factors. There might be other factors causing arterial stiffening leading to atherosclerosis especially in HIV patients. Schillaci et al (2005) in their study showed that aortic stiffness is increased in HIV-positive individuals receiving antiretroviral therapy including a protease inhibitor. The study also showed that pulse wave velocity increased with longer exposure to protease inhibitors. They also hypothesized that atherosclrosis is a side effect of antiretroviral treatment including a protease inhibitor.

Aortic and large artery stiffening which are hallmarks of vascular aging, are predictive of cardiovascular events, particularly in subjects with hypertension and components of the metabolic syndrome (Cecelja et al. 2010). One hypothesis is that stiffening is a consequence of the combined insult of risk factors on the vascular wall (Cecelja et al. 2010). However, some cross-sectional studies show little association of arterial stiffness with conventional risk factors other than blood pressure. An elevation in mean arterial pressure stretches the arterial wall, leading to a functional increase in stiffness. Stiffening associated with a chronic elevation of blood pressure (ie, hypertension) may be because of distention of the arterial wall but could also be explained by structural changes of the arterial wall. The effect of increased stiffening is to increase pulse pressure and systolic blood pressure. An increased arterial stiffness can increase the risk of stroke through several mechanisms, including an increase in central PP, influencing arterial remodeling both at the site of the extracranial and intracranial arteries. Increased arterial stiffness can also increase carotid wall thickness and the development of stenosis and plaques, the likelihood of plaque rupture, and the prevalence and severity of cerebral white matter lesions (Van der Heijden et al. 2002).

The assessment of arterial stiffness is increasingly used in the clinical assessment of patients. The amplification of pulse pressure between central and peripheral arteries has made it inaccurate to use brachial pulse pressure as a surrogate for aortic or carotid pulse pressure, particularly in young subjects. In younger subjects, the central arteries are usually more elastic than peripheral arteries. However, this gradient can be reversed with ageing or hypertension. The stiffness of the common carotid artery is six-fold higher in a 70-year-old normotensive subject than at the age of 20 (Laurent et al 2006). In elderly patients with hypertension or diabetes, the carotid artery may become stiffer than either the common femoral or radial arteries, which stiffen little with age or hypertension.

The aorta is strong and elastic; it expands to contain the blood ejected during systole. It then contracts and pushes the blood forward, helping to maintain an even flow throughout the cardiac cycle. As the aorta gets stiffer and less elastic and distensible, it can't expand as well during systole so it can't hold as much blood in reserve so its contribution to forward flow is diminished. This contributes to the widening pulse pressure that is often seen in older adults. A widened pulse pressure is increasingly viewed as an important cardiovascular risk factor.

2.5. Cytokines

Cytokines are a category of signaling molecules that are used extensively in cellular communication. Cytokines can be classified as proteins, peptides, or glycoproteins; the term "cytokine" encompasses a large and diverse family of regulators produced throughout the body by cells of diverse embryological origin. The term "cytokine" has been used to refer to the immunomodulating agents, such as interleukins and interferons. They are proteins produced by the white blood cells that act as chemical messengers between cells. They are also produced by many different cells of the immune system which act upon other cells.

They attach to receptors on the outside of cells causing the target cell to produce a certain reaction, depending on the cell and the cytokine. Often the target cell produces other cytokines in response to the initial cytokine. This complicated relationship is called the cytokine network, and it is one of the most important ways that the immune system communicates and orchestrates appropriate responses to various challenges, including viruses, bacteria, fungi and even tumours.

Most cytokines are produced by T-lymphocyte cells and to a lesser degree by monocytes and macrophages (Babakhanian 1995). Cytokines have been classed as lymphokines, interleukins, and chemokines, based on their presumed function, cell of secretion, or target of action. The term chemokine refers to a specific class of cytokines that mediates chemoattraction (chemotaxis) between cells.

Biochemists disagree as to which molecules should be termed cytokines and which hormones. Classic protein hormones circulate in nanomolar (10^{-9}) concentrations that usually vary by less than one order of magnitude. In contrast, some cytokines (such as IL-6) circulate in picomolar (10^{-12}) concentrations that can increase up to 1,000-fold during trauma or infection. The widespread distribution of cellular sources for cytokines may be a feature that differentiates them from hormones. Virtually all nucleated cells, but especially endo/epithelial cells and resident macrophages (many near the interface with the external environment) are potent producers of IL-1, IL-6, and TNFα (Dowlati et al. 2010, Tien at al. 2006). In contrast, classic hormones, such as insulin, are secreted from discrete glands (e.g., the pancreas). As of 2008, the current terminology refers to cytokines as immunomodulating agents. However, more research is needed in this area of defining cytokines and hormones.

Cytokines can stimulate or inhibit the growth and activity of various immune cells in response to the particular type of disease present. They are major determinants of the make-up of the cellular infiltrate, the state of cellular activation, and the systemic responses to inflammation. Most cytokines are multifunctional. They are involved in extensive networks that involve synergistic as well as antagonistic interactions and exhibit both negative and positive regulatory effects on various target cells.

2.5.1.Cytokines and HIV

There is growing evidence that initial HIV infection disrupts the normal balance of cytokines by causing the levels of certain cytokines to rise. As the disease progresses to AIDS, the production of these cytokines declines whilst the production of another group of cytokines increases. Some scientists think that this change from one group of cytokines to the other group directly causes many of the symptoms associated with AIDS including wasting, lymphomas, neurological damage and dementia (Babakhanian 1995). Cytokine imbalances may also help HIV to target CD4 cells and the lymph nodes, leading to the progressive immunosuppression and the opportunistic infections that follow (Babakhanian 1995).

This theory - that the shift in cytokine balances causes the progression from HIV infection to AIDS - is called the Th-1/Th-2 theory. Th-1 refers to the group of cytokines that seem to be produced by T-lymphocyte or T helper cells in response to the initial HIV infection. This group of cytokines includes gamma interferon and interleukins 2 and 12 (IL-2 and IL-12). During the asymptomatic period of HIV infection, the levels of these cytokines remain high. This may itself help to suppress levels of Th-2 cytokines; for example gamma interferon appears to inhibit the production of Th-2 cytokines. For those people in whom HIV progresses to AIDS, a shift begins to occur during this asymptomatic period. Levels of the Th-1 cytokines start to fall and levels of the Th-2 cytokines - interleukins 4, 5, 6 and 10 (IL-4, IL-5, IL-6 and IL-10) and Tumour Necrosis Factor (TNF) alpha - start to rise. Precisely why this shift occurs is not known. But one thing that seems to contribute to and amplify this shift is the way that high levels of one cytokine can affect the production of others. For example, high levels of IL-4 (a Th-2 cytokine) can stimulate production of more Th-2 cytokines (including IL-4 itself) creating a continuous loop, or vicious circle (Babakhanian 1995). Th-1 cytokines are associated with a cellular immune response in which immune cells such as CD8

cells are used to fight infections. Th-2 cytokines are associated with a humoral immune response, which relies more on the use of antibodies (Janeway et al. 2001).

2.5.2. Interleukin -6

The term interleukin was initially used by researchers for those cytokines whose presumed targets are principally leukocytes. It is now used largely for designation of newer cytokine molecules discovered every day and bears little relation to their presumed function. The vast majority of these are produced by T-helper cells. IL-6 is a protein that in humans is encoded by the IL-6 gene. It acts as both a pro-inflammatory and anti-inflammatory cytokine. It is secreted by T cells and macrophages to stimulate immune response, e.g. during infection and after trauma, especially burns or other tissue damage leading to inflammation.

2.5.2.1. The structure of Interleukin -6

The structure is composed of a four helix bundle linked by loops and an additional mini-helix. 157 of 185 residues are well defined in the final structure, with 18 N-terminal and 8 A–B loop amino acids displaying no interpretable electron density (Figure 19).

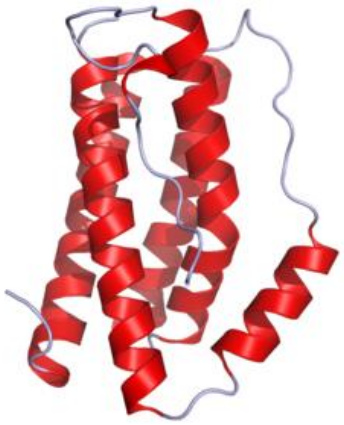

Figure 19. Structure of IL6. Source**:** A crystal structure of interleukin 6: implications for a novel mode of receptor dimerization and signaling (Somers at al 1997 EMBO J. v16 pp.989-997, 1997).

IL-6 is one of the most important mediators of fever and of the acute phase response. It is capable of crossing the blood brain barrier and initiating synthesis of prostaglandin E2 in the hypothalamus, thereby changing the body's temperature setpoint. In muscle and fatty tissue, IL-6 stimulates energy mobilization which leads to increased body temperature. IL-6 can be secreted by macrophages in response to specific microbial molecules, referred to as pathogen associated molecular patterns (PAMPs). IL-6 is responsible for the production of neutrophils in the bone marrow. It supports the growth of B cells and is antagonistic to regulatory T cells. Smooth muscle cells in the tunica media of many blood vessels also produce IL-6 as a pro-inflammatory cytokine. IL-6's role as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-alpha and IL-1, and activation of IL-10 (Janeway et al. 2001). Interleukin 6 is both an anti-inflammatory and a pro-inflammatory cytokine and it is produced in response to infection and tissue injury. IL-6 exerts its effects on multiple cell types and can act systemically.

IL-6 stimulates liver secretion of acute phase proteins. IL-6 stimulates B-lymphocytes to produce antibodies. IL-6 in concert with IL-1 causes T-cell activation. IL-6 is released in response to IL-1 and TNF-b. The IL-6 receptor is found on many cell surfaces, including resting normal T-cells, activated normal B-cells, myeloid cell lines, hepatoma cell lines, myeloma cell lines, and on Epstein-Barr virus (EBV) modified B-cells, in which it promotes proliferation. (Hirano et al. 1988, Tosato & Pike, 1989). IL-6 can lead to the transcription of a wide variety of proteins through all three major signal transduction pathways; Protein kinase C, cAMP/protein kinase A, and the calcium release pathway (Nakajima et al. 1989). IL-6 has

many molecular forms and each molecule has a different function when secreted by different cells in distinct situations activated through diverse stimuli (Lee et al. 1989).

2.5.2.2. IL-6 and acute phase reaction

Il-6 stimulates the acute-phase reaction, which enhances the innate immune system and protects against tissue damage (Abbas et al. 1997). It results in the release of certain proteins, called acute-phase proteins into the blood plasma by liver cells and the decrease in rate of synthesis of other proteins. Acute phase proteins mimic antibodies but have a very broad specificity. The acute phase proteins have been defined as a set of plasma proteins with concentrations that increase (positive acute phase proteins) or decrease (negative acute phase proteins) by at least 25% in inflammatory disorders. These inflammation associated cytokines include IL-6, IL-1β, tumour necrosis factor-α, interferon-γ, transforming growth factor-β and, possibly, IL-8. They are produced by a variety of cell types, but the most important sources are macrophages and monocytes at inflammatory sites. Acute phase changes reflect the presence and intensity of inflammation, and they have long been used as a clinical guide to diagnosis and management.

Overall, in a normal host, one function of inducible IL-6 during acute responses is to suppress the level of proinflammatory cytokines without compromising the level of anti-inflammatory cytokines. In addition, IL-6 stimulates the production of IL-1 receptor antagonist, which is an anti-inflammatory mediator. IL-6 therefore can have a protective effect. IL-6 increases the synthesis of the two major acute-phase proteins, C-reactive protein (Crp), which increases the rate of phagocytosis of bacteria, and serum amyloid A (SAA) by regulating changes in the gene transcription rate of these proteins. In the same way it also increases the synthesis of fibrinogen, an important clotting agent. Albumin and transferrin levels are decreased in the

presence of IL-6 (Abbas et al. 1997, Kushner et al. 1993). The acute phase local reaction leads to a systemic reaction which includes: fever, increased erythrocyte sedimentation rate, increased secretion of glucocorticoids, and the activation of the complement and clotting cascades (Castell et al. 1990).

2.5.2.3. IL-6 and cellular immune response

In chronic diseases, typically exemplified by immune stressors such as chronic intracellular infections and tumours, IL-6 not only serves as an inducer of acute phase reactions but also is an important player in eliciting cellular immune responses to affected cells and mucosal humoral responses directed against reinfection. The main switch from acute to chronic inflammation is the recruitment of monocytes to the area of inflammation. IL-6 is important to the transition between acute and chronic inflammation. Interleukin-6 is especially important in the early stages of T-cell differentiation. In this phase, it reinforces the effect of IL-2 and promotes the differentiation of CD4 cells into T-helper 2 cells. (Janeway et al. 2001). It controls the growth and proliferation of early progenitor cells in the thymus and bone marrow and is later important in both T-cell and Natural Killer (NK) cell activation (Lee et al. 1989). Interleukin-6 is very important in the stimulation of differentiation and proliferation of B-cells. The knockout of IL-6 has severe effects on the immune system, including a major decrease in the acute phase immune reaction and in the production of IgA antibodies (Janeway et al. 2001). An uncontrolled or defective production of this protein most often leads to disease and is involved in the pathogenesis of many diseases and autoimmune disorders (Tovey et al. 1989).

In chronic inflammation, IL-6 has a detrimental role that favours mononuclear cell accumulation at the site of injury, through continuous MCP-1 secretion, angioproliferation

and anti-apoptotic functions on T cells . This may increase serum levels of IL-6 and provide the basis for the amplification step of chronic inflammatory proliferation. Levels of circulating IL-6 may be elevated in several inflammatory diseases.

2.5.3. Tumor necrosis factor (TNF)

Cachexin or cachectin formerly known as tumor necrosis factor-alpha or (TNFα) is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. It is produced chiefly by activated macrophages, although it can be produced by other cell types as well. TNFα stimulates the production of free radicals. Moreover, enhanced levels of free radicals are likely to increase TNFα in various cells. TNFα is expressed on all somatic cells, particularly on the cell membrane where it becomes hydrolyzed to its soluble form. TNFα is considered as one of the most highly studied pro-inflammatory cytokines because it plays a critical role in the origin and progression of diseases such as HIV-1 (Bahia & Silakari 2010). The primary role of TNFα is in the regulation of immune cells. TNFα, being an endogenous pyrogen, is able to induce fever, to induce apoptotic cell death, to induce sepsis (through IL-1 & IL-6 production), to induce cachexia, induce inflammation, and to inhibit tumorigenesis and viral replication.

2.5.3.1. Structure of TNFα or cachectin

TNFα exists as a trimer (Beutler & Cerami 1988). It is one of the products of activated macrophages/monocytes, fibroblasts, mast cells, and some T and natural killer (NK) cells (figure 20). TNFα is primarily produced as a 212-amino acid-long type II transmembrane protein arranged in stable homotrimers.

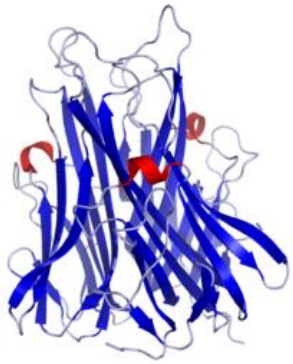

Figure 20. The structure of TNFα. Source: Oxford Journals Life Sciences & Medicine 2010 Transmembrane TNFα: structure, function and interaction with anti-TNF agents. *Rheumatology*. (499) 1215-1228.

2.5.3.2. Synthesis of TNFα.

TNFα was thought to be produced primarily by macrophages, but it is produced also by a broad variety of cell types including lymphoid cells, mast cells, endothelial cells, cardiac myocytes, adipose tissue, fibroblasts, and neuronal tissue. Large amounts of TNFα are released in response to lipopolysaccharide, other bacterial products, and Interleukin-1 (IL-1). In the skin, mast cells appear to be the predominant source of pre-formed TNFα, which can be released upon inflammatory stimulus.

2.5.3.3. Functions of TNFα

It has a number of actions on various organ systems, generally together with IL-1 and Interleukin-6 (IL-6).

On the hypothalamus it has the following functions:

- stimulation of the hypothalamic-pituitary-adrenal axis by stimulating the release of corticotrophin releasing hormone (CRH);
- suppressing appetite;

On the liver:

- it stimulates the acute phase response, leading to an increase in C-reactive protein and a number of other mediators;
- it also induces insulin resistance by promoting serine-phosphorylation of insulin receptor substrate-1 (IRS-1), which impairs insulin signaling;
- it is a potent chemoattractant for neutrophils, and promotes the expression of adhesion molecules on endothelial cells, helping neutrophils migrate;
- on macrophages: stimulates phagocytosis, and production of IL-1 oxidants and the inflammatory lipid prostaglandin E2;
- on other tissues TNFα is related to increase in insulin resistance.

A local increase in concentration of TNFα will cause the cardinal signs of inflammation to occur: heat, swelling, redness, pain and loss of function. High concentrations of TNFα induce shock-like symptoms but the prolonged exposure to low concentrations of TNFα can result in cachexia, a wasting syndrome. This can be found, for example, in cancer patients (Locksley et al. 2001).

2.5.3.4. TNFα, inflammation, atherosclerosis and HIV

TNFα promotes inflammatory response, which, in turn, causes many of the clinical problems associated with autoimmune disorders such as rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease, psoriasis, and refractory asthma. These disorders are sometimes treated by using a TNFα inhibitor. Beneficial functions of TNFα include its role in the immune response to bacterial, and certain fungal, viral, and parasitic infections as well as its role in the necrosis of specific tumors. TNFα is an acute phase protein which initiates a cascade of cytokines and

increases vascular permeability, thereby recruiting macrophage and neutrophils to a site of infection. TNFα secreted by the macrophage causes blood clotting which serves to contain the infection.

High levels of TNFα correlate with increased risk of mortality (Rink & Kirchner 1996). Exogenous and endogenous factors from bacteria, viruses, and parasites stimulate production of TNFα and other cytokines. TNFα exhibits chronic effects as well as resulting in acute pathologies.

TNFα influences many aspects of atherogenesis, including increasing permeability of endothelial cells, promoting monocyte adhesion, inducing macrophage differentiation, and promoting foam cell formation. In vivo, TNFα induces arteriosclerosis-like lesions in coronary arteries (Fukumoto 1997). TNFα also regulates bone turnover, inhibiting osteoblastic function and stimulating bone resorption.

HIV infected monocytes have been shown to exhibit increased adherence to endothelial cells and to transverse the endothelial barrier. TNFα has also been show to be one of the cytokines produced by monocytes in response to stimulation by HIV (Kauschic et al. 2010). According to Kauschic et al (2010) exposure to HIV can directly disrupt cellular barriers whose function is to keep out pathogens. The process discovered accidentally depends on a still-mysterious interaction between an HIV surface protein and mucosal epithelial cells. The interaction causes the cells to release a signaling molecule that damages their tight junctions with each other, allowing HIV and other pathogens to slip though. Kauschic and his colleagues in their experiment also found out that epithelial cells exposed to HIV increased production of signaling molecules, including TNFα, which is known to disrupt the barrier. Although TNFα

might be expected to be a protective component of an antiviral immune response, several lines of evidence suggest that TNFα and other virally-induced cytokines actually may contribute to the pathogenesis of HIV infection (Alfano & Poli 2005). Based on the activation of HIV replication in response to TNFα, HIV appears to have evolved to take advantage of host cytokine activation pathways.

Antibodies to TNFα are present in the serum of normal individuals as well as in certain autoimmune disorders, and may modulate disease progression in the setting of HIV infection. Persistent exposure to HIV and HIV-infected cells may eventually skew the immune system toward producing higher-than-normal levels of chemical signals (cytokines) that foster inflammation of many organs and tissues. Some researchers worry that chronic inflammation associated with HIV may increase the risk for or accelerate the development of complications in HIV-positive people that are traditionally seen in older (HIV-negative) people, including cardiovascular disease, thinning bones, dysfunctional kidneys and diabetes. TNFα is a highly pleiotropic cytokine and plays an important role in regulating HIV-1 replication. It may compromise the integrity of the blood-brain-barrier and, thus, may contribute to the neurotoxicity of HIV-1-infection, (Miller & Krangel 1992).

2.6. Adhesion molecules

All adhesion molecules are integral membrane proteins that have cytoplasmic, transmembrane and extracellular domains. The cytoplasmic tail often interacts with cytoskeletal proteins which serve as the actual anchor within the cell. The extracellular domains of adhesion molecules extend from the cell and bind to other cells or matrix by binding to other adhesion molecules of the same type (homophilic binding), binding to other

adhesion molecules of a different type (heterophilic binding) or binding to an intermediary 'linker' which itself binds to other adhesion molecules (Figure 21).

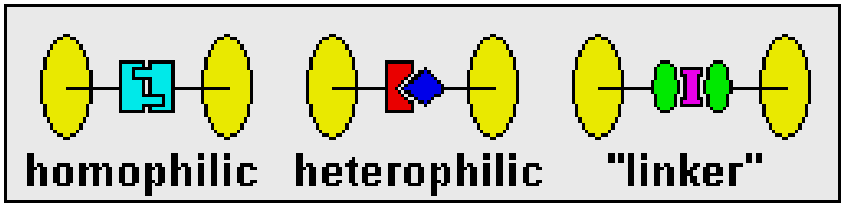

Figure 21. Binding of adhesion molecules. Source lib.bioinfo.pl/courses/view/232.

Dozens of different adhesion molecules have been identified, but, at least so far, they fall into four major families:

- cadherins cause adhesion via homophilic binding to other cadherins depending on the extracellular calcium, (i.e. removal of extracellular calcium disrupts binding). Among other things, cadherins is critical in embryology and acts by segregating embryonic cells into tissues. Cadherins anchor cells through cytoplasmic actin and intermediate filaments in desmosomes and adherens junctions;

- immunoglobulin-like adhesion molecules include a large group of molecules that are generated from a smaller number of genes by alternative RNA splicing. The best-studied members of this group are the neural cell adhesion molecules (N-CAMs) which are expressed predominantly in nervous tissue and the intercellular cell adhesion molecules (ICAMs);

- Integrins are a diverse and large group of heterodimeric glycoproteins. The two subunits, designated as alpha and beta, both participate in binding. Integrins participate in cell-cell adhesion and are of great importance in binding and interactions of cells with components of

the extracellular matrix such as fibronectin. Importantly, integrins facilitate "communication" between the cytoskeleton and extracellular matrix, allow each to influence the orientation and structure of the other. A feature of integrins is that some exist in "active" and "inactive" states. For example, a group of integrins responsible for binding of white blood cells to endothelium are normally inactive, allowing the blood cells to circulate freely, but become activated in response to inflammatory mediators, resulting in the white cells being "pulled" from blood into inflammed tissues. It follows that deficits in expression of certain integrins can result in diseases characterized by abnormal inflammatory responses;

- Selectins are expressed primarily on leukocytes and endothelial cells and, like integrins, are important in many host defense mechanisms involving those cells. In contrast to other cell adhesion molecules, selectins bind to carbohydrate ligands on cells and the resulting binding forces are relatively weak. For example, selectin-mediated interactions between leukocytes and endothelial cells promotes rolling of the leukocytes along the endothelium and integrin-binding allows the leukocytes to be stopped in place.

2.6.1. E-Selectin

E-selectin (Endothelial Leukocyte Adhesion Molecule-1, (ELAM-1) belongs to the selectin family of adhesion molecules (Lasky et al. 1991). E-selectin mediates the initial interactions of leukocytes and platelets with endothelial cells. Selectins provide the first, loose contacts of polymorphonuclear cells with the endothelium in areas of inflammation. E-selectin is expressed on cytokine-activated endothelial cells, and promotes the adhesion of leukocytes to the endothelium. E-selectin, also known as CD62 antigen-like family member E (CD62E), endothelial-leukocyte adhesion molecule 1 (ELAM-1), or leukocyte-endothelial cell adhesion

molecule 2 (LECAM2), is a cell adhesion molecule expressed only on endothelial cells activated by cytokines.

Like other selectins, it plays an important part in inflammation. In humans, E-selectin is encoded by the SELE gene (Morrol et al. 2001). E-selectin is not stored in the cell and has to be transcribed, translated, and transported to the cell surface. The production of E-selectin is stimulated by the expression of P-selectin which is stimulated by tumor necrosis factor α (TNFα), and it can also be stimulated by interleukin-1 (IL-1) and lipopolysaccharide (LPS). It takes about two hours, after cytokine recognition for E-selectin to be expressed on the endothelial cell's surface, with maximal expression of E-selectin occurring around 6-12 hours after cytokine recognition by the cell. E-selectin production levels-out 24 hours after the cytokine signal, and continues to be expressed for three days.

During inflammation, E-selectin plays an important part in recruiting leukocytes to the site of injury. The local release of cytokines IL-1 and TNFα by damaged cells induces the over-expression of E-selectin on endothelial cells of nearby blood vessels. Leukocytes in the blood expressing the correct ligand will bind with low affinity to E-selectin, also under the shear stress of blood flow, causing the leukocytes to "roll" along the internal surface of the blood vessel as temporary interactions are made and broken. As the inflammatory response progresses, chemokines released by injured tissue enter the blood vessels and activate the rolling leukocytes, which are now able to tightly bind to the endothelial surface and begin making their way into the tissue (Morrol et al. 2001). .

CHAPTER 3.

METHODS

3.1.Study design

The design of the study was a descriptive and analytical cross sectional study. The study was from October 2008 to December 2012.

3.2. Study population:

The proposed study was carried out amongst diagnosed HIV participants referred to the Infectious Disease Clinic, Stamford Terrace Clinic, Gateway clinic and Ngagalizwe clinic in Mthatha. The HIV seronegative participants were people with minor complaints from Stamford terrace clinic in Mthatha, Gateway clinic and Walter Sisulu University in Mthatha (Figure 22). They were all of African descent.

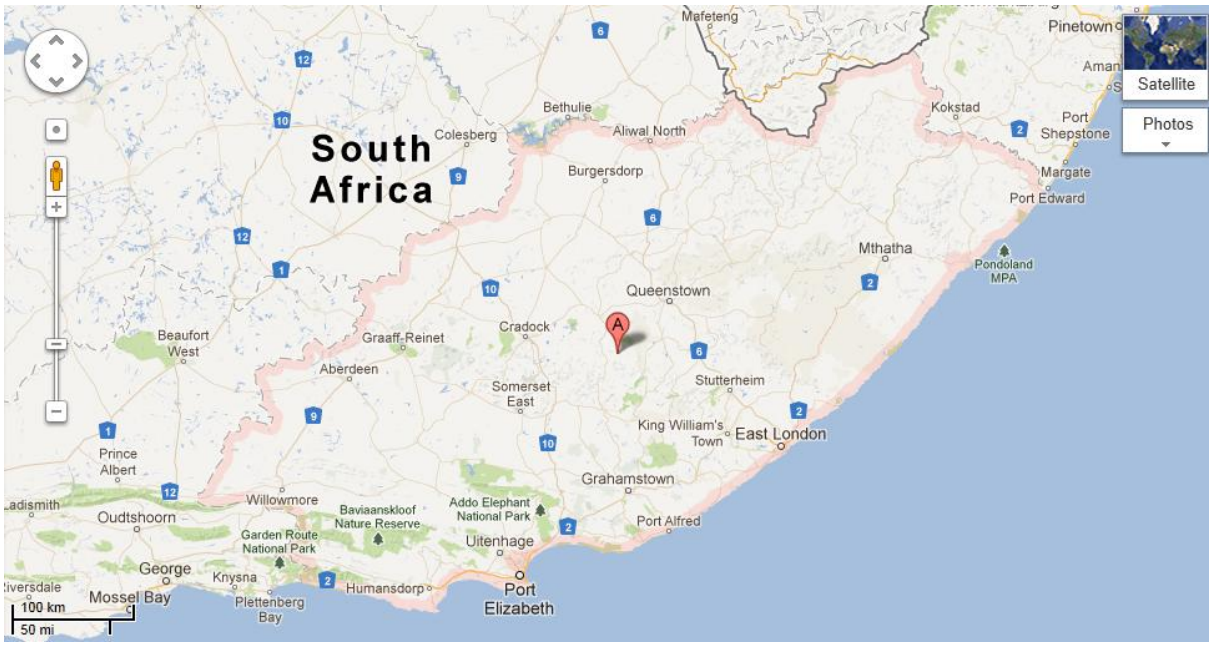

Figure 22. The Map of the Eastern Cape showing Mthatha.

Mthatha is a town in the old Transkei with a relatively poor community. The population of Mthatha and its environs is about 500,000. The few well to do go to the private clinics, while majority of the people go to these Government clinics in which the study took place. Most of them were unemployed eating mostly carbohyrate food once a day.

A convenient sampling method was used.

Out of the 169 participants, 63 were HIV negative, 52 were HIV positive not on treatment and 54 were HIV positive on treatment. Of the 169, there were 62 males and 107 females. There were 88 participants below the age of 35, and 81 above the age of 35 years.

The first group of participants were people who are HIV negative.

The second group are people who are HIV positive and antiretroviral naïve.

The third group of participants are those who are HIV positive and on treatment.

3.3. Calculation of Sample size

There was insufficient information from literature for the sample size(Ni) calculation.

The dichotomous outcome with up to a frequency (p) of 0.05 (5%), is usually one of the best ways to increase power.The general formula for p (expected proportion), the desired total (W) of the confidence level and the confidence interval of (1-α), was as follows:

Zα= the standard normal deviate for a two sided α, where (1- α) is the confidence level of 95%.

Since α=0.05, Zα=1.95;

Then the total number of participants was $Ni=4x(Z\alpha)^2x\ P(1-P)/W^2$;

$=4(1.96)^2\ x0.50\ x0.50/(0.15)^2$;

=3.84/0.0225;

=171 participants required for evaluation.

3.4. Participant selection

300 participants were recruited because of envisaged drop out rate, with 169 completing the study. There was a high drop out rate of 41% because many of the participants who were willing to take part in the study did not have a steady source of income. Death, drug side effects, change of address and phone numbers, lack of compliance and drop out of patients from the study reduced the number of patients from 300 to 169.

3.4.1. Inclusion criteria for HIV positive participants

The following participants were included:

- those on antiretroviral therapy for at least 6 months;
- newly diagnosed HIV positive patients not on antiretroviral therapy;

- documentation of HIV 1 infection by any licensed ELISA test kit and confirmed by a second method;
- able to provide informed consent.

3.4.2. Exclusion criteria for HIV positive participants

The following criteria served to exclude certain individuals:

- participants who had high blood pressure, diabetes mellitus or jaundice before starting treatment;
- use of slidenafil (Viagra) within 12 hours of the vascular (Sphygmocor) studies;
- recent life threatening opportunistic infections;
- history of liver disease;
- inability to provide informed consent;
- pregnant women;
- breastfeeding women;
- HIV positive participants not on first line antiretroviral;
- those on ARV for less than 6 months and have detectable RNA copies of the virus;
- those who were not willing to fast overnight.

3.5. Consent from the participants

Informed consent was obtained from the participants after due explanation of the procedures to them. They were informed that they may refuse to participate or withdraw from the study at any time without fear of victimization. Those who agreed to be tested for their HIV status were counseled and post counseled. Participants were informed of the purpose of the research as well as of any other implications of the research. Participants were assured of total confidentiality and there will be no information that identified them in any manner. The

information will only be utilized for the purposes as stipulated and all possible steps will be taken to ensure that the information remained confidential. They were informed in the local language making sure they understood. After these they signed the consent form. They were referred to the necessary specialist clinic if they wanted to.

3.6. Ethical approval

Ethical approval was obtained from the Ethics Committee, Faculty of Health Sciences, Walter Sisulu University for ethical and bio-safety clearance. Ethical clearance certificate number 0043/009.

3.7. Administration of questionnaires and follow up

Translated questionnaires were administered to the participants by someone who spoke fluent Xhosa. The administrator of the questionnaire was trained by me. After administration of the questionnaire, those participants selected were informed and given appointments. First visit involved taking their baseline blood profile and their anthropometric measurements. The second appointment required them to fast. They fasted overnight from 8pm to 8 am before their blood samples were taken for assays and their vasular function was evaluated. For all these appointments they were contacted on phone and given incentives for transport.

3.8. Anthropometric measurements

Height was measured using the Harpenden stadiometer (Holtain Ltd, Crymych, Dyfed, United Kingdom). Participants were bare- footed with heels together, arms to the side, legs together, legs straight, shoulders relaxed. Head was positioned in the Frankfort horizontal plane. The heels, buttocks, scapulae and back of the head were placed against the vertical

board of the stadiometer. The measurement was to the nearest 0.1 cm. the eye leveling with the headboard to avoid errors. Averages of two readings were taken.

For weight measurement the participant stood in the middle of the scale without touching anything. They had minimal clothing. The weight was read to the nearest 100gm (0.1kg) and recorded immediately. The average of the weights measured was taken. Body Mass Index (BMI) was calculated using the following formula. Weight (in Kilograms) divided by height (in meters) squared=wt (kg)/(ht (m)^2.

Waist circumference was measured in cm at a level midway between the lower rib margin and iliac crest with the tape all around the body in horizontal position in cm.
Hip circumference was measured as the maximal circumference over the buttocks in cm.

Blood pressure was measured using the Omron sphygmomanometer (Omron, Guangdong, China). Three blood pressure measurements were taken 5 minutes apart and the average of these readings was used in the analysis. The first appearance of sound (phase 1) is used to define systolic blood pressure (Sbp). One intern whom I supervised took all the anthroprometric measurements .

3.9. Blood Sample collection

20 ml of blood was collected after fasting overnight and divided into aliquots into plain tubes centrifuged at 3000rpm for five seconds and stored at -80^{0}C until when needed for the estimation of serum HDL-C, TC, and Triglycerides. C-Reactive protein was assayed in the NHLS laboratory using the Hitachi/Cobas C system.

Elisa was used for determining the levels of the following, TNFα, IL-6 and E-selectin in serum. 5 ml of blood was collected in EDTA bottles and sent to the National Health Laboratory Service (NHLS) for the determination of HIV antibodies. Full blood count, including monocytes and platelets were also analyzed in the NHLS laboratory using the Coulter counter (Model Sysmex XT 2000 i).

3.9.1. Centrifugation protocol

Blood was spun at a speed of 3000 rpm in a cold centrifuge for 5 minutes to obtain serum samples.

3.10. Total cholesterol analysis

3.10.1. Principles

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed affects the oxidative coupling of phenol and 4-aminophenzone to form a red quinone-imine dye. The colour intensity of the dye formed is directly proportional to the cholesterol concentrations. It is determined by measuring the increase in absorbance.

3.10.2. Quantitative Colorimetric determination of Total cholesterol

The determination of total cholesterol was by the CHOP-PAP method (Enzymatic colorimetric Determination of serum cholesterol). Reference number CHOL-MC-0530 N.S BIOTEC combined with the method of Trinder (1969). It is as follows:

Cholesterol esterase

Cholesterol esters + H_2O ----------→ cholesterol + RCOOH

Cholesterol oxidase

Cholesterol +O_2 ----------→ 4 Cholestenone + H_2O_2

Peroxidase

$2H_20_2$ + 4-aminophenazone + phenol----------→ 4-(p-benzoquinone-monoiminophenazone+ $4H_2O$.

3.10.3. Procedure

The tubes for reagent solution, standard and serum were labeled in duplicate. The wavelength of the spectrophotometer was set at 500nm. 20µl of sample was pipetted into sample tubes. 2 ml of reagent solution was pipetted into reagent blank (RB) and sample tubes. The contents in the tubes were mixed and incubated for 10 minutes at 20-25^{0}C. The absorbance was read against RB within I hour = A $_{sample}$. The concentrations of cholesterol in the sample was calculated as follows; 14.9mmol/l xA sample.

3.10.4. Measurement and test priciple of Triglycerides by Enzymatic colometric method

To quantitate the amount of triglycerides in a serum sample the following method was used. (Peridochrom Triglycerides GPO-PAP method) by (Medichem) Clinical Chemistry Reagents. A modification of Trinders method (1969).

Triglycerides are hydrolyzed to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red substance. The colour intensity of the red substance formed is directly proportional to the triglyceride concentrations and measured photometrically.

The reaction takes place according to the following equations:

Lipase

Triglycerides + $3H_20$ ----------.> glycerol + 3 RCOOH

GK

Glycerol + ATP ----------→ glycerol-3-phosphate + ADP

Mg++

Glycerol -3 –phosphate + O_2 __GPO_____>dihydroxyacetone phosphate +H_2O_2

Peroxidase

H_2O_2 + 4 aminophenazone + 4-chlorophenol-→ 4 – (p-benzoquinone- monoimino) phenazone+ $2H_2O_2$ + HCL.

3.10.5. Procedure for the measurement of triglyceride level

The stored serum was thawed. The tubes for reagent solution, standard and serum samples were labeled in duplicate. The wavelength of the spectrophotometer was set at 546nm. 20 µl of standard solution was pipetted into tube 1 and 20µl of serum into the remaining labeled tubes. 2 ml of reagent solution was pipetted into each of the labeled tubes including the standard solution. The content in each tube was mixed and incubated for 10 minutes at 20-

25^0C. The absorbance of serum sample (A_{sample}) and Standard ($A_{standard)}$ was read against reagent solution within 60 minutes.

The concentrations of triglycerides was calculated as follows: (mmol/l)

$$= 2.29 \times \frac{(A \text{ sample})}{(A \text{ standard})}$$

Where (A_{sample}) is absorbance of the serum sample and ($A_{standard}$) is the absorbance of the standard from the colorimetic reading where 2.29 is a constant.

3.10.6. Measurement and Test Principle of HDL cholesterol by CHOD-PAP method (cat No. 543004, Boehringer Mannheim; Mannheim, Germany).

On adding phosphotungstic acid and magnesium ions to the serum samples, chylomicrons, VLDL, and LDL are precipitated. HDL is then left in the supernatant after centrifugation. The HDL cholesterol is then determined enzymatically.

3.10.7. Procedure for HDL-C

The tubes for reagent solution, standard and serum samples were labeled in duplicate. The wavelength of the spectrophotometer was set at 500nm. 200µl of serum sample and 500µl of precipitant were pipetted into labelled centrifuge tubes except for tube 1 which contained 200µl standard and 500µl precipitant. The contents of the tubes were mixed and allowed to stand for 10 minutes at room temperature. The contents were centrifuged for 10 minutes at 4000rpm. After centrifugation, the clear supernatant was separated within 2 hours. 100µl distilled water was pipetted into reagent blank (RB) and 100µl supernatant into each of the

sample tubes. The content in the tubes were mixed and incubated for 10 minutes at 20-25^{0}C. The absorbance of sample (A sample) at 500nm against RB was measured within I hour.

The value of HDL cholesterol was calculated using the formula: 5.67xA $_{sample.}$

3.10.8. Determination of LDL cholesterol (Optimized colorimetric assay method)

LDL was calculated from the values of Total cholesterol, Triglycerides and HDL cholesterol for each sample. This was according to Friedewalds formula (1972).

LDL cholesterol in mmol/l = total cholesterol—triglycerides - HDL cholesterol/5.

3.11.1. Cytokines

3.11.1.1. Interleukin- 6

IL-6 is a multi functional cytokine. The method for the assay is The RayBio ® Human IL-6 using the Elisa technique (Cat #ELH-IL6-001).

3.11.1.2. Test principles

ELISA (Enzyme linked Immunosorbent Assay) which is an in vitro enzyme –linked immunosorbent assay for the quantitative measurement of human IL-6 in serum was utilized. This assay employs an antibody for human IL-6 coated on a 96 –well plate. Standards and samples were pipetted into the wells. IL-6 present on a sample is bound to the wells by the immobilized antibody. A tetramethyl-benzidine (TMB) substrate solution is added to the wells and a color develops in proportion to the amount of IL-6 bound after washing the wells.

3.11.1.3. Procedure

The levels of Il-6 were measured in sera derived from 169 participants using a quantitative ELISA.

3.11.1.4. Reagents

The following reagents were used:

- IL-6 Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-human IL-6;
- wash buffer concentrate (20x) item B: 25ml of 20x concentrated solution;
- standards (Item C) : 2 vials, recombinant human IL-6;
- assay diluent A (Item D): 30 ml, 0.09% sodium azide as preservative.

3.11.1.5. Reagents for standard/Sample(serum/plasma) diluent

Reagents for standard diluent used were as follows:

- detection Antibody IL-6 :2 vials of biotinylated anti-human IL-6;
- HRP-Streptavidin concentrate : 8µl 30000x concentrated HRP- conjugated streptavidin;
- TMB One–Step Substrate Reagent:12ml of 3,3',5,5' tetramethyl-benzidine(TMB) in buffered solution;
- Stop Solution: 8ml of 2M sulphuric acid.

3.11.1.6. Reagent preparation

All reagents and samples were brought to room temperature. The vials containing the standards were spun and 400µl assay diluent A was added into the item C standard vial to prepare a 50 ng/ml standard solution. The powder was dissolved by gently mixing it. 20µl of IL6 standard from the vial of Item C was added into a tube with 980µl Assay Diluent A to prepare a 1000 pg/ml stock standard solution. 400µl of assay diluent A was pipetted into each tube. The stock standard solution was used to produce a dilution series. Each tube was mixed thoroughly before the next transfer. The tubes were gently vortexed and mixed. Assay Diluent A served as the zero standard.

3.11.1.7. Assay procedure:

All the standards and samples were ran in duplicates. 100µl of each standard and sample were added into appropriate wells. They were covered and incubated for 2.5 hours at room temperature after gentle shaking. The solution was discarded and washed four times. It was washed by filling each well with wash buffer (300µl) using a multi-channel pipette. There was complete removal of liquid at each step. After the last wash, remaining wash buffer was removed by aspirating or decanting. The plate was inverted and blotted against clean paper towels. 100µl of 1x prepared biotinylated antibody was added to each well. These were incubated for an hour at room temperature after gentle shaking. The solution was discarded and washed again for four times.

100µl of prepared Streptavidin solution was added to each well and incubated for 45 minutes at room temperature after gentle shaking. Solution was discarded and again washed four times. 100µl of TMB substrate reagent was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking. 50µl of stop solution was added to each well and read at 450nm immediately.

3.11.1.8. Calculation of results

The mean absorbance for each set of duplicate standards, controls and samples were calculated and the average zero standard opical density was substracted. The standard curve was plotted on log-log graph paper with standard concentration on the x-axis and absorbance on the y- axis.

3.11.2. Measurement of TNFα (Tumour Necrosis Factor) using ANOGEN (Canada) method from Catalogue number EL10019.

3.11.2.1. Principle of the assay

According to the testing system, the provided standard was diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. A standard curve of Optical Density (O.D) versus TNF-α concentration (pg/ml) was produced. The concentration of TNF-α in the samples was determined by comparing the O.D. of the samples to the standard curve. Human TNFα Elisa Kit was used for the determination of human tumor necrosis factor alpha concentrations in the serum samples (Catalogue no EL 10019). Elisa technique applied is called a quantitative sandwich immunoassay.

The microtiter plate provided in the kit was pre-coated with a monoclonal antibody specific to TNF-α. The plate had 96 wells. Reagents provided included the following;

- 7 ml of biotin conjugate;
- 12 ml of avidin conjugate (avidin conjugated to horseradish peroxidase);
- 2 vials of TNFα standard ;
- 22ml of calibrator diluent 1, ;
- 60 ml Wash buffer(29 fold concentrated solution of buffered surfactant);
- 11 ml Substrate A (Buffered solution with H_2O_2);
- 11 ml substrate B (Buffered solution with TMB),
- 14 ml Stop solution.

3.11.2.2. Preparation of reagents

To prepare the reagents all the kit reagents were allowed to reach room temperature (20-25^{0}C). Wash buffer was reconstituted by adding 1 volume of wash buffer to 19 volumes of

distilled water. Substrate A and substrate B were mixed in equal volumes 15 minutes before use (Since there are 96 wells with 12 strips, 7 ml of substrate A was mixed with 7 ml of substrate B).

TNFα standard was reconstituted with 4.0 ml of calibrator diluent 1 which produced a stock solution of 1000 pg/ml. This stock solution was used to produce a serial 2-fold dilution series within the range of 15.625 to 1000pg/ml. 0.5ml of the calibrator dluent was added to each test tube. The contents of each test tubes were mixed thoroughly. The undiluted TNFα stock solution served as the high standard (1000pg/ml) and the calibrator diluent serving as the zero standard (0pg/ml). The tubes then ranged from 1000 to 500, 250, 125, 62.50, 31.25 to 15.625pg/ml. 50 µl of Biotin Conjugate was added to the antibody precoated microtiter plate. 100µl of standard or sample was added to the appropriate wells. It was mixed well. The plate was covered and incubated for 1 hour at room temperature. The microtiter plate was washed five times. After the final wash the plate was inverted and blot dried by hitting the plate onto adsorbent paper until no moisture appeared. 100 µl of Avidin conjugate was added to each well. Avidin was added in order to quantify the amount of TNF-α present in the sample. (Avidin is a tetramer containing four identical subunits in which each has a high affinity-binding site for biotin.) The plate was covered and incubated for 1 hour at room temperature.

Washing was repeated. 100µl substrate solution was added into each well. The plate was again covered and incubated for 20-30 minutes at room temperature. 100µl stop solution was added to each well and mixed. The enzyme-substrate reaction was terminated by the addition of the stop solution which contained a sulphuric acid solution. The colour change was measured spectrophotometrically at a wavelength of 450 nm. The standard curve was used to determine the amount of TNFα in an unknown sample. The standard curve was generated by

plotting the average O.D.(450nm) obtained for each of the standard concentrations on the vertical axis(Y) versus the corresponding TNFα concentration (pg/ml) on the horizontal axis(X) axis.

3.11.3. E-Selectin with test principle

Method used for the determination of the level of E Selectin in the samples is Elisa. (Cat /ELA-E Selectin-od by RayBiotech.Inc).

Anti-E-selectin monoclonal antibody is adsorbed onto microwells. The pair of monoclonal antibodies used in this ELISA detects the soluble form of E Selectin present in the serum. Soluble E-selectin present in a sample or standard binds to antibodies adsorbed to the microwells. A second, HRP-conjugated monoclonal anti-E-selectin antibody is added and binds to E-selectin captured by the first antibody. Unbound enzyme-conjugated anti-E-selectin is removed with washing and HRP substrate solution is added to the wells. An amount of colored product is formed, proportional to the amount of soluble E-selectin present in the sample. The reaction is terminated by addition of acid. Absorbance is measured at 450 nm. A standard curve is prepared from six E-selectin standard dilutions and the E-selectin sample concentration is determined.

3.11.3.1. The components of the Kit

One aluminum pouch with a Microwell Plate coated with Monoclonal Antibody (murine) to human E-Selectin:

- 1 vial (150 µL) HRP-Conjugated Anti-E-Selectin Monoclonal (murine) Antibody;
- 2 vials (100 ng/mL each, reconstituted) soluble E-selectin standard, lyophilized;
- 1 vial control (high), lyophilized;

- 1 vial control (low), lyophilized;
- 1 bottle (50 mL) wash buffer concentrate 20X (PBS with 1% Tween 20);
- 1 vial (5 mL) assay buffer concentrate 20X (PBS with 1% Tween 20 and 10% BSA);
- 1 bottle (12 mL) sample diluent (buffered protein matrix);
- 1 vial (15 mL) substrate Solution;
- 1 vial (15 mL) stop solution (1M phosphoric Acid);
- 1 microwell strip holder;
- 2 adhesive plate covers.

Stored serum samples in cryo tubes were thawed. Prior to this all reagents except for HRP-Conjugate was prepared in advance before starting the test procedure.

3.11.3.1.1. Preparation of Assay Buffer

Just prior to use a 1:1000 dilution of assay buffer solution was prepared in a clean plastic tube. Contents of assay buffer concentrate (%ml) was added to 95 ml of deionized water and mixed gently. It was stored in the fridge.

3.11.3.1.2. Preparation of reagents

In the preparation of E-selectin standard, distilled water was added to the vial labelled E-selectin standard. The vial was mixed. 20 minutes was allowed for the standard to reconstitute.

3.11.3.1.3. Preparation of controls

The positive controls were reconstituted by adding 200µl of distilled water. It was mixed gently to ensure complete and homogenous solubilisation. The controls were treated like a sample in the assay. Phosphoric acid was supplied as a stop solution.

3.11.3.1.4. Preparation of the wash buffer

All the 50 ml of the wash buffer was poured into a clean 1000ml graduated cylinder. The final volume was brought to 1000 ml with deionized water. It was mixed gently to avoid foaming. The pH of the final solution was 7.4. The final solution was transferred to a clean wash bottle and stored between 2^0C and 20^0C.

3.11.3.2. Procedure

All reagents were thoroughly mixed before use, to avoid foaming. Each sample, standard, blank and optional controls were assayed in duplicate. Microwell strips coated with monoclonal antibody to human E-selectin were removed from their aluminium pouches immediately prior to use. The microwell strips were loaded into the 96 microwell strip holder making sure that the first microwell strip is in row 1.

The microwell strips were washed twice with approximately 300µL wash buffer per well with thorough aspiration of microwell contents between washes. After the last wash the wells were emptied and microwell strips were tapped on a paper towel to remove excess wash buffer. 100µl of sample diluent was added in duplicate to all standard wells. Standard dilutions were prepared by pipetting 100µl of soluble E-selectin standard in duplicate , into wells A1 and A2. 100µl was transferred to well B1 and B2 respectively. The contents were

mixed by repeated aspiration and ejection and 100µl was transferred to well C1 and C2 respectively.

The procedure was repeated to create two rows of E-selectin standard solutions ranging from 50 to 0.8ng/ml. 100µl of the contents from the last microwells (G1 and G2) used was discarded. 100µl of sample diluent was added to all blank wells. 80µl of sample diluent was added to all sample wells. 20µl of each sample was added in duplicate to the designated wells and mixed. 50µl of diluted (1:100) HRP conjugate was added to each well including blank wells. The plate was covered with a plate cover and incubated at room temperature (18^0 to 25^0C) for 2 hours. The plate cover was removed and the wells emptied. The microwell strips was washed 3 times.

100 µL of mixed TMB Substrate Solution was pipetted into all wells including the blank wells. The microwell strips was incubated at room temperature (18° to 25°C) for about 10 minutes, on a rotator set at 100 rpm. Direct exposure to intense light was avoided. The enzyme reaction was stopped quickly by pipetting 100 µL of Stop Solution into each well, including the blank wells. Results were read immediately. The absorbance of each microwell was read on a spectrophotometer using 450 nm as the primary wavelength. The absorbance of both the samples and the E-selectin standards was determined.

3.11.3.3. Calculation of Results

The average absorbance values for each set of duplicate standards and samples were calculated. A standard curve was created by plotting the mean absorbance for each standard concentration on the ordinate against the soluble E-selectin concentration on the abscissa. A

best fit curve through the points of the graph was drawn.The concentration of circulating E-selectin for each sample was then determined from the graph.

3.12. Measurement of Pulse wave analysis and pulse wave velocity using the The Sphygmocor(Atcor Medical, Australia)

3.12.1. Principles

The SphygmoCor is one of the recently-developed computerized, portable and simple-to-use devices used to assess pulse waveforms. The probe incorporates a high-fidelity strain-gauge transducer at the tip, which has a small pressure-sensitive ceramic sensor area with a frequency response of >2 kHz that is coplanar with a longer area (7 mm diameter) of flat surface in contact with the skin overlying the arterial pulse (Millar Instruments). The probe's technology is based on the principle of applanation tonometry, as used in ocular tonometry for the assessment of intraocular pressure. The SphygmoCor provides a continuous calibrated blood pressure waveform at the ascending aorta, using a recording of the radial artery blood pressure waveform (figure 23).

3.12.1.1. The Sphygmocor

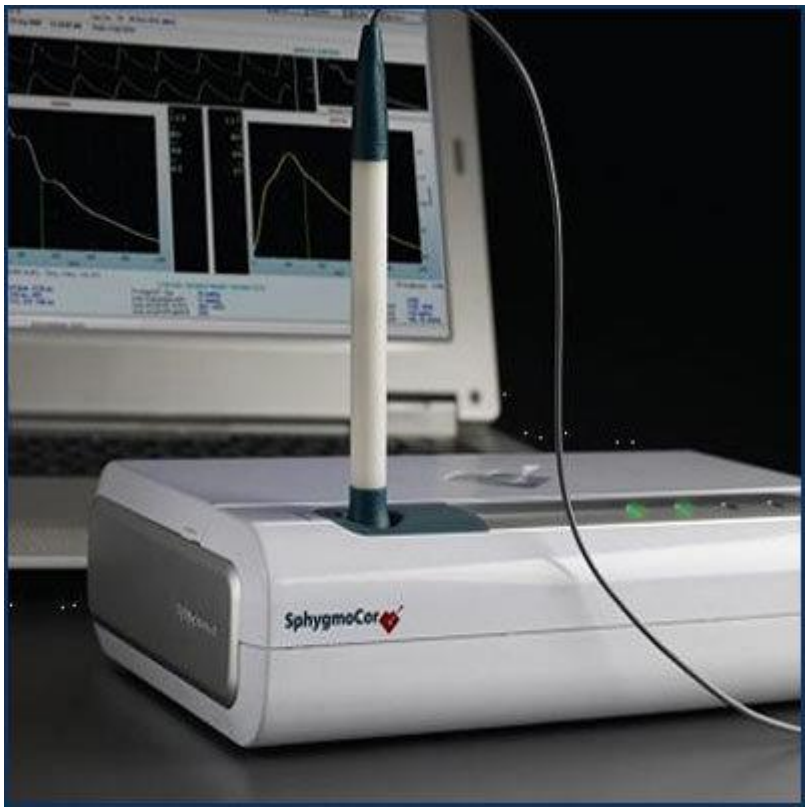

Figure 23. The Sphygmocor. source: http;//www.atcormedical.com.

The Sphygmocor Vx system performs analysis to provide a quantitave aortic cardiovascular data such as:

- systolic pressure;
- diastolic pressure
- augmentation pressure;
- ejection duration and
- subendocardial viability index.

3.12.2.1 PWA (Pulse wave analysis)

This is a reproducible non invasive method for assessing central blood pressure and augmentation index (AIx(75)). Augmentation pressure (AP) is defined as the

difference between the second and the first systolic peak. Augmentation index (AIx(75)) is expressed as a percentage of the pulse pressure. The pulse pressure is the difference between the systolic and diastolic pressure. Augmentation index is a measure of the contribution that wave reflection makes to the arterial pressure waveform. The amplitude and timing of the reflected wave ultimately depends on the stiffness of the small preresistance and large arteries and thus AIx provides a measure of systemic arterial stiffness (Wilkinson et al 1998).

Carotid pressure waveform is recorded by applanation tonometry. The height of the late systolic peak (P1) above the inflection (P2) defines the augmentation pressure, and the ratio of augmentation pressure to PP defines the AIx (75) (in percent) (Figure 24).

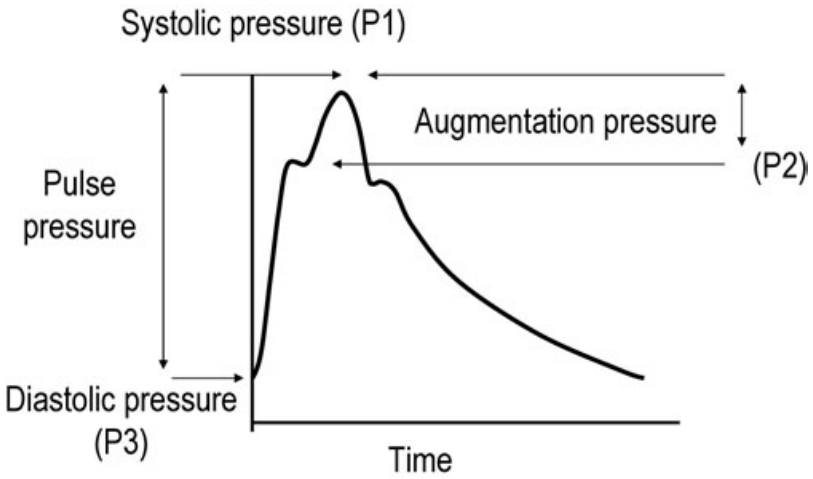

Figure 24. The Carotid pressure waveform. Source: O' Rouke& Yahima 1984. Wave reflection and the arterial pulse. Arch. Intern. Med.

Figure 25. and Figure 26. showing the investigator measuring the pulse wave velocity of a participant

Figure 25. Atonometer applied to the carotid artery for the measurement of PWV.

Figure 26. Waveforms as seen on the screen.

3.12.2.2. Pulse wave analysis measurement

The SphygmoCor system (Pulse wave velocity system PWV, SphygmoCor Vx version 7.01 software Atcor Medical, Sydney, Australia) was used for pulse wave analysis; The participants having fasted, abstained from caffeine, smoking and using viagra for at least three days. The procedure took place in a quiet room and participants were not allowed to talk. The participant's personal details such as the name, date of birth and gender were entered into the patient screen. The height, weight and blood pressure of the participants were measured and also entered into the corresponding Sphygmocor software fields. The

participants sat comfortably beside a table with their arm on the table and their palm facing upward in a quiet room.

The location of the strongest pulse was felt on top of the skin and the tonometer placed directly on that location. Peripheral pressure waveforms were recorded from the right radial artery using applanation tonometry. After 30 sequential waveforms had been acquired, a validated generalized transfer function was used to generate the corresponding central aortic pressure waveform, from which the augmentation index was obtained (AIx(75)). This was calculated as the ratio between augmentation pressure and pulse pressure.

Because AIx is influenced by heart rate, an index normalized for heart rate of 75 bpm AIx(75) was applied. Larger values of AIx(75) indicate increased wave reflection from the periphery or early return of the reflected wave as a result of increased pulse wave velocity due to increased arterial stiffness. Only high-quality recordings, defined as an in-device quality index 80% (derived from an algorithm including average pulse, height, pulse height variation, diastolic variation, and the maximum rate of rise of the peripheral waveform and acceptable curves on visual inspection) were included in the analysis.

3.12.2.3. Measurement of Pulse wave velocity

Three main arterial sites can be evaluated, mainly the aortic trunk (carotid-femoral) (Figure 27) and the upper (carotid-brachial) and lower (femoral-dorsalis pedis) limbs.

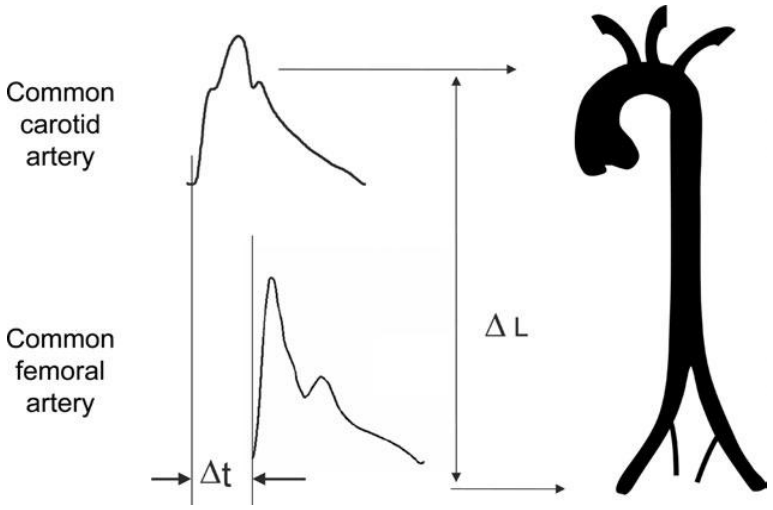

Figure 27. Measurement of carotid-femoral PWV with the foot to foot method. Source: O' Rouke & Yahima 1984. Wave reflection and the arterial pulse. Arch Intern. Med.

To perform a Sphygmocor control measurement of the pulse wave velocity, the participant was told to abstain from alcohol at least 12 hours prior to the measurement and abstain from tobacco and caffeine 4 hours prior to the measurement. Participants had their blood pressure taken 5 minutes before the measurements. The participants laid on their supine position with the limbs cleaned with an alcohol swab. Electrodes were placed on the participant's wrists and on the left leg. For carotid artery measurements the participant laid on a bed with his or her head tilted slightly to the back and to one side (either left or right) in the absence of a pillow (Figure 25 and 26). The position of the strongest pulse was felt using the index finger and the tonometer placed directly on top of the skin at the base of neck. Femoral artery measurements involves the participant lying supine and the femoral pulse felt by pressing directly backward at a point that is midway between the anterior superior iliac spine and the front of the pubic bone, with the thigh slightly flexed at the hip joint, moved away from the midline of the body and rotated away from the body. The probe was held on the skin over the maximal arterial pulsation by hand and pressed down on the artery against the underlying bone. Arterial PWV was determined by the foot-to-foot flow wave velocity method. The foot

of the flow wave was identified between the two recording sites as the beginning of the sharp systolic up-stroke. The time delay was measured between the feet of the flow waves recorded at these different points and designated as pulse transmit time (All these were displayed on the machine).

The distance traveled by the pulse wave was measured over the surface of the body with a tape measure. ECG gating permitted the time lapse between pulse waves at the carotid and femoral sites to be calculated from sequential rather than simultaneous measurements. PWV was calculated as the distance: transit time ratio and is expressed as meters per second. (This was also recorded automatically on the sphygmocor machine).

3.13. DATA ANALYSIS

Data was analyzed using the statistical program SPSS version 19. All quantitative variables in the text are expressed as means +_ SD. Univariate and multivariate (logistic regression model) analyses was used to analyze the data. (P values <0.05 will be significant for statistical differences).

Data was tested for normal distribution. Statistically, ANOVA was used for variables with normal distribution and Non parametric tests were used for variables with skewed distribution. Post Hoc test (tukey and LSD) were administered to establish which of the 3 groups is the cause of the significance difference observed within and between the 3 groups. Kruskal-Wallis Non parametric test was used for variables with skewed distribution to establish significant difference. Data were expressed as means and standard deviations. Results were expressed as mean ± SD or as proportion (%) for the different parameters.

CHAPTER 4.

4.1. RESULTS

A total of 169 participants completed the study

4.1.1. The three groups of participants:

Among 169 participants 63 were HIV negative, 52 were HIV positive not on treatment and 54 were HIV positive on treatment (Figure 28).

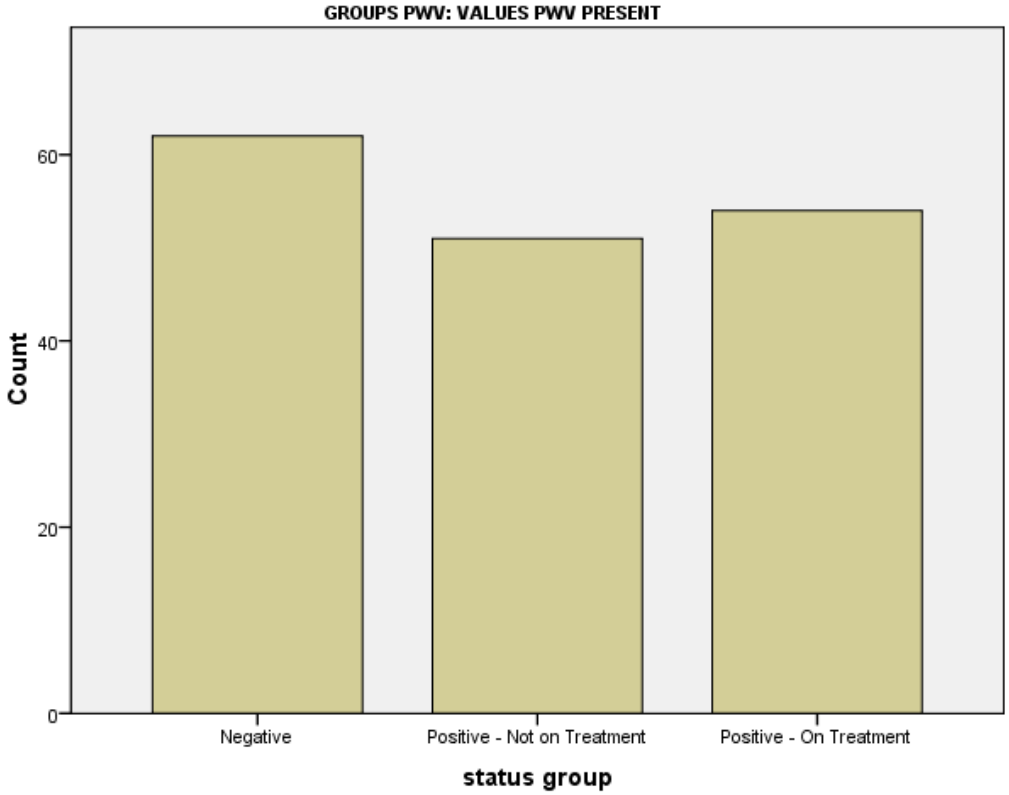

Figure 28. Proportion of participants in each group.

Group 1 HIV Negative, Group 2 HIV posititve not on treatment, Group3 HIV positive on treatment.

4.1.2. Age

The mean ages were significantly different (P<0.001) across the study groups (Figure 29). For HIV negative participants the mean age is 30.34±10.65 years, HIV positive participants not on treatment it is 36.25±10.79 years and for HIV positive on treatment it is 40.21±9.56 years.

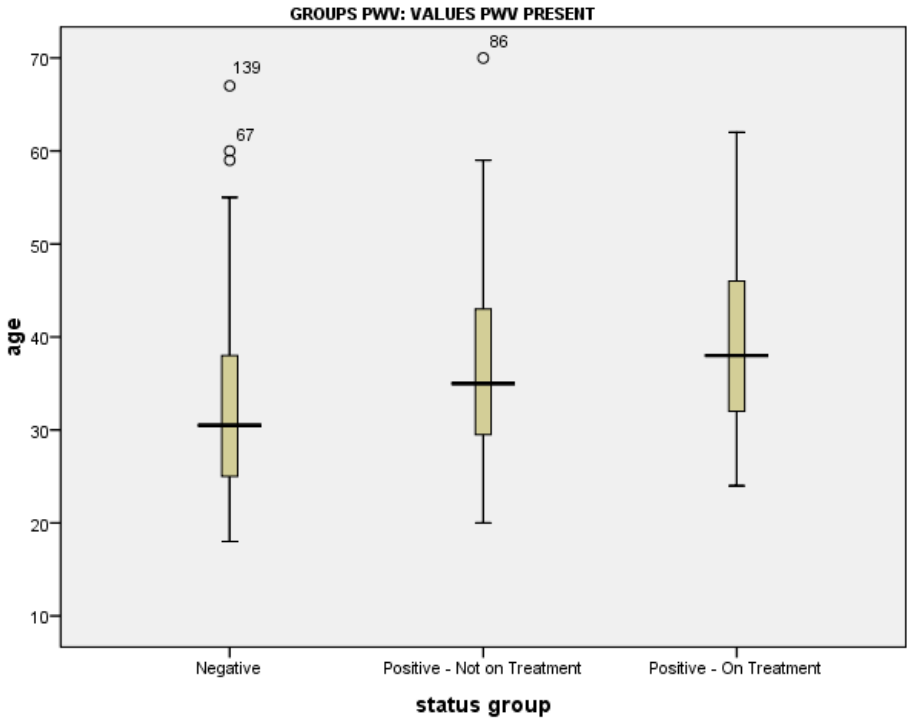

Figure 29. The mean ages of the different groups.

The mean values of all anthropometric parameters were similar (P>0.05) across the HIV status groups (Table 7). The highest mean age was in HIV positive participants on treatment. Post hoc the age group was most significant between the HIV negative and HIV positive on Treatment (p<0.001).

Table 7. Comparisons of mean levels of age and anthropometric variables according to HIV status groups

variables	HIV -ve n=63	HIV +ve not on treatment n=54	HIV+ve on treatment n=52	P-value ANOVA
Age in years	30.34±10.646	36.25± 10.785	40.21± 9.554	<0.000*
Weight(Kgm)	71.35± 15.45	71.31± 19.64	68.50±13.72	0.583
Height(cm)	164.77± 8.40	162.87± 7.61	162.72±8.03	0.322
WC (cm)	85.43± 15.32	85.84± 21.41	90.39±10.58	0.209
HC (cm)	105.44±14.02	102.12±13.94	103.69±10.30	0.439
BMI (Kgm/m^2)	33.10± 38.57	26.38± 5.80	25.78± 4.79	0.170
WHR (%)	0.83±0.09	0.80±0.19	0.88±0.21	0.130

WC Waist circumference.

HC hip circumference.

WHR Waist to hip ratio

BMI Body Mass Index

4.1.3. Participant gender distribution within the three groups

In total there were 169 participants with 63 males and 106 females, there were 35 (57%) male participants who were HIV negative, 15 (23%) HIV positive not on treatment, and 13 (20%) HIV positive on treatment. 27 (25,5%) HIV female participants who were HIV negative 37, (34.9%) were positive but not on treatment, 42 (39.6%) were positive and on treatment (Figure 30).

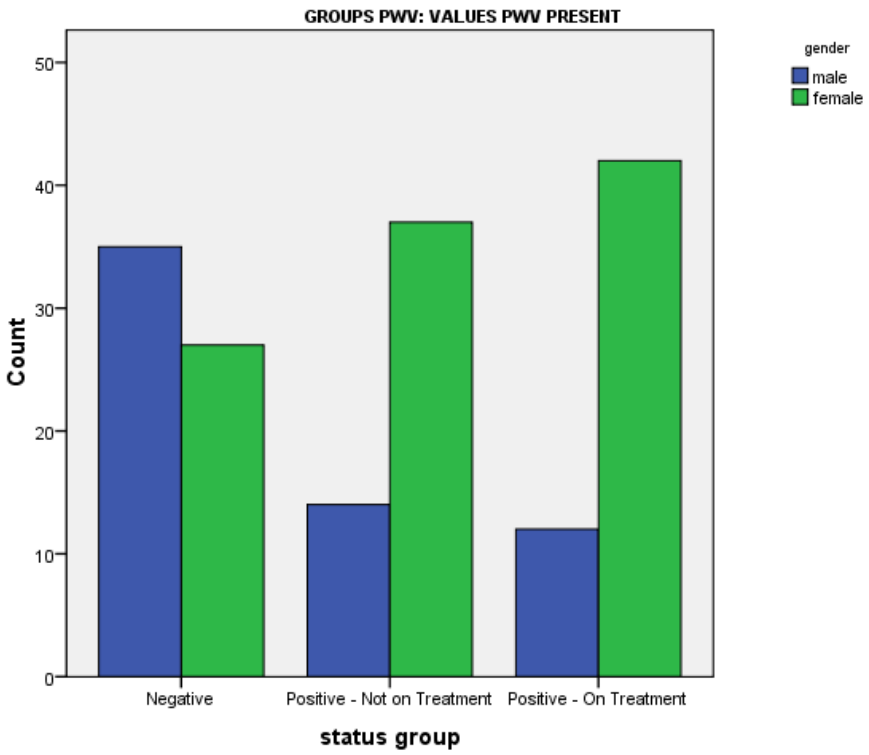

Figure 30. The HIV status and the gender.

One can see that females were more in all the three groups especially with the HIV positive participants. This is because most of the willing participants who are HIV positive participants were females and our public health hospitals were mostly patronized by females.

4.1.4. Haemodynamic profile across the study groups

The aortic and brachial blood pressures are as below Table 8**.** The mean heart rate in the group of participants not on treatment was significantly higher in the three groups (p=0.017) . Post hoc this was more significant between the HIV treatment naïve groups and the HIV negative groups (p= 0.034). However the levels of other variables did not vary (P>0.05) across the study group.

Table 8. Chararacteristics of both the brachial and aortic blood pressure in all the 3 groups of participants

variables	HIV -ve n=63	HIV +ve not on ANOVA treatment n=54	HIV+ve on treatment n=52	P-value

Spa(mm/Hg)	110.60±14.90	115.41± 14.72	111.63± 18.06	0.257
Dpa(mm/Hg)	80.21± 9.88	83.75± 9.90	84.80± 12.62	0.053
MP(mm/Hg)	92.76± 15.34	98.22± 11.25	97.33± 12.62	0.063
Ppa(mm/Hg)	30.39± 8.79	31.69± 10.06	28.09± 10.49	0.162
Sbp(mm/Hg)	121.25±14.52	123.40± 21.99	126.44± 17.83	0.312
dbp(mm/Hg)	79.72± 9.57	82.88± 12.05	84.63± 11.60	0.059
Pbp(mm/Hg)	75.43± 11.88	77.29± 18.27	79.33± 18.69	0.458
HR(b/ min)	67.58± 9.50	73.31± 11.54	72.83± 14.40	0.017*

Spa Systolic centra(aortic) blood pressure

Dpa Diastolic central(aortic blood pressure

MP This is MAP or mean arterial pressure

Ppa Aortic(central) pulse pressure

Sbp Systolic brachial pressure

Dbp Diastolic blood pressure

Pbp Bracial pulse pressure

HR Heart Rate

4.1.5. Lipid profile by study groups

Lipid profile by study groups is shown in (Table 9). Both the means of High Density Lipoprotein cholesterol (HDL-C) and Total Choleterol (TC) levels in the HIV participants on treatment were significantly higher than the two other groups (p=002 and p=0.005) repectively. However, the values of triglycerides did not vary (p>0.05) across the study group. Post hoc there was a marked significance between the levels of HDL-C in those who are treatment naïve and those on treament (p<0.05). Post hoc the cholesterol levels in

participants who are treatment naïve differed significantly from those who are on treatment (P=0.06).

Table 9. The lipid profile of the 3 groups of participants

Variables	HIV -ve n=63	HIV +ve not on treatment n=54	HIV+ve on treatment n=52	P-value ANOVA
HDL-C(mmol/l)	1.39± 0.42	1.14± .34	1.56±.74	0.002
TC (mmol/l)	4.23± 1.09	3.64± 1.14	4.38± .96	0.005
TG (mmol/)	0.97± 0.40	1.06± 0.46	1.19±.51	0.092
LDL-C (mmol/l)	2.40± 1.16	2.50± 2.76	2.34± .90	0.919

TC Total cholesterol, TG Tryglyceride

HDL-C High density lipoprotein chlesterol

TG Triglyceridess

LDL-C Low density lipoprotein chholeterol

4.1.6. Values of cytokine levels across study groups

The cytokine levels exhibited variability across the study groups (Table 10). The participants who were HIV negative had the lowest figure of IL-6, E-Selectin and C-reactive protein (Crp) but these were not significant in these study groups. IL-6 and TNFα showed a marked increase in participants who are HAART naïve compared to the other two groups whilst Crp was higher in the HIV positive participants on treatment. However, the levels of IL-6 and Crp were similar (P> 0.05) between the study groups.

Table. 10. Cytokine levels in the three groups of participants

variables	HIV -ve n=63	HIV +ve not on treatment n=54	HIV+ve on treatment n=52	P-value ANOVA
Tnf-α (pg/ml)	106.61± 10.0	161.30±16.73	81.30±10.09	0.468
IL-6(pg/ml)	1.59±2.33	6.70± 18.57	1.90±1.61	0.122
Crp(mg/ml)	3.79±5.48	4.84±6.43	13.14± 3.64	0.122
E-Selectin(ng/ml)	66.73±31.21	76.81± 33.93	70.74± 35.12	0.498

Tnfα Tumour Necrosis factor alpha

IL-6 Interleukin 6

Crp C reactive protein

4.1.7. The value of CD4 count across study group

The CD4 count and white blood results exhibited variabiliy as shown below (Table 11). The CD4 counts of the participants who are HIV negative is the highest followed by those participants who are on treatment ($p<0.001$). The neutrophil count of participants who are on treatment is also the highest($p=0.034$). The lymphocyte counts of the HIV positive participants in both groups are lower than the HIV negative participants ($p=0.013$) (Table 11). However, the values of platelets and monocytes were not significantly different ($p>0.05$) across the groups.

Table 11. CD4 count and blood count results in the three groups of participants

Variables	HIV -ve n=63	HIV +ve not on treatment n=54	HIV+ve on treatment n=52	P-value ANOVA
CD4(cells/cm^3)	946.12±298.09	434.26±307.27	457.85± 255.60	<0.001*
Platelets(gm/L)	280.07± 59.74	280.88± 91.73	275.12± 63.25	0.925
Neutrophills(%)	47.26±9.85	48.74±12.83	53.83±8.25	0.034*
Monocytes(%)	7.99± 2.29	8.84± 2.33	7.96± 2.22	0.157
Lymphocytes(%)	41.23± 8.98	38.26±12.81	38.70±10.54	0.013*

The PWV and other cardiac functions as shown exhibited marked significant differences across the study group (Table 12). The ED% was lowest n the HIV negative participants and highest in those that are not on treatment. The AIx(75) is lowest in the HIV negative participants and highest in those that are on treatment. The SEVR% is highest in the HIV negative participants and lowest in HAART naïve participants. The PWV is highest in HIV participants who are HAART naïve but lowest in HIV negative participants. Post hoc there was stastical difference in the levels of AIx(75) between HIV negatives and HIV positive participants on treatment (p= 0.025) whilst there was a significant differece in the values of SEVR in HIV positive on treatment and HIV negative participants (p= 0.06). PWV was stastically significant between treatment naïve participants and HIV negatives (p=0.033).

Table 12. PWV and other cardiac functions in the three groups of participants

variables	HIV -ve n=63	HIV +ve not on treatment n=54	HIV+ve on treatment n=52	P-value ANOVA
ED(%)	33.71±3.80	36.31± 3.56	36.15± 4.431	0.001*
AIx(75)	15.56±13.20	19.92± 12.20	21.87± 12.26	0.024*
SEVR(%)	176.16±29.55	155.04± 24.55	159.80±28.559	0.000*
PWV(m/s)	6.38 ±1.67	7.21± 2.17	6.84±1.17	0.037*

ED Ejection duration

AIx(75) Augmentation index at a heart rate of 75 beats per minute

SEVR Subendocardial viability ratio

PWV Pulse Wave velocity

4.1.8. Pulse wave velocity (PWV)

The box plot (figure 31) below demonstrated that the mean of the Pulse Wave Velocity of those participants who are HIV negative is 6.38 , those participants who are d HIV positive and not on treatment have a mean value of 7.21 and those on treatment have a mean value of 6.84. This is significant ($P<0.05$). The high pulse wave velocity of the antiretroviral naïve group is clearly shown in this box plot. Post hoc the PWV of the HIV negatives was significantly different from that of the treatment naïve HiV participants ($p=0.033$).

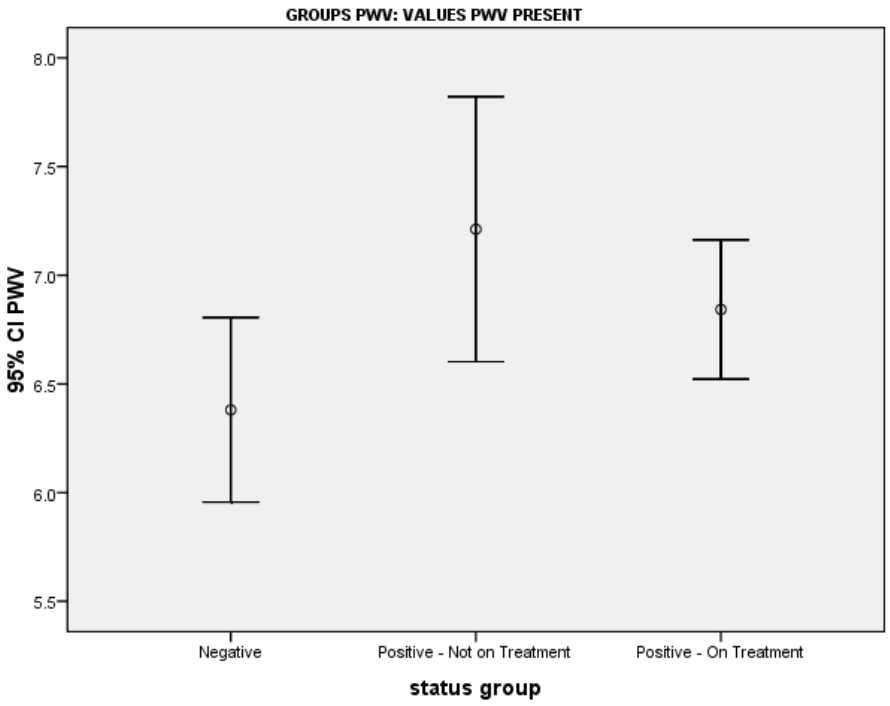

Figure 31. PWV of the three study groups using a box plot.

4.1.9. The augmentation index in the three groups of participants

The ratio of augmentation and central pulse pressure (difference between central systolic and diastolic pressure) varied significantly ($p<0.05$) across the groups (Figure 32). The augmentation index which is also a measure of arterial stiffness is greatest in the HIV positive partcipants on antiretrovirals. Post hoc this is significant between those on treatment and and HiV negatives ($p=0.025$).

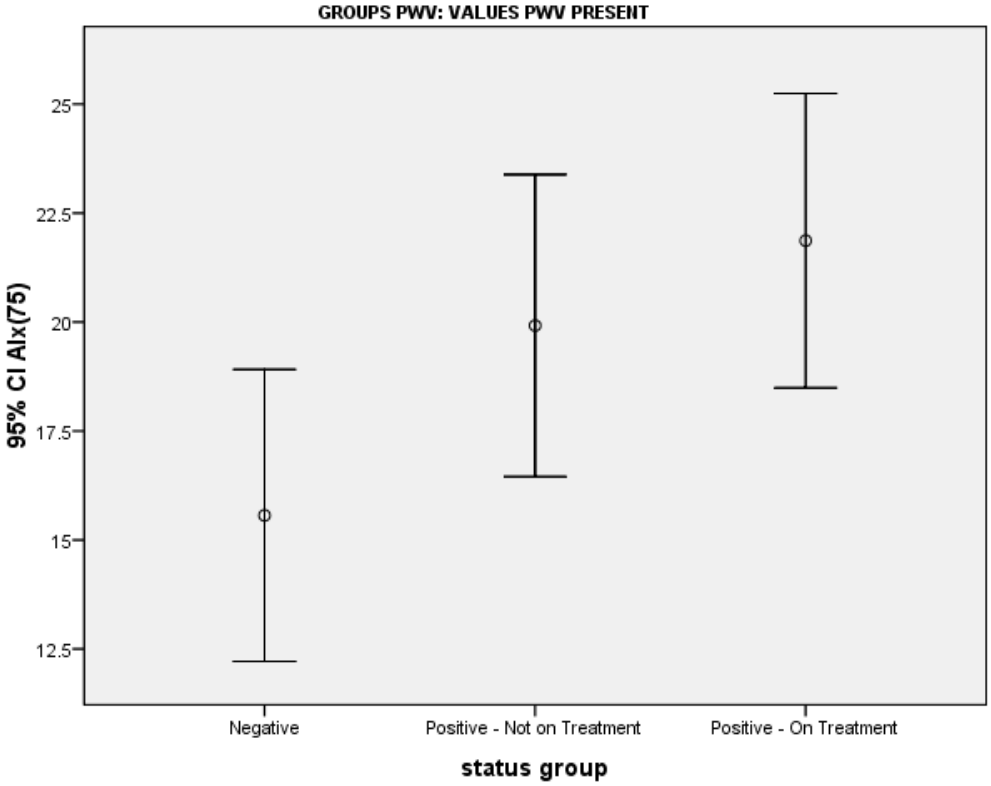

Figure 32. Augmentation index in the three groups of participants.

4.1.10. Waist circumference

Figure 33. shows the variations of waist circumference by the study groups.The highest level of waist circumference was in positive participants on HAART but this was not significant (P>0.05).

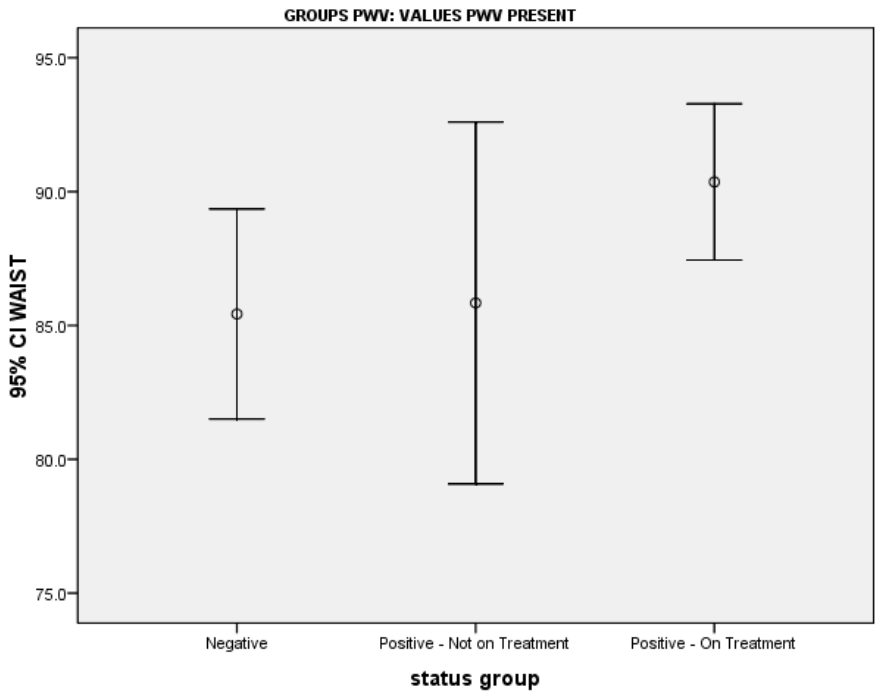

Figure 33. The waist circumferences in the three groups of participants.

4.1.11. Waist to hip ratio

Waist to hip ratio is highest in the group on treatment (figure 34). The highest, intermediate and lowest levels of the waist to hip ratio were in HIV positives on HAART; negatives; and in positives not on HAART ($p<0.05$) respectively.The values are not significant ($p>0.05$).

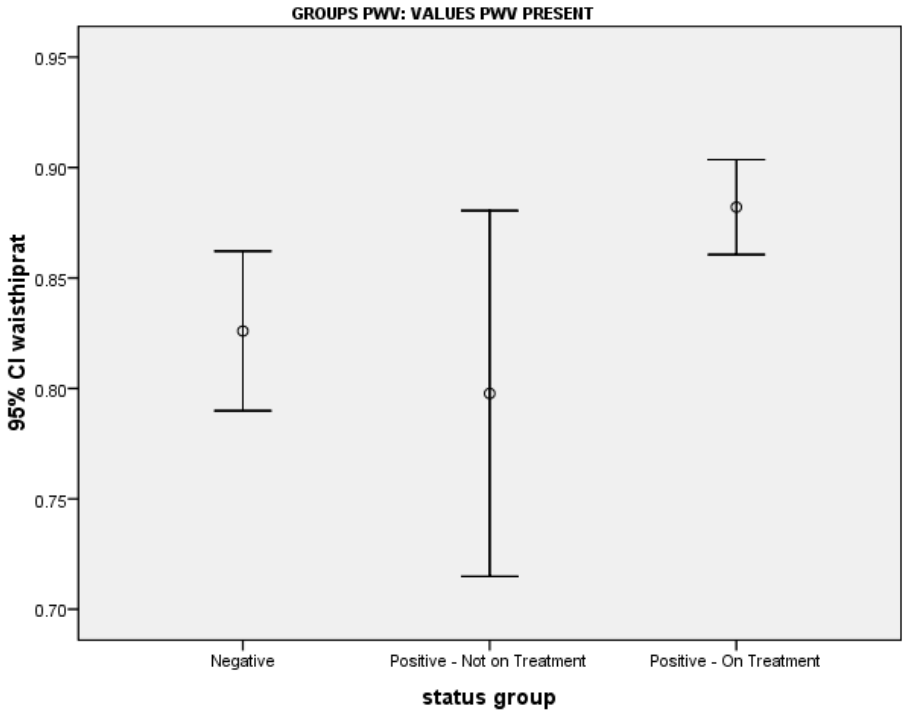

Figure 34. The waist to hip ratio in the three groups of participants.

4.1.12. Systolic brachial blood pressure (Sbp)

The HIV positive participants on treatment had the highest brachial systolic blood pressure (Figure 35). The levels of systolic brachial blood pressure across the study groups were not statistically significant ($p>0.05$).

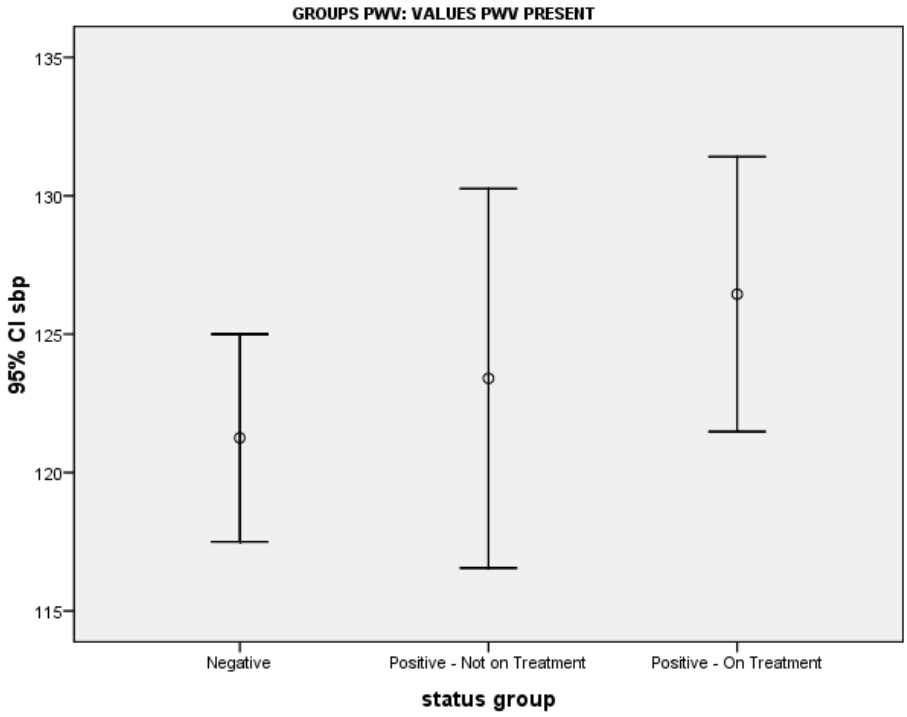

Figure 35. The systolic brachial blood pressure in the three groups.

4.1.13. Systolic aortic (central) blood pressure

This is the blood pressure measured at the level of the aorta when the heart is contracting (Figure 36).The highest level of systolic aortic pressure was amongst the Haart naïve HIV positive participants, compared to the levels in those who are on HAART and those who are HIV negative. The values are not stastically significant ($p>0.05$).

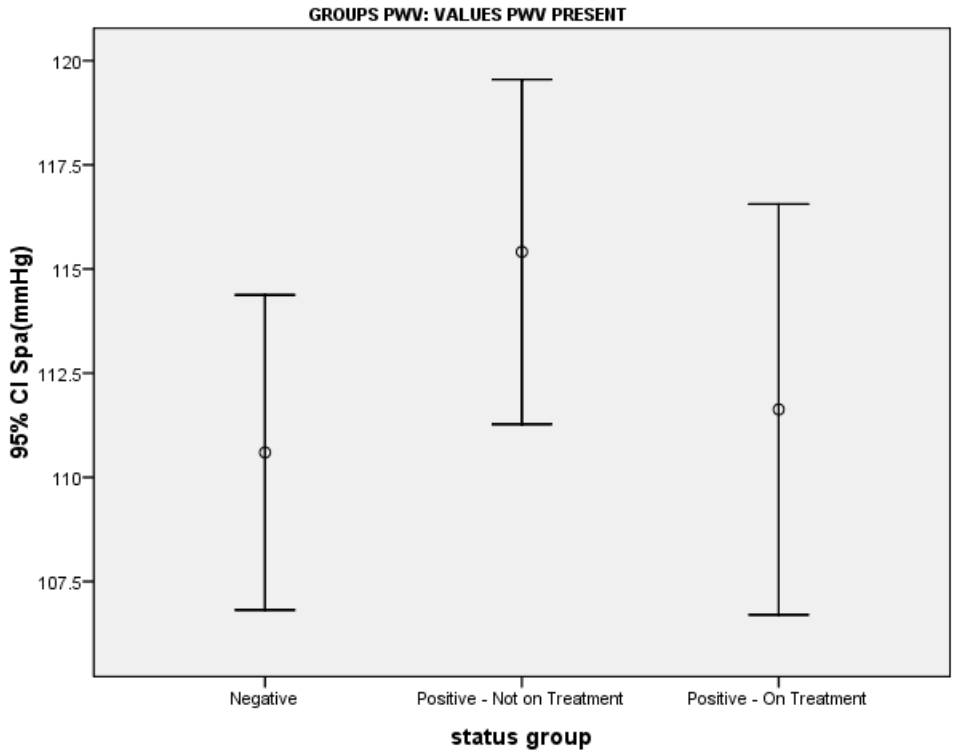

Figure 36. The systolic aortic blood pressure in the three groups.

4.1.14 Diastolic brachial blood pressure

The participants on treatment had the highest diastolic brachial blood pressure (figure 37).There was no statistical difference ($p>0.05$) in comparing the values between the study groups.

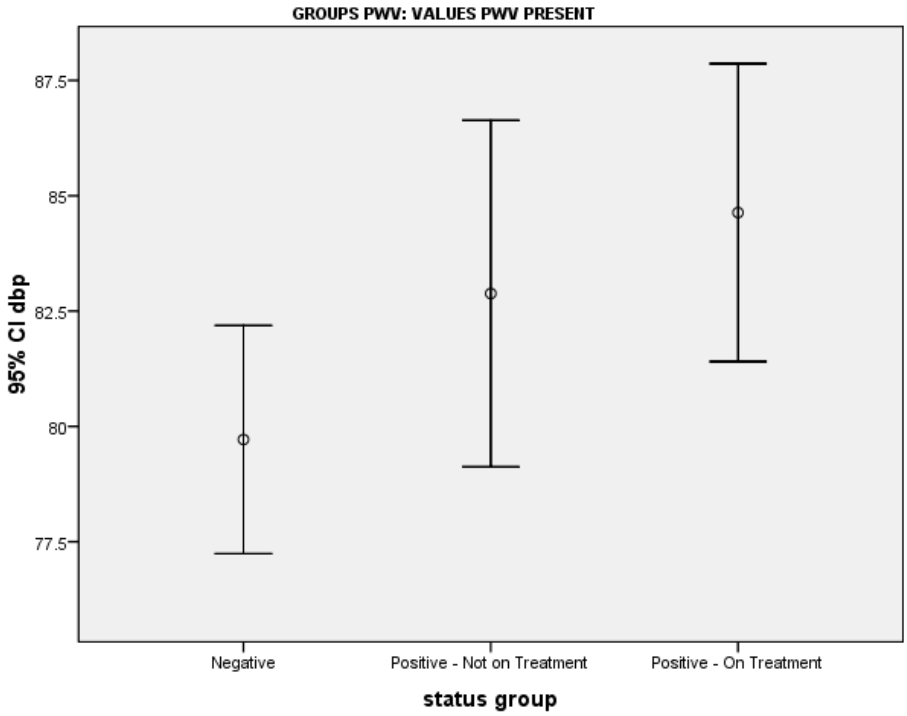

Figure 37. The diastolic brachial blood pressure in the three groups.

4.1.15. Diastolic aortic (central) blood pressure

The diastolic aortic blood pressure was highest in participants who are HIV positive but slightly higher in those who are on treatment (Figure 38). The difference was not statistically significant ($p>0.05$) across the study groups.

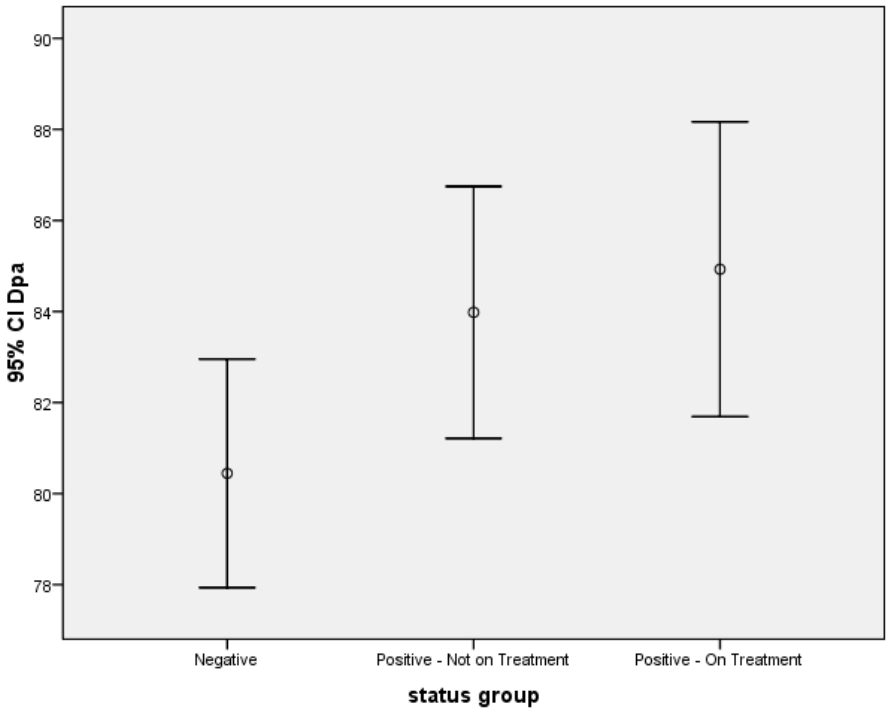

Figure. 38. The diastolic aortic blood pressure of the three groups of participants.

4.1.16. Pulse pressure (brachial)

The brachial pulse pressure was highest in the group on antiretroviral therapy (Figure 39). The difference was statistically not significant (P>0.05) across the study groups.

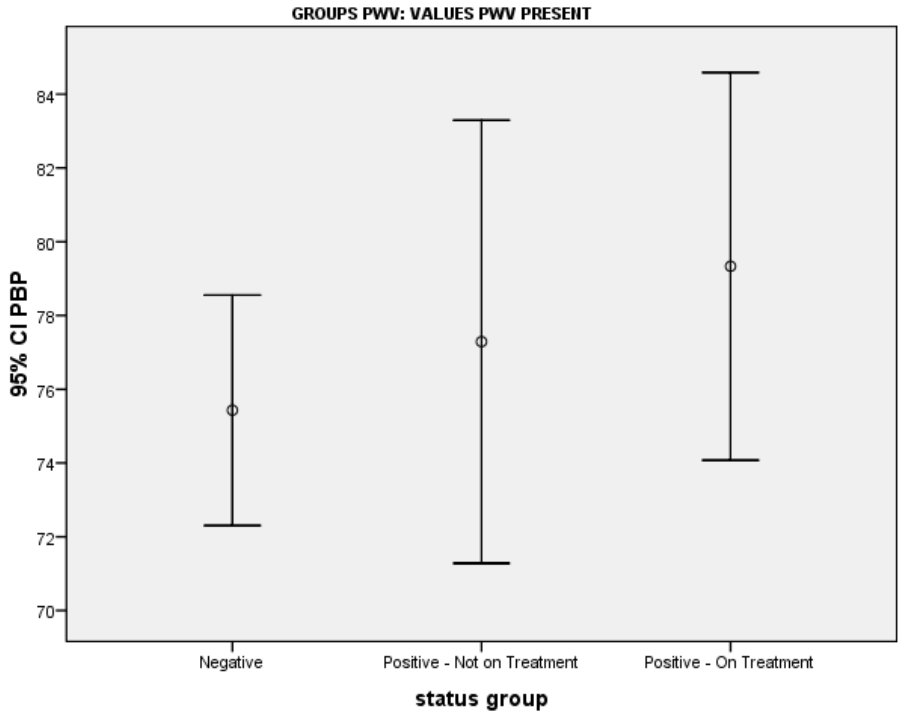

Figure 39. The brachial pulse pressure in the three groups of participants.

4.1.17. Pulse Pressure (aortic)

The aortic (central) pulse pressure is highest in the group of antiretroviral naïve HIV positive participants (Figure 40). Their values were not significant ($p>0.05$) across the study groups.

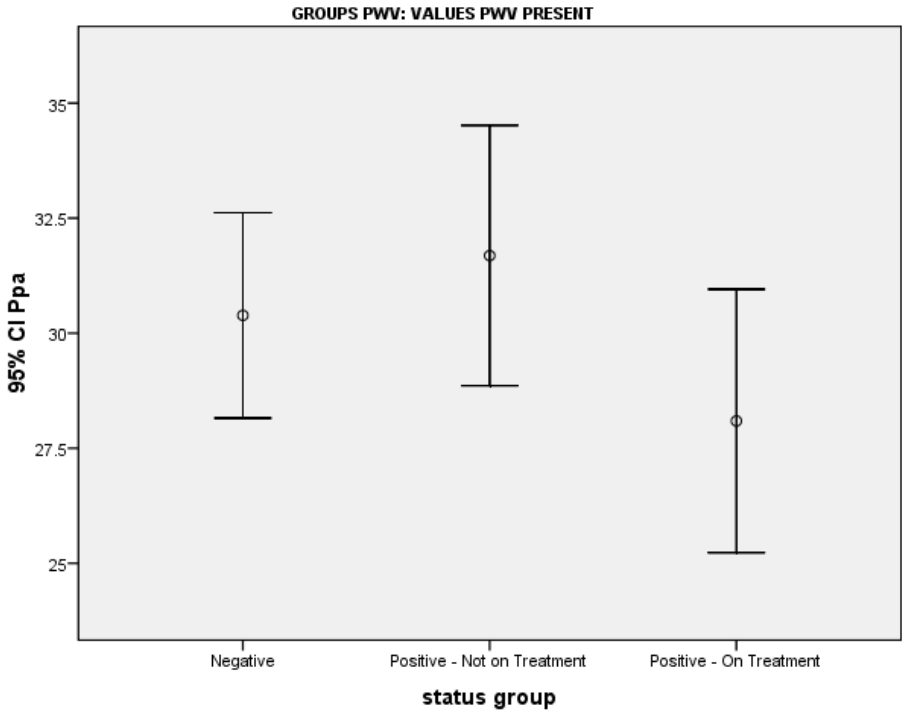

Figure 40. Box plot of aortic (central) pulse pressure in the participants.

4.1.18. Mean arterial pressure (MAP or MP)

MAP is highest in the antiretroviral naïve group (figure 41). The difference was not statistically significant ($p>0.05$) in all the study groups.

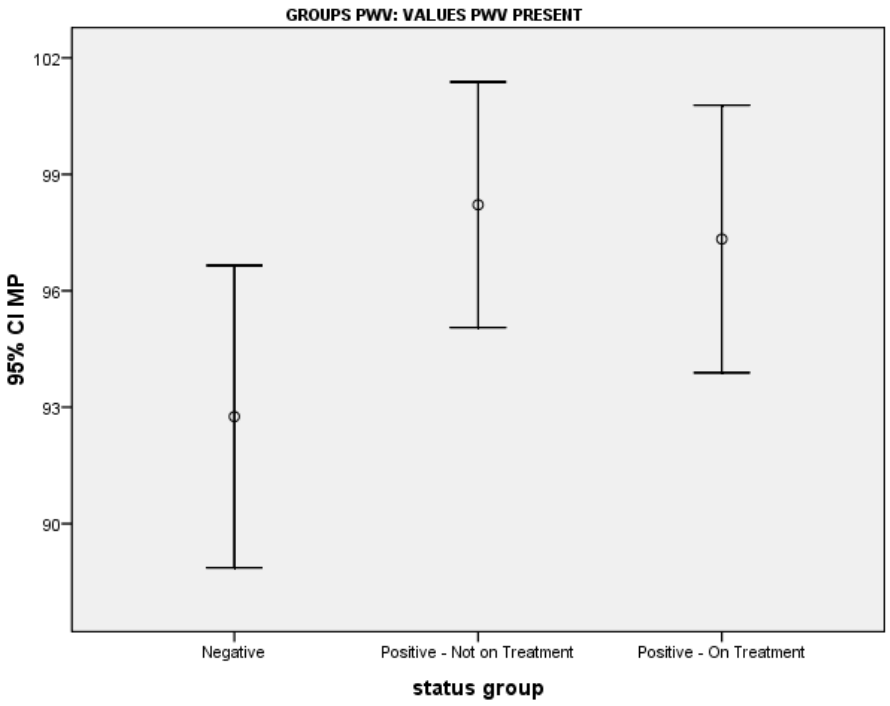

Figure 41. The mean arterial pressure of the three group of participants.

4.1.19. Heart rate

Figure 42. presents a significant and unequal variation of heart rate across the study groups. Post hoc there is a significant difference between participants on treatment and HIV negatives (p=0.034).

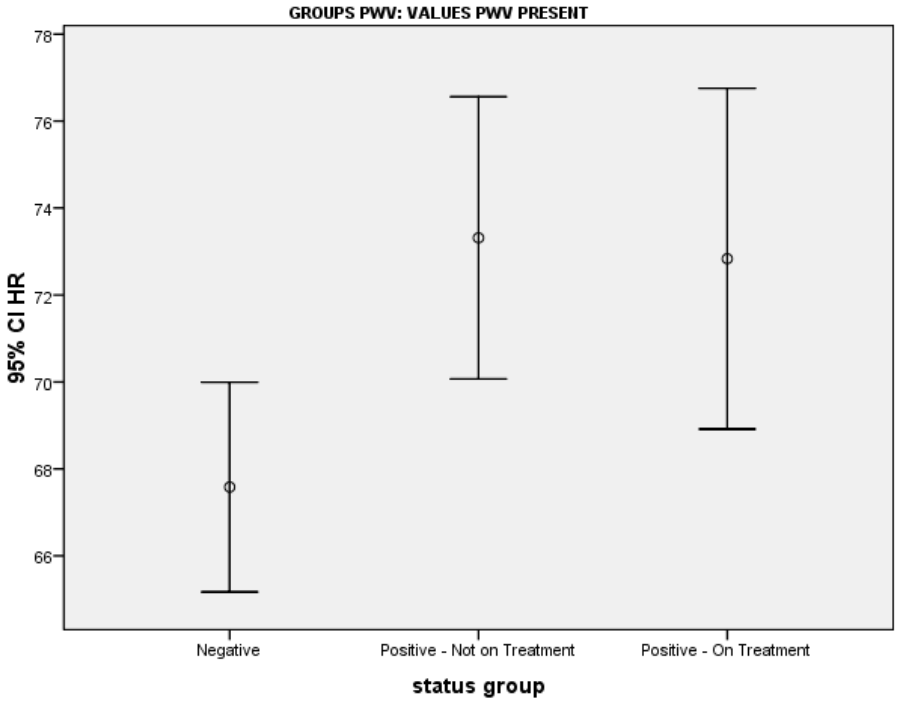

Figure 42. Graph of the heart rates in the three groups of participants.

4.1.20. Ejection duration index (ED %)

The mean values of ED% varied significantly ($p<0.05$) across the study groups. Post hoc, there is significant difference between Negatives and treatment naïve participants ($p<0.002$) and between Negatives and those on treatment ($p<0.003$), (figure 43).

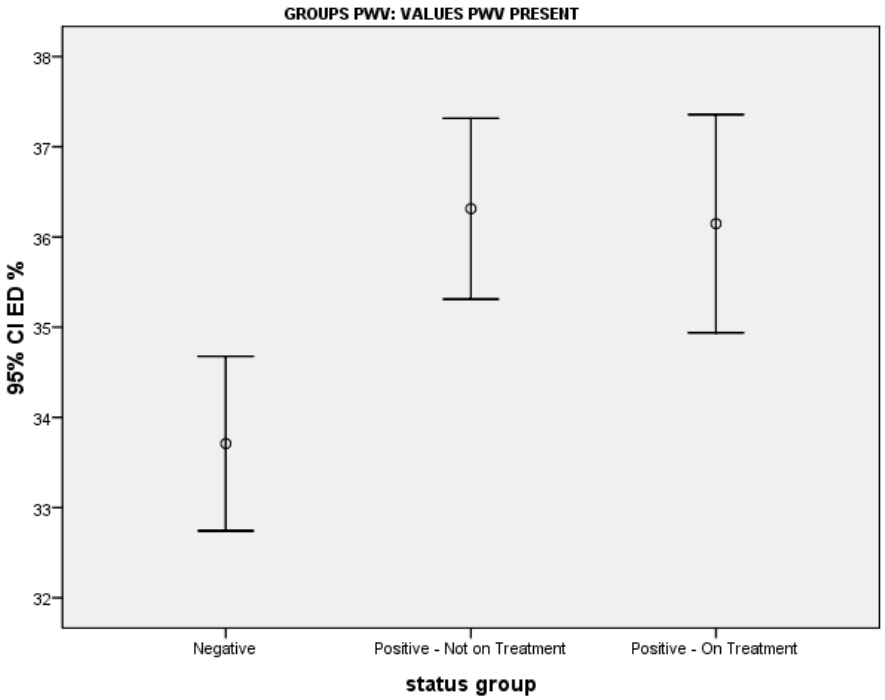

Figure. 43. Ejection duration in the three groups of participants.

4.1.21. Subendocardial viability ratio

The antiretoviral naïve group of HIV positive participants has the lowest values for SEVR%, whilst the HIV negative participants has the highest level (figure 44). These values varies significantly ($p<0.05$) across the study groups. Post hoc there is significant difference between HIV positive participant on treatment and HIV negatives.($p=0.033$).

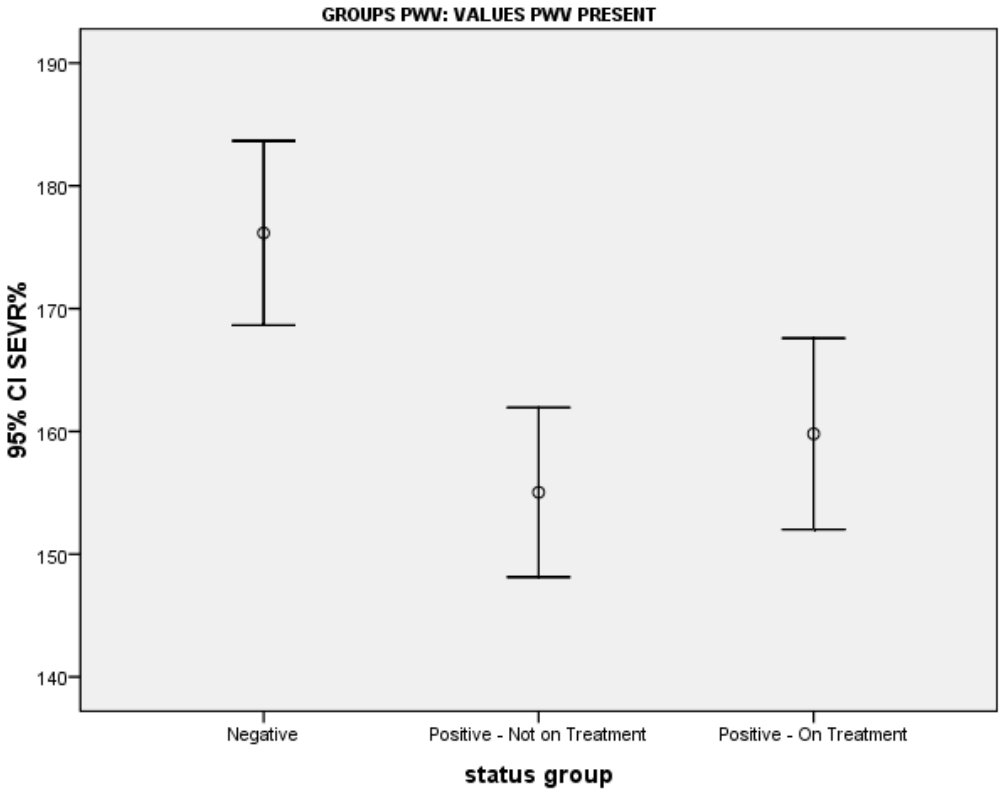

Figure 44. SEVR% in the three study groups.

4.1.22. E- Selectin

Levels in the three groups of participants are shown (Figure 45). E selectin level is highest in the antiretroviral naïve group of HIV positive participants. However, the differences are not statistically significant ($p>0.05$) across the study groups.

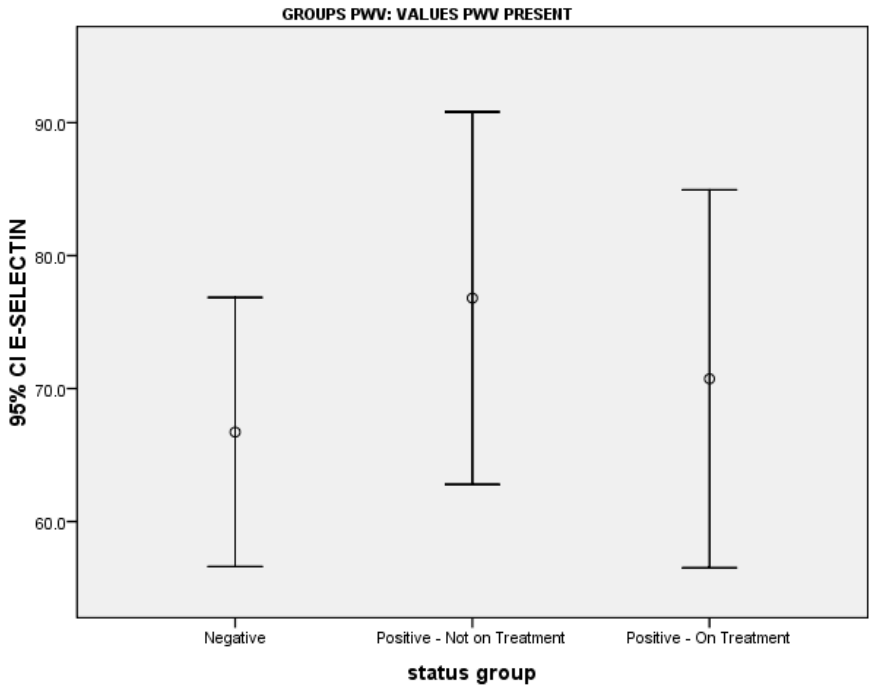

Figure. 45. The E-selectin levels in the three groups of participants.

4.1.23. IL-6

IL-6 levels in the three groups of participants (Figure 46). IL- 6 level is highest in the antiretroviral naïve group. However, the difference across the study groups was not significant ($p>0.05$).

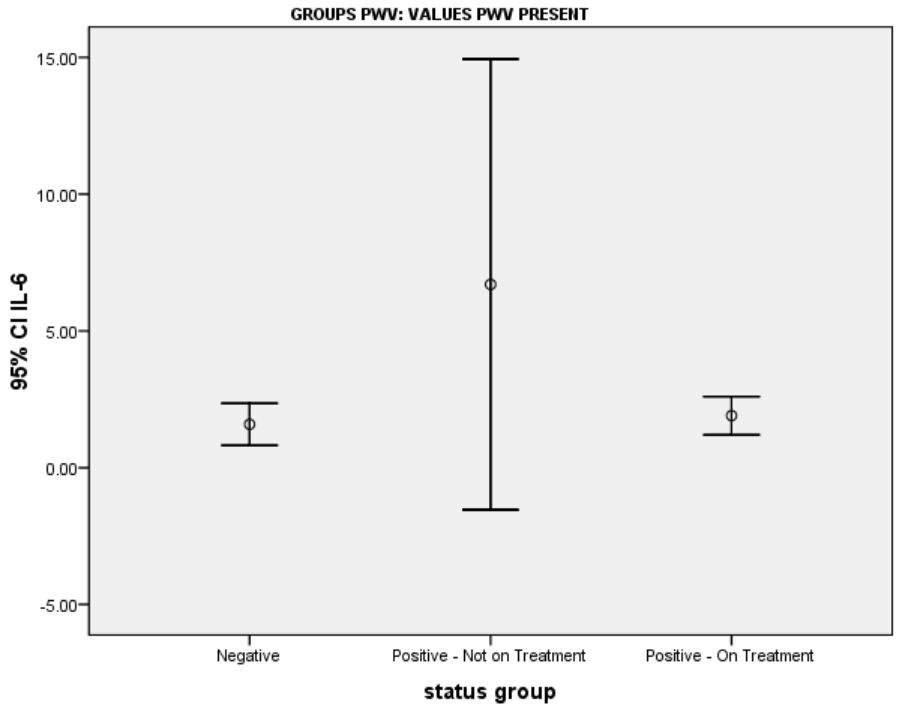

Figure. 46. IL-6 levels in the three groups of participants.

4.1.24. TNFα

TNFα levels in the three gropus of participants (Figure 47). The TNFα level in the HAART naïve group is highest, whereas the difference was not statistically significant ($p>0.05$) across the three groups.

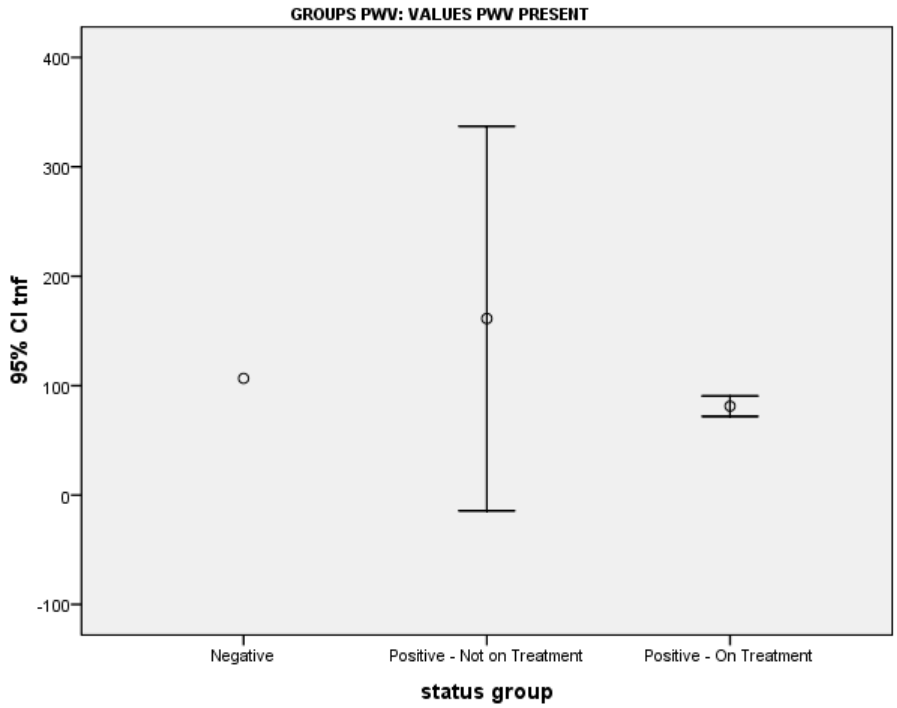

Figure 47. TNFα in the three groups of participants.

4.1.25. Cholesterol

Box plot below depicting the cholesterol levels of the the three groups of participants (Figure 48). The level of cholesterol is highest in HIV positive participants on treatment. Post hoc there is a statistical differece between Negatives and participants on treatment (p=0.036), and between participants who are on treatment and those who are treatment naïve (p=0.006).

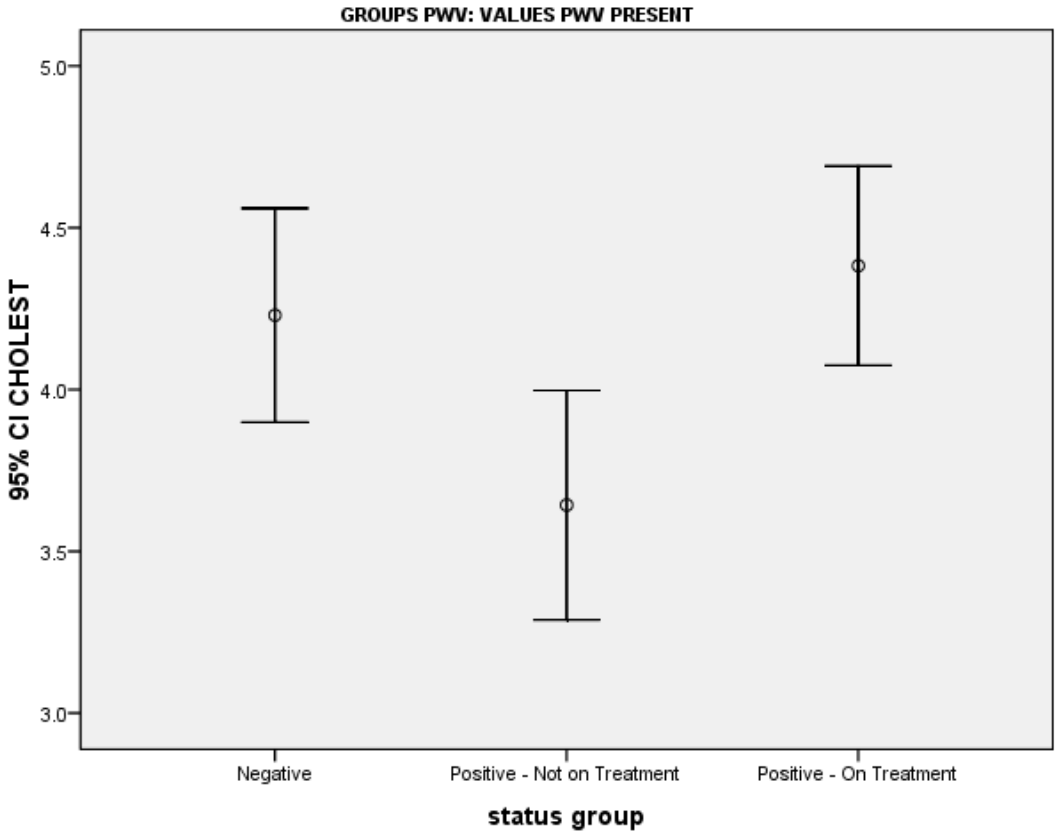

Figure 48. Cholesterol levels in the three groups.

4.1.26. Triglycerides

Triglyceride levels in the three groups of participants (Figure 49). The highest level was in HIV positive participants on HAART but the diffrence is not significant ($p>0.05$).

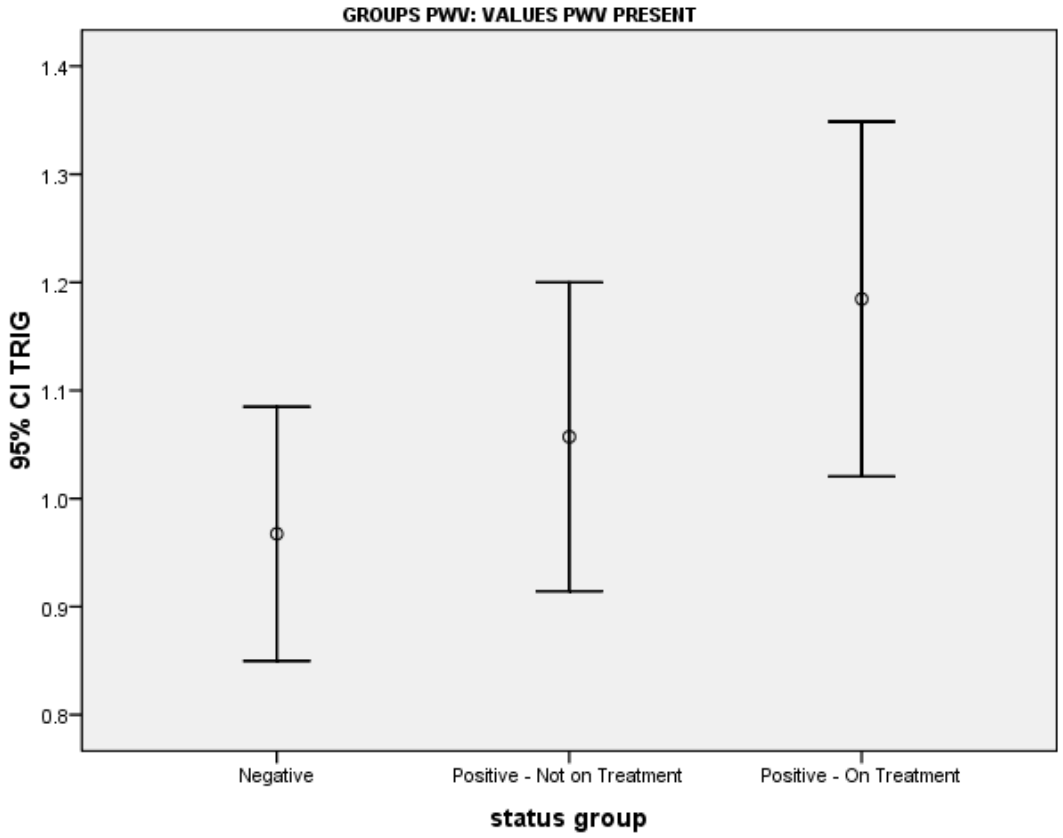

Figure 49. Levels of triglycerides in the three groups of participants.

4.1.27. HDL-C level

The box plot compares the levels of HDL-C in the three groups of paticipants (figure 50). Post hoc there is a stastistical difference between those paricipants who are on treatment and those who are not on treatment (p=0.001).

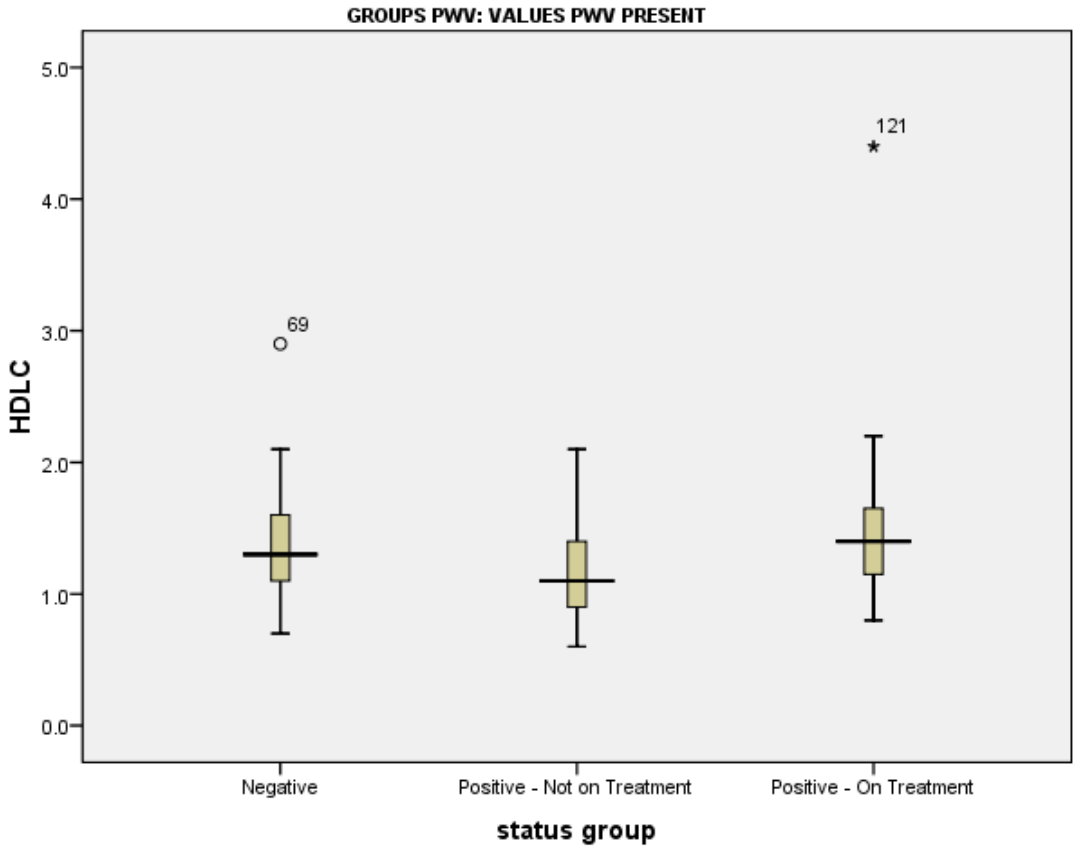

Figure 50. HDL-C levels in the three groups of participants.

4.1.28. LDL-C

The levels of LDL-C is similar ($p>0.05$) in the three groups of participants as shown by the box plot (Figure 51).

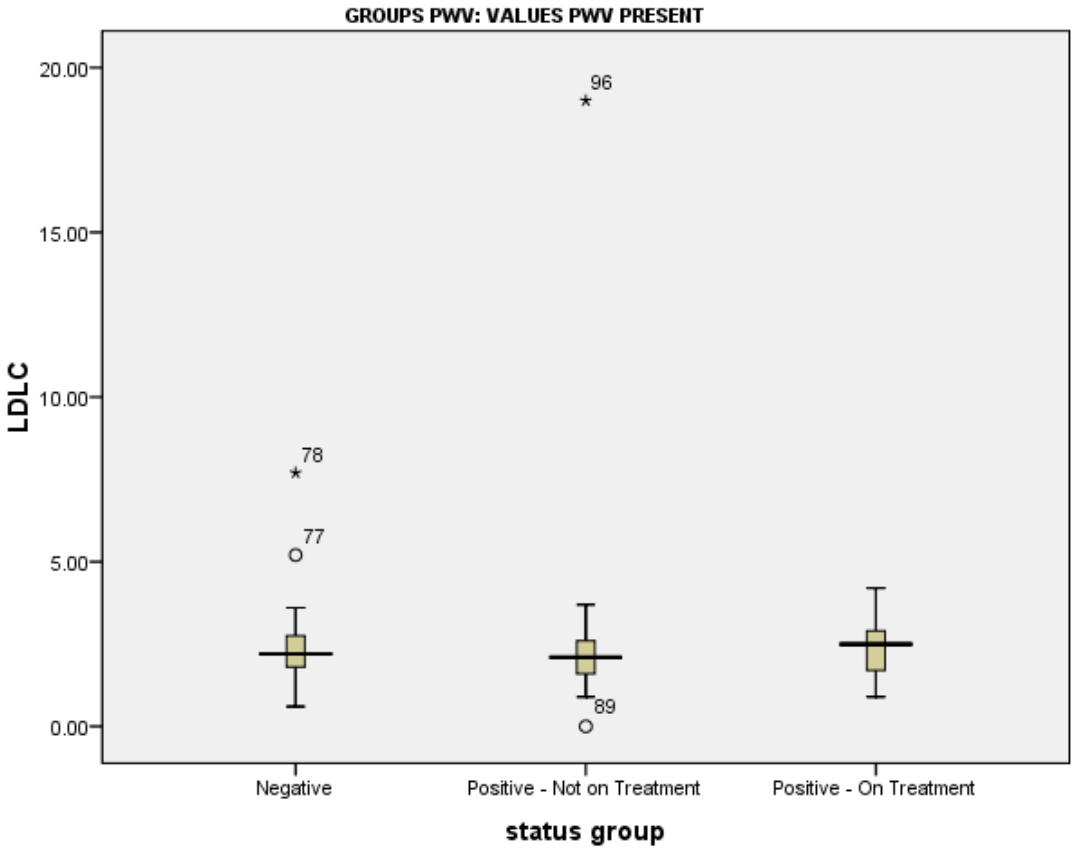

Figure 51. LDL-C levels of the three group of participants.

4.1.29. Monocytes

There is no stastistical difference in the level of monocytes in the three groups of participants ($p>0.05$), with the antiretroviral naïve group having the highest monocyte level (Figure 52).

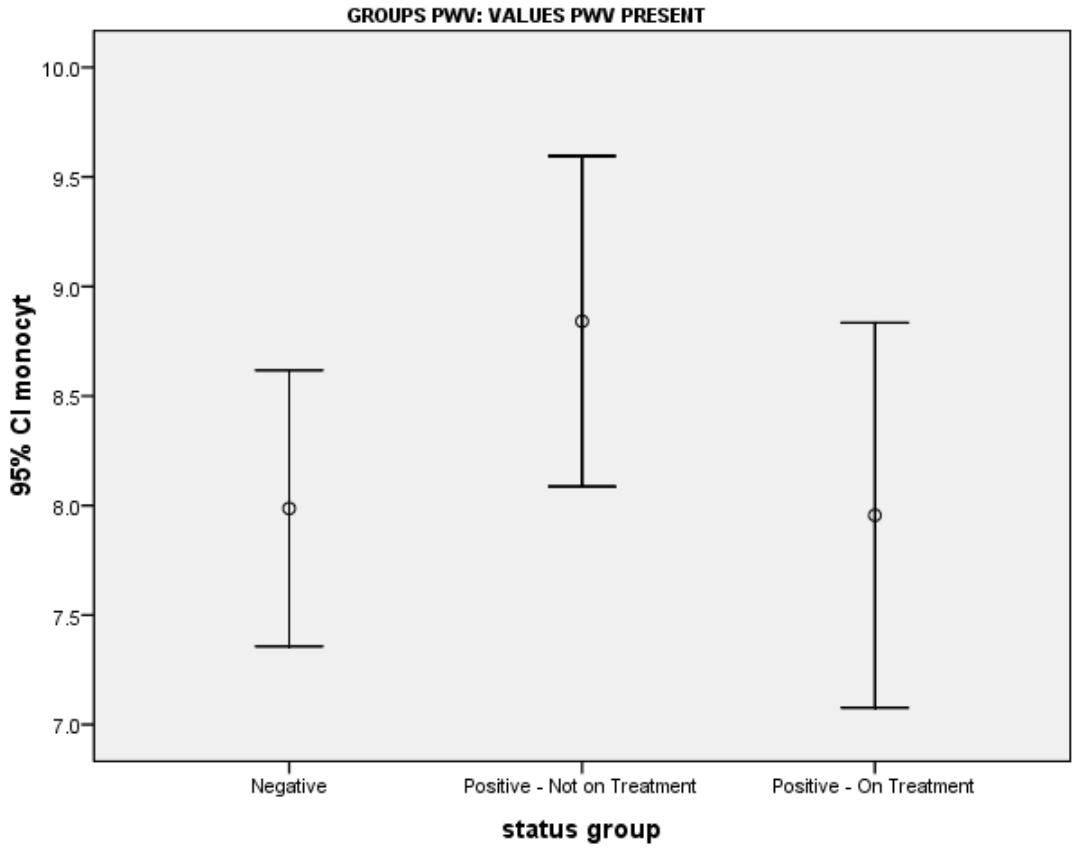

Figure 52. Monocyte count levels in the three groups of participants.

4.1.30. Neutrophils

The values of the neutrophils are compared using a box plot (Figure 53). The netrophil levels of HIV positive participants on treatment are the highest and there is a statistically difference ($p<0.05$) across the study groups. Post hoc there is a sifnificant difference between the Negatives and HIV positive on treatment (p=030).

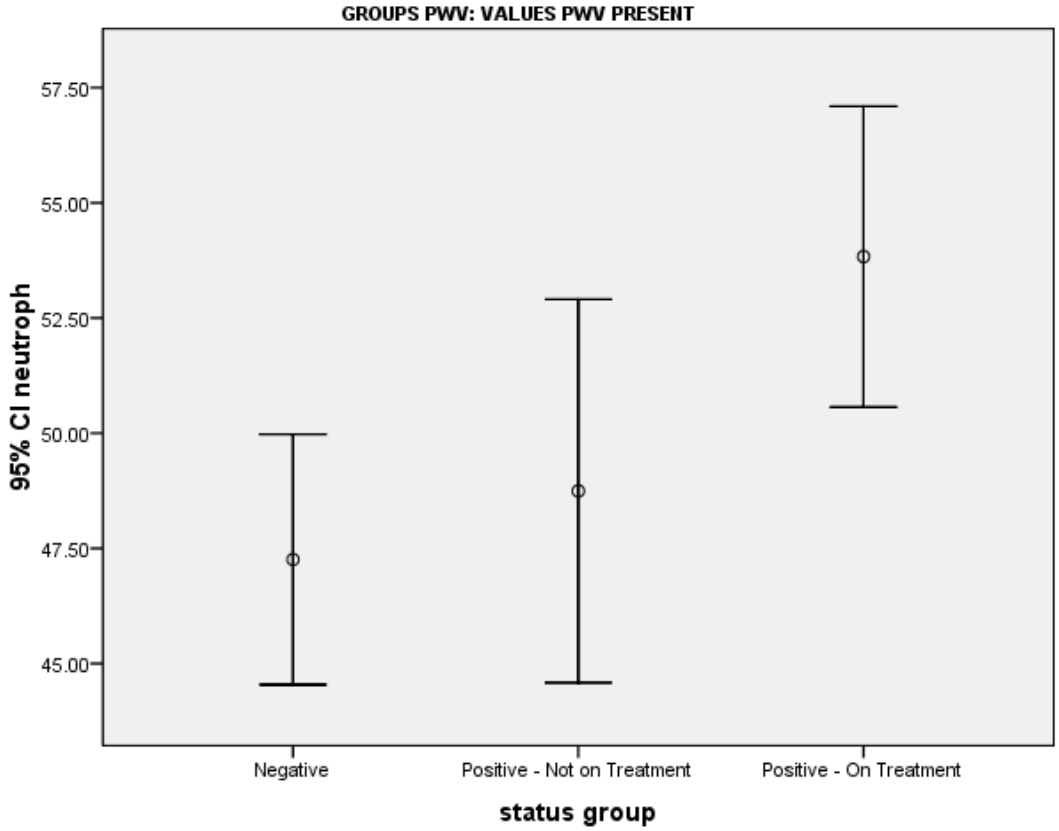

Figure 53. Levels of the Neutrophil count in the three groups of participants.

4.1.31. Platelets

The levels of platelets, was similar ($p>0.05$) across the study groups as shown in figure 54.

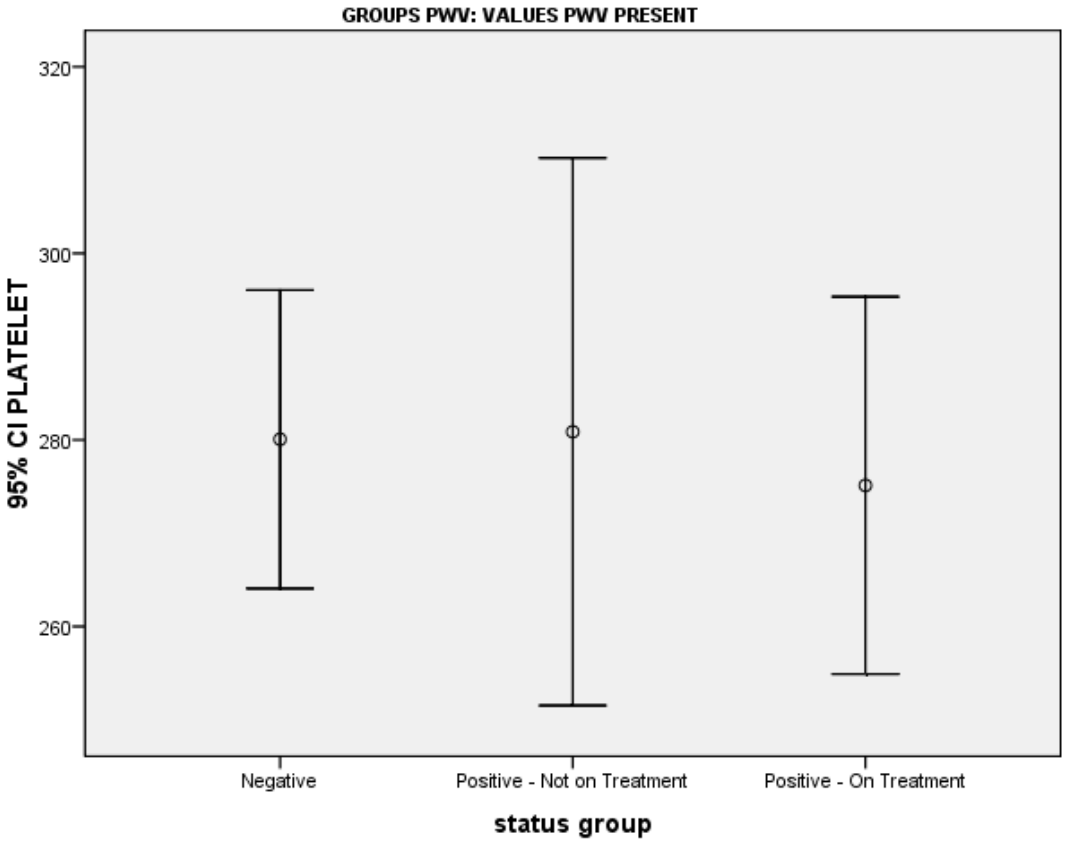

Figure 54. The platelet count in the three groups of participants.

4.1.32. Lymphocytes

Comparing the lymphocyte levels across the three groups of participant, their values varied significantly ($p<0.05$) with the lowest level in positives on HAART (figure 55). Post hoc significant difference is observed between the negatives and participants who are on treatment ($p=0.010$).

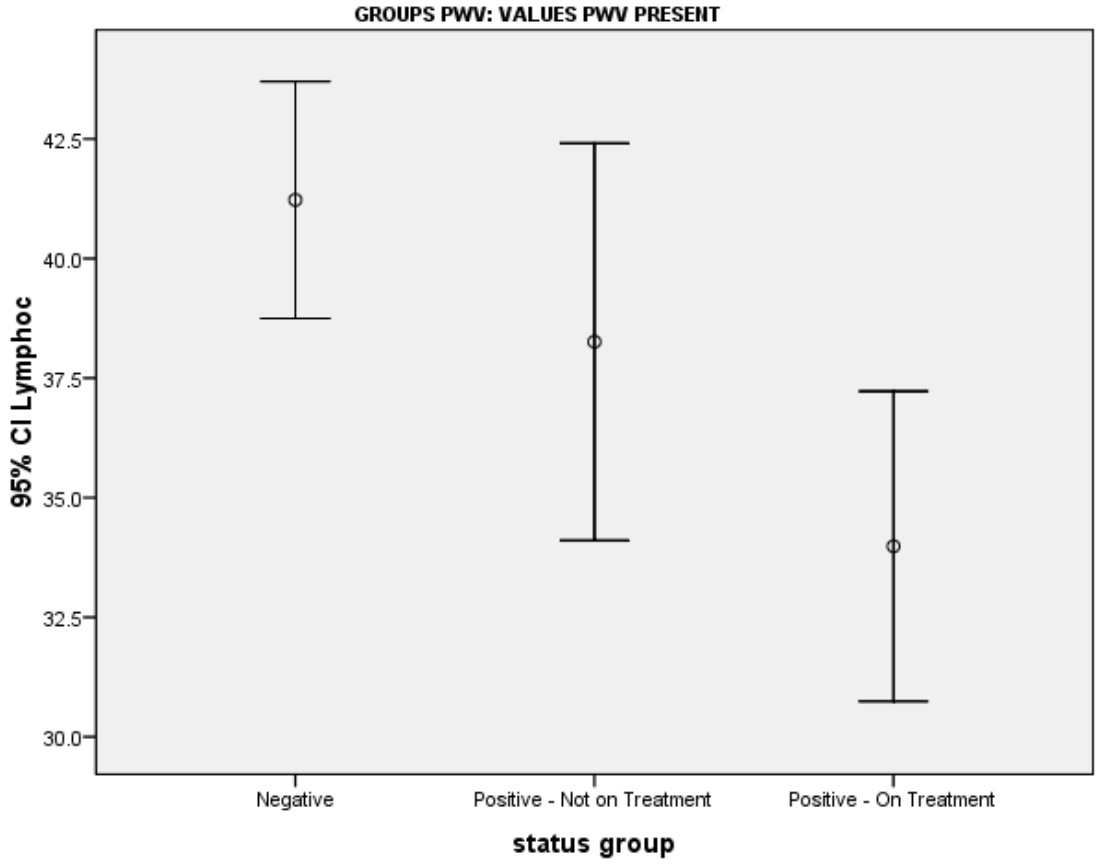

Figure 55. Lymphocyte counts in the three groups of participants.

4.1.33. C-reactive protein (Crp)

The box plot graph was used to demonstrate the lack of significant diffrence ($p>0.05$) for the values of Crp across the study groups (figure 56).

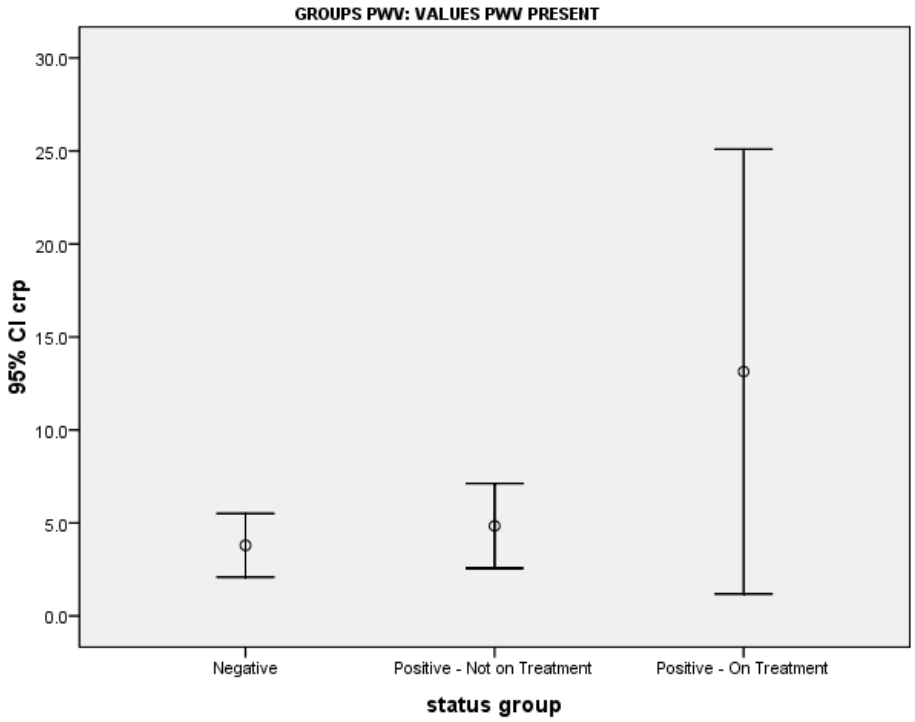

Figure 56. Crp values in the three groups of participants.

4.2. Influence of advanced age in all anthropometric parameters

4.2.1. Anthropometric parameters

High values of waist and waist to hip ratio were significantly associated with advanced age ($p<0.05$) whereas values for weight and height were similar between older and younger participants ($p>0.05$) (Table 13).

Table 13. The means of the age and anthropometric measurements of participants aged below 35 years and above

variables	Age more than 35 years	Age less than 35 years	P-value ANOVA

Age (years)	45.47± 8.55	27.71± 4.38	0.000*
smoke cigarettes(n)	1.87± 0.34	6.88 ±13.85	0.334
weight(Kgm)	72.25± 18.47	62.90± 8.00	0.217
height(cm)	164.26± 8.14	185.95± 7.98	0.313
WC(cm)	92.68± 15.87	82.95± 14.39	0.000*
WHR(%)	0.88 ±0.07	0.81± 0.14	0.001*

WC waist circumference

WHR waist to hip ratio

4.2.2. Haemodynamic parameters according to age groups

The comparisons of the mean values of haemodynamic data. The comparisons of the mean values of haemodynamic data according to age groups are presented for all participants in Table 14. The levels of brachial pulse pressure and HR are similar ($p>0.05$) between participants with age>35 years and those with age <35 years while the values of Sbp,Dbp,Spa,Dpa,MP,and Ppa are significant ($p<0.005$) in participants with advanced age than those from participants aged less than 35 years.

Table 14. The means of the aortic and brachial blood pressure of participants aged below 35 years and above.

Variables	Age more than 35 years	Age less than 35 years	P-value ANOVA

Sbp(mmHg)	127.78±20.10	120.30±15.13	0.007*
Dbp(mmHg)	84.82± 10.58	80.31± 10.97	0.012*
Pbp(mmHg)	78.54±19.74	76.36±12.88	0.403
Spa (mmHg)	116.37±18.74	109.35±12.36	0.003*
Dpa(mmHg)	84.55±11.21	81.58±9.94	0.050*
MP(mmHg)	99.04± 12.42	93.68 ±13.43	0.006*
Ppa(mmHg)	32.77± 12.00	27.77±6.95	0.001*
HR(beats/secs)	71.08± 13.96	70.91±10.56	0.934

Sbp bracial systolic blood pressure

Dbp brachial diastolic blood pressure

Pbp brachial pulse pressure

Spa Aortic (central) blood pressure

Dpa Aortic (central) diastolic bloood pressure

MP Mean aortic (central) blood pressire

Ppa Aortic (central) blood pressure

HR heart rate

4.2.3. Cytokines

There was not a significant association ($p>0.05$) between the levels of TNFα ,IL-6, E-Selectin and aging (Table 15)

Table 15. The means of some cytokine levels of participants aged below 35 years and above 35 years.

variables	Age more than 35 years	Age less than 35 years	P-value ANOVA

Tnfα(pg/mL)	87.25 ±16.48	171.64± 184.69	0.185
IL-6(pg/mL)	2.58± 2.46	3.22± 12.31	0.755
E-Selectin(ng/mL)	77.88 ±36.75	65.37± 29.51	0.091

Tnfa Tumour necrosis alpha

IL-6 Interleulin 6

4.2.4. Immmunity and haematological data

Table 16. summarizes the mean values of CD4 count, platelets, neutrophils, monocytes and lymphocytes according to age groups. The influence of advanced age is neutral and indifferent ($p>0.05$) with monocytes and with CD4 ($p>0.05$). Platelets, neutrophils and lymphocytes has significant value ($p<0.05$) between the two groups of participants. The values of CD4 count and lymphocytes are lower in participants with advance age compared to participants who are less than 35 years, whereas the levels of platelets and neutrophils of participants aged more than 35 years are higher than those aged less than 35 years. The levels of monocytes in the two groups are almost the same.

Table 16. The means of CD4 and blood counts of participants aged below 35 years and above 35 years.

variables	Age more than 35 years	Age less than 35 years	P-value ANOVA

CD4(cells/cm^3)	505.54±358.29	743.82±362.80	0.059
Platelets(gm/L)	290.10±86.32	269.34± 53.86	0.018*
Neutrophils(%)	52.65±10.39	47.11±10.59	0.018*
Monocytes(%)	8.32 ±2.53	8.26±2.17	0.793
Lymphocytes(%)	35.81± 10.58	40.74± 10.10	0.016*

4.2.5. Cardiac functions using the Sphygmocor

The AIx(75) and PWV in participants older than 35 years are higher ($p<0.05$) than those aged less than 35 years, whereas the levels of ED% and SEVR% were similar ($p>0.05$) between both examined participants according to age groups(Table 17).

Table 17. The means of PWV and some cardiac functions using some spygmocor variables in participants aged below 35 years and above 35 years.

Variables	Age more than 35 years	Age less than 35 years	P-value ANOVA

ED (%)	35.30± 3.99	35.30±4.21	0.888
Alx (75)	24.52± 11.06	14.34 ±12.22	0.000*
SEVR (%)	162.71± 26.72	165.7±30.95	0.420
PWV(m/s)	7.46± 2.15	6.2±1.02	0.000*

ED(%) Ejection duration index

AIx(75) Augmentation index at a heart rate of 75 beats per minute

SEVR(%) Subendocardial viability ratio

PWV(m/s) Pulse Wave Velocity in metres per second

4.3. Influence of gender

4.3.1. Anthropometric data

There was no significant ($p>0.05$) influence of gender on the variations of age, weight, waist, BMI and WHR, while females were shorter ($p<0.05$) and heavier ($p<0.05$) by BMI levels than males (Table 18).

Table 18. The means of age and anthropometric characteristics in the male and female groups of participants

Variables	Males	Females	P-value

Age (years)	35.53± 12.08	35.02± 10.49	0.773
weight (kgm)	69.73± 12.83	70.855±17.81	0.671
height(cm)	170.00±7.501	159.75±5.64	0.000*
WC(cm)	84.24± 11.95	88.96± 17.66	0.071
HC (cm)	97.64± 10.42	107.17±12.78	0.000*
BMI (kgm/m^2)	24.11± 4.07	30.18± 24.96	0.069
WHR(%)	0.87± 0.07	0.84± 0.13	0.320

WC waist circumference

HC hip circumference

WHR waist to hip ratio

BMI body mass index

4.3.2. Haemodynamic data in males and females

The mean values of Sbp,Dbp,Spa,Dpa,HP,Ppa,and HR did not vary($p>0.05$) between males and females (Table 19).

Table 19. The means of the aortic and brachial blood pressure measurements in both male and females participants

Variable	Males	Females	P-value

Sbp(mmHg)	126.22±18.34	121.96± 17.58	0.151
Dbp(mmHg)	81.42± 10.64	82.78± 11.39	0.459
Pbp(mmHg)	74.91± 16.79	78.60± 15.72	0.177
Spa (mmHg)	112.89± 14.94	111.99± 16.48	0.725
Dpa(mmHg)	82.81± 10.86	82.79± 10.65	0.990
MP(mmHg)	96.58± 11.77	95.50± 14.28	0.613
Ppa(mmHg)	30.08± 8.84	29.85± 10.35	0.883
HR(b/secs)	69.53± 11.80	71.88 ± 12.13	0.223

Sbp bracial systolic blood pressure
Dbp brachial diastolic blood pressure
Pbp brachial pulse pressure
Spa Aortic (central) blood pressure
Dpa Aortic (central) diastolic bloood pressure
MP Mean aortic (central) blood pressire
Ppa Aortic (central) blood pressure
HR heart rate

4.3.2. Lipid profile in males and females

Table 20 presents no significant variations ($p>0.05$) in LDL-C and total cholesterol but significant ($p<0.05$) variations of HDL-C and triglycerides between males and females. Females had lower levels of HDL-C and triglycerides than males.

Table 20. The means of the lipid profiles between male and female groups in the participants

Variable	Male	Female	P-value

LDL-C (mmol/l)	2.58± 2.57	2.32± 1.02	0.441
HDL-C (mmol/l)	1.49± 0.77	1.27± 0.32	0.027*
TG (mmolo/l)	1.17± 0.48	0.99± .43	0.026*
TC (mmol/l)	4.20± 1.02	4.02±1.15	0.372

TG Triglycerides.

TC Total cholesterol

LDL-C Low density lipoprotein cholesterol

HDL-C High density lipoprotein cholesterol

4.3.3. Cytokine values in males and females

There were no significant difference ($p>0.05$) in the levels of TNFα, IL-6, E-selectin and Crp in both males and females (Table 21).

Table 21. The means of some cytokine levels between male and female participants.

Variables	Males	Females	P-value
Tnfα (pg/mL)	82.95± 16.50	131.17± 130.83	0.487
IL-6(pg/mL)	4.18± 14.56	2.07± 2.06	0.332
E-Selectin(ng/mL)	77.26± 33.77	65.74± 31.58	0.098
Crp (mg/L)	4.58± 6.44	8.91± 7.45	0.301

Tnfa Tumour necrosis factor alpha

IL-6 Interleukin 6

E-Selectin

Crp C-reactive protein

4.3.4. Immunity and haematological data in males and females

In Table 22, there were no significant differences (P>0.05) in the values of CD4 count, platelets, lymphoctes and neutrophils in both males and females while monocytes were lower significantly (P<0.001) in females than males.

Table 22. The means of the CD4 count and blood counts between male and female participants

Variables	Male	Female	P-value
CD4count(cells/mm^3)	680.13±391.20	621.22±369.41	0.398
Platelets $x10^9$(gm/L)	283.42± 77.48	283.05±66.79	0.441
Lymphocytes (%)	36.89± 11.42	39.94 ±9.78	0.120
Neutrophils (%)	49.12± 10.16	48.12±9.60	0.962

Monocytes (%)	9.17± 2.43	7.60 ±1.97	<0.000*

4.3.5. Cardiac functions in males and females

Compared with males, females had similar levels of PWV (P>0.05), higher levels of ED% and AIx(75), (P<0.05), but lower values for SEVR% (Table 23).

Table 23. The means of PWV and some sphygmocor variables between male and female participants

Variables	Males	Females	P-value
ED (%)	33.73± 4.16	36.15± 3.81	< 0.000*
AIx (75)	14.64± 11.78	21.15 ±12.81	0.001*
SEVR (%)	177.10±31.02	157.52±25.47	<0.000*
PWV(m/sec)	6.99± 1.64	6.65± 1.77	0.220

ED(%) Ejection duration index

AIx (75) Augmentation index at a heart rate of 75 beats per minute

SEVR Subendocardial viability ratio

PWV Pulse wave velocity in metres per second

4.4. Bivariate (Univariate) analysis

4.4.1. Simple correlations between age, anthropometric measurements and PWV

Table 24, shows correlations between ages, anthropometric parameters, number of cigaretes smoked per day and PWV in each study group. In HIV –negatives, there is a significant (P<0.05) positive correlation between age, number of cigarettes, weight, waist circumference and PWV, but a (P>0.05) negative correlation between height and PWV. In HIV positive not on HAART there is a significant (P<0.05) positive correlation between weight, waist and PWV. With positives on HAART there is a significant positive correlation between age WHR and PWV and anegative correlation with height. On the other hand the other variables in each group were not significantly correlated with PWV (P>0.05).

Table 24. Simple linear regression analysis of PWV with biometry by the study groups

variables	HIV-ve		HIV+ve not on treatment		HIV+ on treatment	
	r	p-value	r	p-value	r	P-value

Age(years)	0.606	<0.000*	0.269	0.057	0.641	<0.000
HC(cm)	0.086	0.512	0.012	0.389	-0.033	0.816
BMI(Kgm/m^2)	0.164	0.202	0.195	0.229	0.069	0.629
weight(kgm)	0.123	0.003*	0.439	0.004	0.096	0.495
WHR(%)	0.479	0.585	0.211	0.345	0.319	0.037*
Cigarettes(smk/day)	0.071	0.015*	0.094	0.439	0.024	0.866
height(cm)	-0.012	0.928	0.046	0.770	-0.399	0.003
waist(cm)	0.252	0.050*	0.406	0.008	0.185	0.185

BMI Body mass Index

HC Hip circumference

WHR Waist to Hip ratio

4.4.2. Haemodynamic correlates of PWV by the study groups

The haemodynamic parameters were differently correlated with PWV across the study groups (Table 25). In HIV negatives and except for HR ($P>0.05$) there is a significant ($P<0.05$) correlation between Ppa, MP,Spa, Dbp,and PWV. In HIV positive participants not on HAART and HIV positive participants on HAART Ppa, MP and Spa are significantly and positively correlated with PWV, respectively. In both HIV positive groups, HR and Dbp are not significantly ($P>0.05$) correlated with PWV.

Table 25. Correlations between haemodynamic data and PWV

variables	HIV-ve		HIV+ve not on treatment		HIV+ on treatment	
	r	P-value	r	P-value	r	P-value
HR(b/min)	0.075	0.561	0.212	0.173	0.057	0.693
Ppa (mmHg	0.472	<0.000	0.338	0.012	0.338	0.012
MP(mmHg)	0.446	<0.000	0.515	<0.000	0.400	0.003
Spa (mmhg)	0.635	<0.000	0.369	0.012	0.369	0.006
Dbp(mmHg)	0.436	<0.000	0.114	0.423	0.114	0.413

Dbp brachial diastolic blood pressure

Spa Aortic (central) blood pressure

MP Mean aortic (central) blood pressire

Ppa Aortic (central) blood pressure

HR heart rate

4.4.3. Lipid correlates of PWV

Only total cholesterol in HIV negatives and triglycerides in HIV positives not on HAART are significantly and positively correlated with PWV ($P<0.05$), whereas the other lipid parameters are not significantly ($P>0.05$) correlated with PWV in each study group (Table 26).

Table 26. Correlation between lipid profile and PWV

Variables	HIV-ve		HIV+ve not on treatment		HIV+ on treatment	
	r	P-value	r	P-value	r	P-value

TC (mmol/L)	0.148	0.339	0.004	0.999	0.370	0.019*
TG (mmol/L)	0.233	0.119	0.028	0.861	0.490	0.002*
HDL-C (mol/L)	0-.106	0.501	-0.124	0.143	0.239	0.143
LDL-C(mmol/L)	0.136	0.384	0.006	0.971	0.229	0.166

TC total cholesterol

TG triglycerides

HDL-C High density Lipoprotein cholesterol

LDL-C Low density Lipoprtein cholestrol

4.4.4.Correlation between CD4 count, haematologic data and PWV

Only platelets in HIV positive participants not on HAART and neutrophils in HIV positives on HAART are significantly and positively correlated with PWV (P<0.05), while monocytes and CD4 count in HIV positives on HAART are significantly but negatively correlated with PWV (P<0.05). Both CD4 count and haematologic data are not significantly correlated (P.0.05) with PWV in HIV negatives (Table 27).

Table 27.Correlation between haematologic data and PWV

variables	HIV-ve		HIV+ve not on treatment		HIV+ on treatment	
	r	p-value	r	p-value	r	p-value

Platelet(gm/L)	0.148	0.339	0.370	0.019*	.013	0.940
Lymphocyte(%)	-0.145	0.299	-0.235	0.237	-0.232	0.155
Monocyte(%)	-0.091	0.591	-0.054	0.745	-0.320	0.047*
Neutrophil(%)	0.186	0.182	0.212	0.228	0.341	0.034*
CD4 (cell/mm^3)	-0.159	0.271	0.149	0.423	-0.446	0.009*

4.4.5. Inflammatory markers as correlates of PWV

All inflammatory markers in HIV negatives and HIV positives on HAART are not significantly correlated with PWV, TNFα and CRP in HAART naive HIV positives are also not significantly correlated with PWV, While IL-6 and E-selectin in HAART naïve HIV positive participants are significantly and positively correlated with PWV($P<0.05$) (Table 28).

Table 28. Correlation between cytokines and PWV

variable	HIV-ve		HIV+ve not on treatment		HIV+ on treatment	
	r	P-value	r	P-value	r	P-value

Tnfα(pg/mL)	-	-	-0.235	0.654	-0.235	0.654
IL-6(pg/mL)	0.282	0.179	0.889	0.031	0.083	0.712
E-Selectin(ng/ml)	0.260	0.110	0.843	0.029	0.296	0.142
Crp(mg/mL)	0.136	0.391	- 0.153	0.360	0.601	0.095

Tnfa Tumour necrosis alpha

IL-6 Interleukin 6

Crp C reactive protein

4.4.6. Correlation between indices of cardiac function and PWV

Aix(75), ED%,and SEVR% in HIV positives not on HAART and HIV positives on HAART are not significant in relation to PWV (P>0.05) and same applies to ED% and SEVR% which are not significantly related to PWV (P>0.05). Only AIx(75) is significantly and positively related to PWV (P>0.05) (Table 29).

Table 29. Correlation between Aix(75), ED%,SEVR%,and PWV

variables	HIV-ve		HIV+ve not on treatment		HIV+ on treatment	
	r	P-value	r	P-value	r	P-value
Alx(75)	0.850	0.004	0.027	0.027	0.109	0.451
ED(%)	0.015	0.906	-0.151	0.275	-0.151	0.275

SERVR%	-0.043	0.742	-0.031	0.825	-0.205	0.150

AIx(75) Augmentation index at a heart rate of 75 beats per minute

ED(%) Ejection duration index

SEVR Subendocardial viability ratio

4.5. Multivariate analysis

Different independent determinants of the variations (total R^2 and adjusted R^2) of PWV and AIx(75) as the dependent variables using multiple linear regression models.

4.5.1. Most important determinants of PWV

4.5.5.1. HIV negatives

Table 30 identifies Spa, age and triglycerides as the only independent and significant determinants of PWV among HIV negatives.

Table 30. Independent determinants of PWV in HIV negative participants

Variables	Beta	P-value

Spa	0.509	‹0.0001
Age	0.386	‹0.0001
Triglycerides	0.196	0.027

R^2 =56.9% (adjusted R^2=54.7%). Model adjusted for gender, anthropometric parameters, HDL-C, TC, LDL-C, haematologic data, haemodynamic data, cytokines, smoking, alcohol.

The positive equations or regression plots of these explanatory variables are plotted in Figures 57-59.

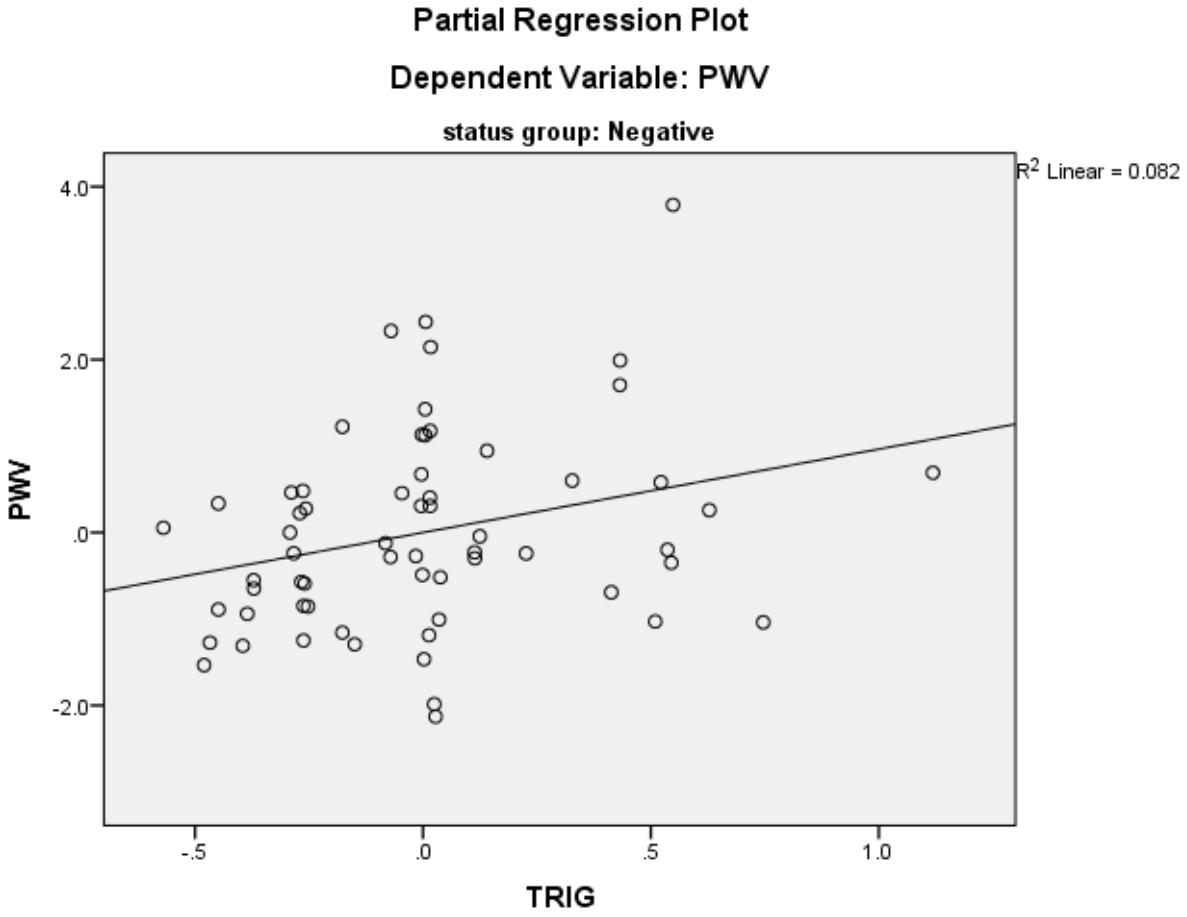

Figure 57. Partial Regression plot for PWV as the dependent variable and triglycerides as the independent variable among HIV negative participants.

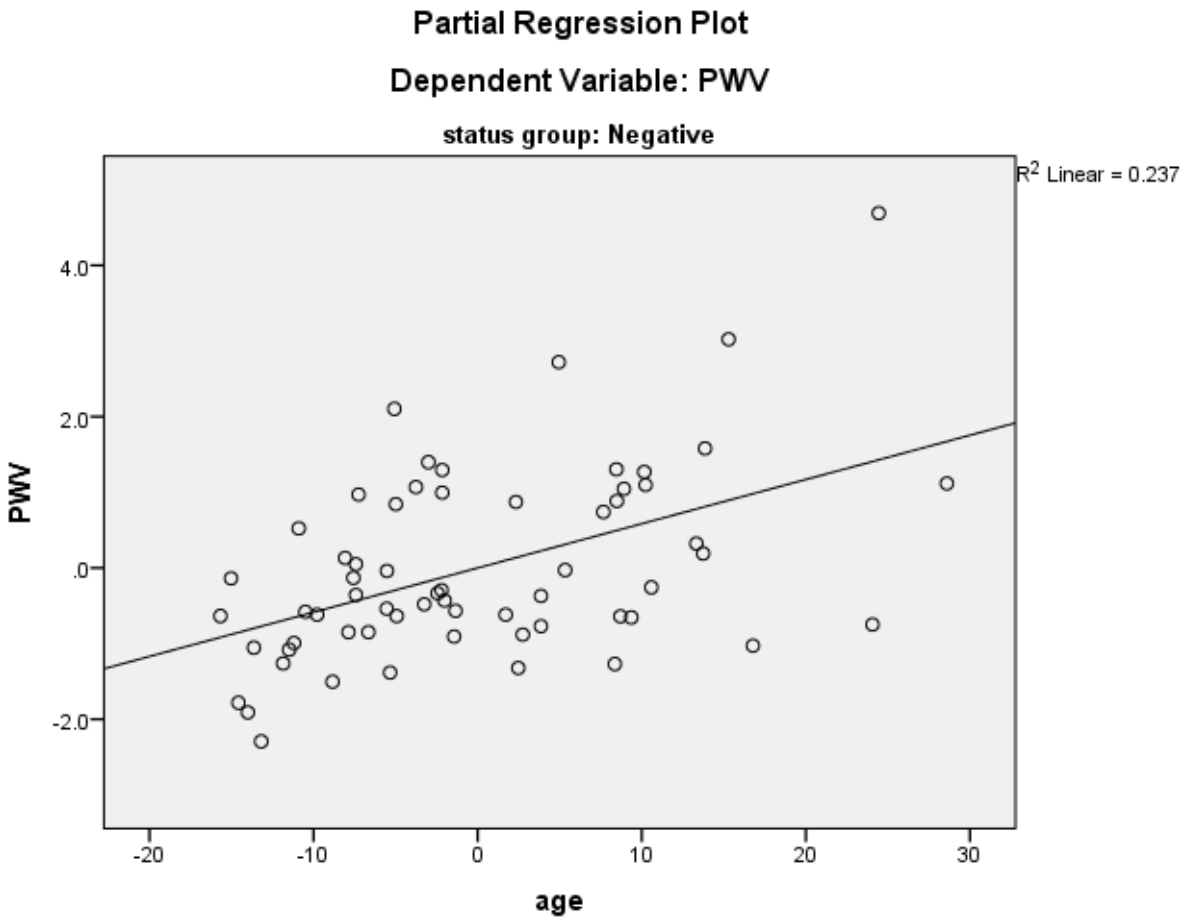

Figure 58. Partial Regression plot for PWV as the dependent variable and age as the independent variable among HIV negative participants.

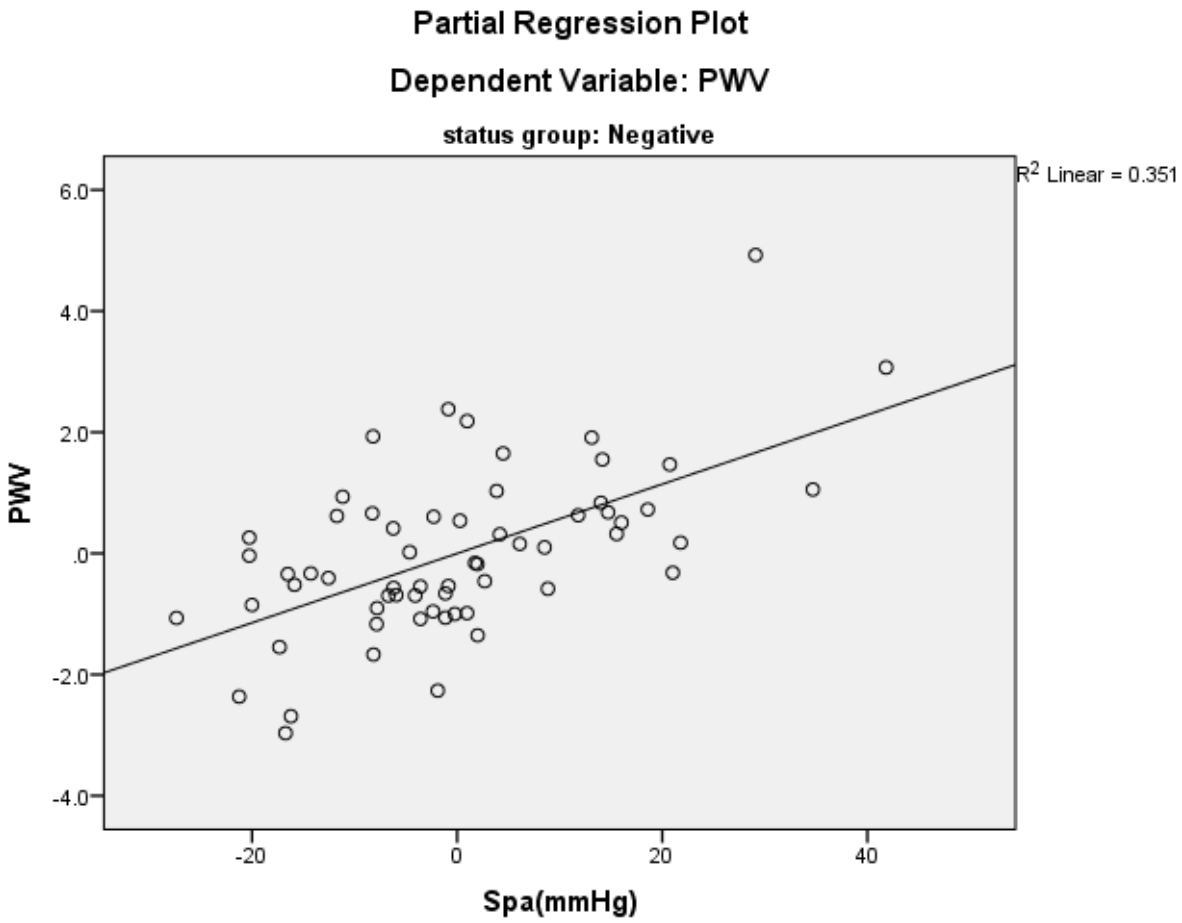

Figure 59. Partial Regression plot for PWV as the dependent variable and Spa as the independent variable among HIV negative participants..

4.5.1.2. HIV positive participants not on HAART

Only MP and waist circumference are identified as the most important and significant independent determinants of PWV in HIV positive participants not on treatment (Table 31)

Table 31. Independent determinants of PWV in HIV positive participants not on HAART

Variables	Beta	P-value
MP	0.443	‹0.001
Waist circumference	0.272	0.031

R^2 =33.4% (adjusted R^2 =30.6%). Model adjusted for gender, weight, height, hip circumference, waist to hip ratio, Lipid profiles, haematological data, cytokines adhesion molecules, smoking, alcohol, other haemodynamic parameters and CD4 count.

The positive associations of both partial regression plots are shown in Figures 60 and 61.

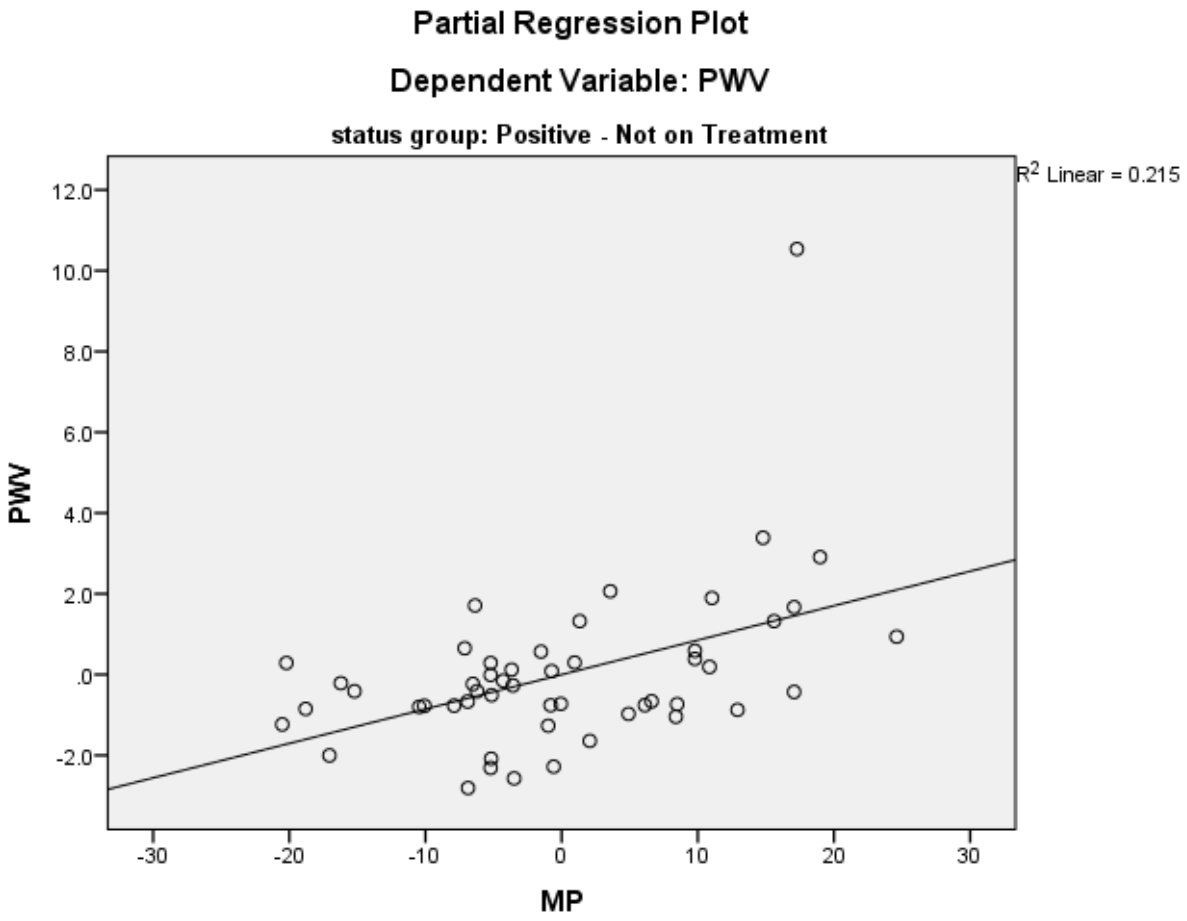

Figure 60. Partial Regression plot for PWV as the dependent variable and MP as the independent variable among HIV positives not on HAART.

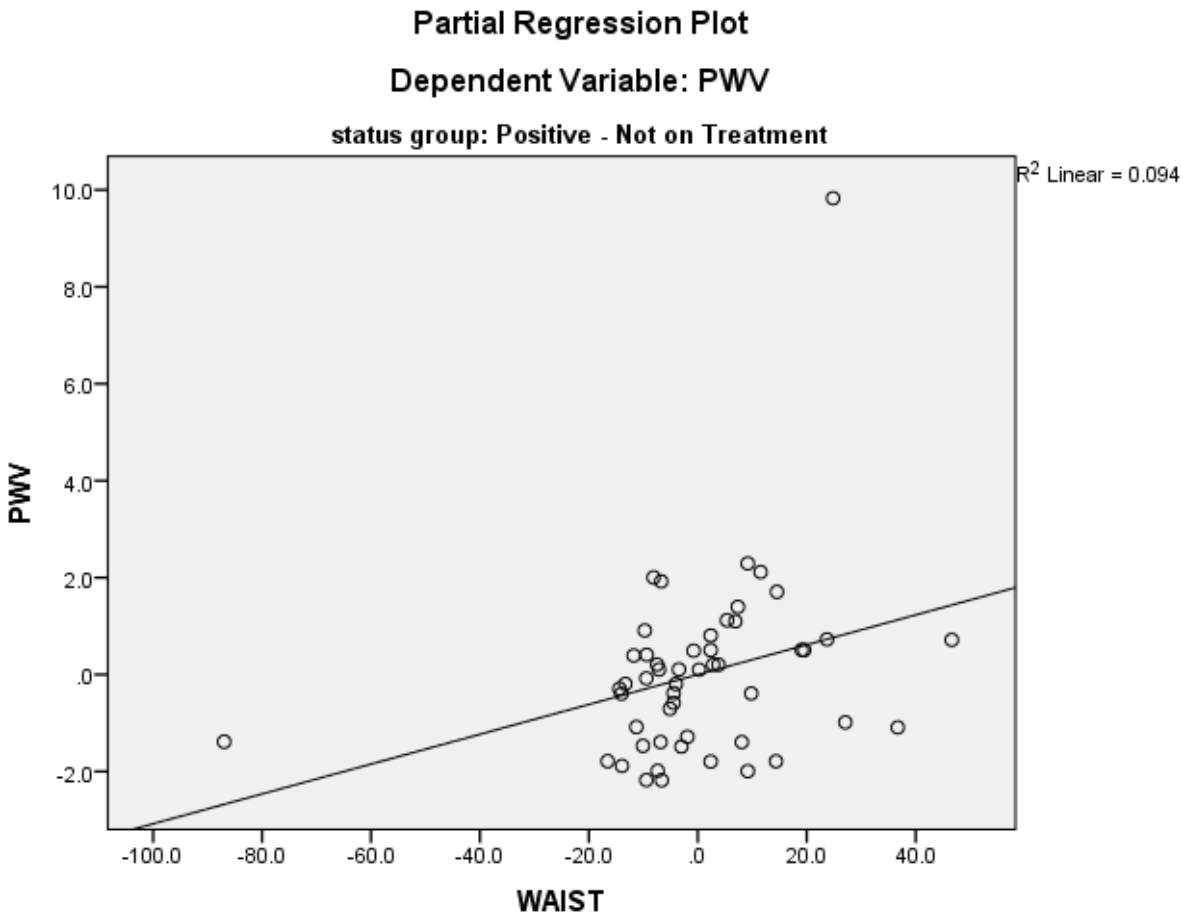

Figure 61. Partial Regression plot for PWV as the dependent variable and waist circumference as the independent variable in HIV positives not on HAART.

4.5.1.3. HIV positive participants on HAART

Age, MP, HDL-C, and triglycerides were identified as the significant independent determinants of the variations of PWV in HIV positive participants on HAART (Table 32).

Table 32. independent determinants of PWV in HIV positives on HAART

Variables	Beta	P-value
Age	0.506	‹0.0001
MP	0.294	0.003
HDL-C	0.272	0.006
Triglycerides	0.212	0.043

R^2 =57 % (adjusted R^2 =53.5%). Model adjusted for gender, anthropometric data, smoking, alcohol, cytokines, adhesion molecules, total cholesterol, LDL-C, haematological data, CD4 count, and other haemodynamic parameters.

The positive association between identified explanatory and PWV in HIV positives on treatment are highlighted in Figures 62-65.

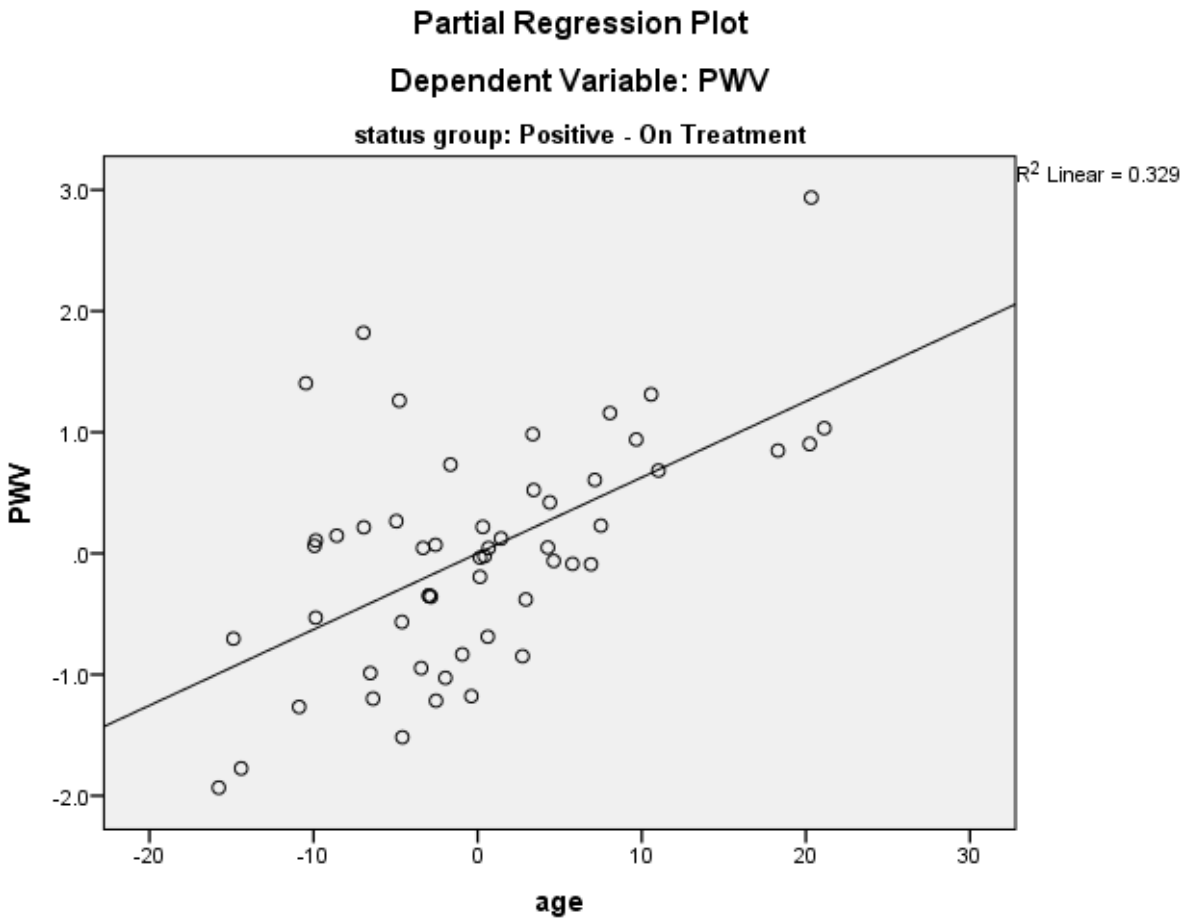

Figure 62. Partial Regression plot for PWV as the dependent variable and age as the independent variable in HIV positives on HAART.

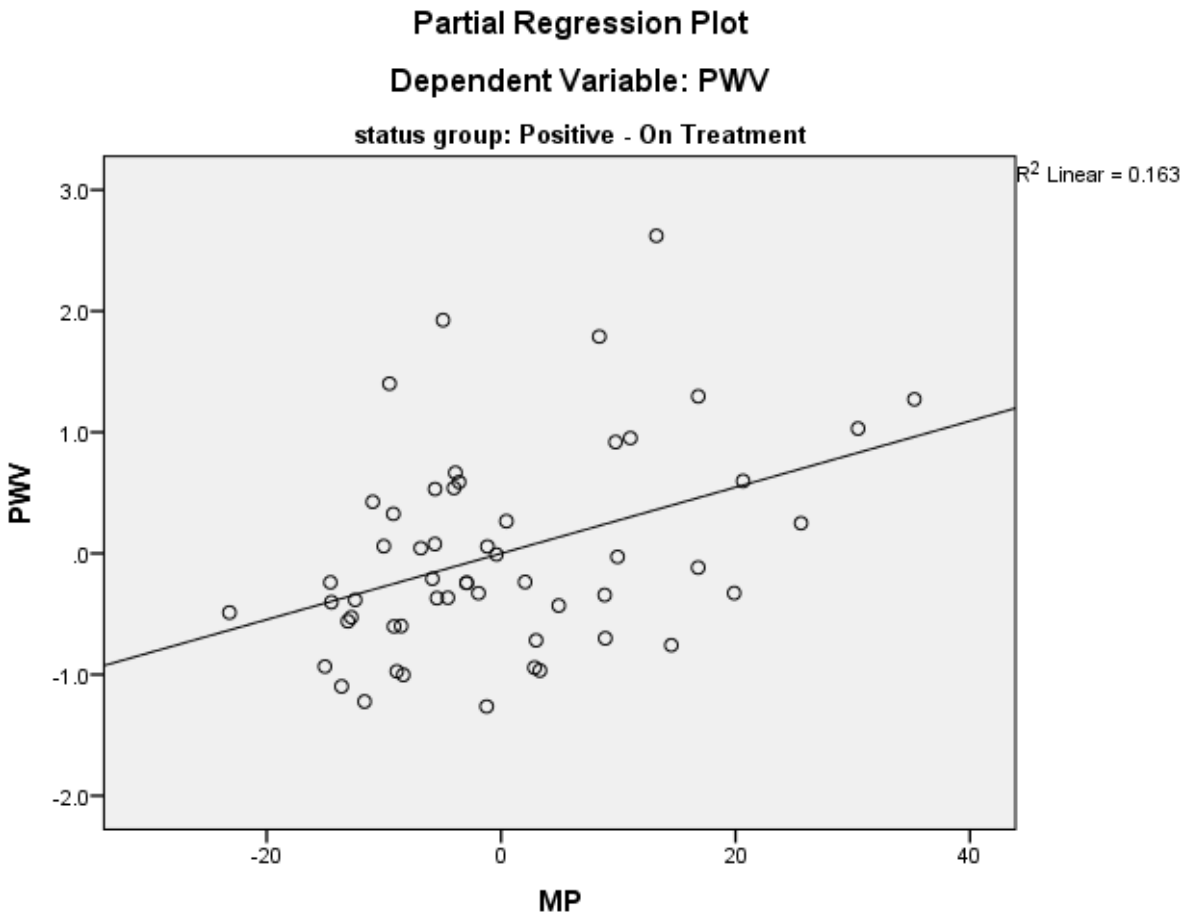

Figure 63. Partial Regression plot for PWV as the dependent variable and MP as the independent variable in HIV positives on HAART.

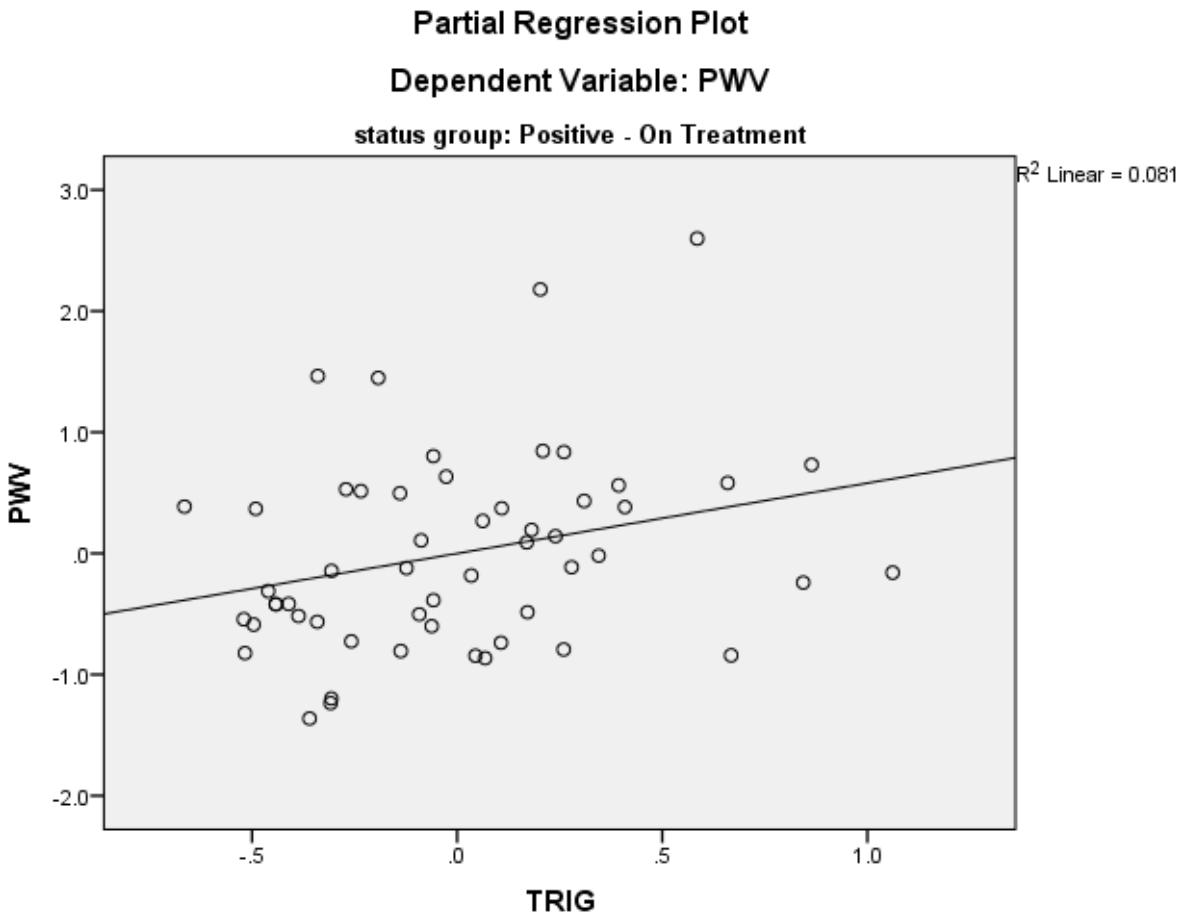

Figure 64. Partial Regression plot for PWV as the dependent variable and triglyceride as the independent variable in HIV positives on HAART.

4.5.2. Significant determinants of AIx(75)

4.5.2.1. HIV negatives

In HIV negatives the multiple linear regression model, identified age (positive correlation), height (negative correlation), CD4 (positive correlation) and MP (positive correlation) as the independent and significant determinants of AIx (75) among HIV negatives (Table 33).

Table 33. Independent determinants of AIx(75) in HIV negatives

Variables	Beta	P-value
Age	0.506	‹0.0001
MP	0.294	0.003
HDL-C	0.272	0.006
Triglycerides	0.212	0.043

R^2 =46.3% (adjusted R^2 =42.5%). Model adjusted for gender, smoking alcohol, weight, waist circumference, hip circumference, waist to hip ratio, lipid profiles, cytokines, adhesion molecules, haematological data, and other haemodynamic parameters.

Figure 66-69 demonstrate the partial regression plot or equations between identified independent variables and the dependent variable PWV among HIV negatives.

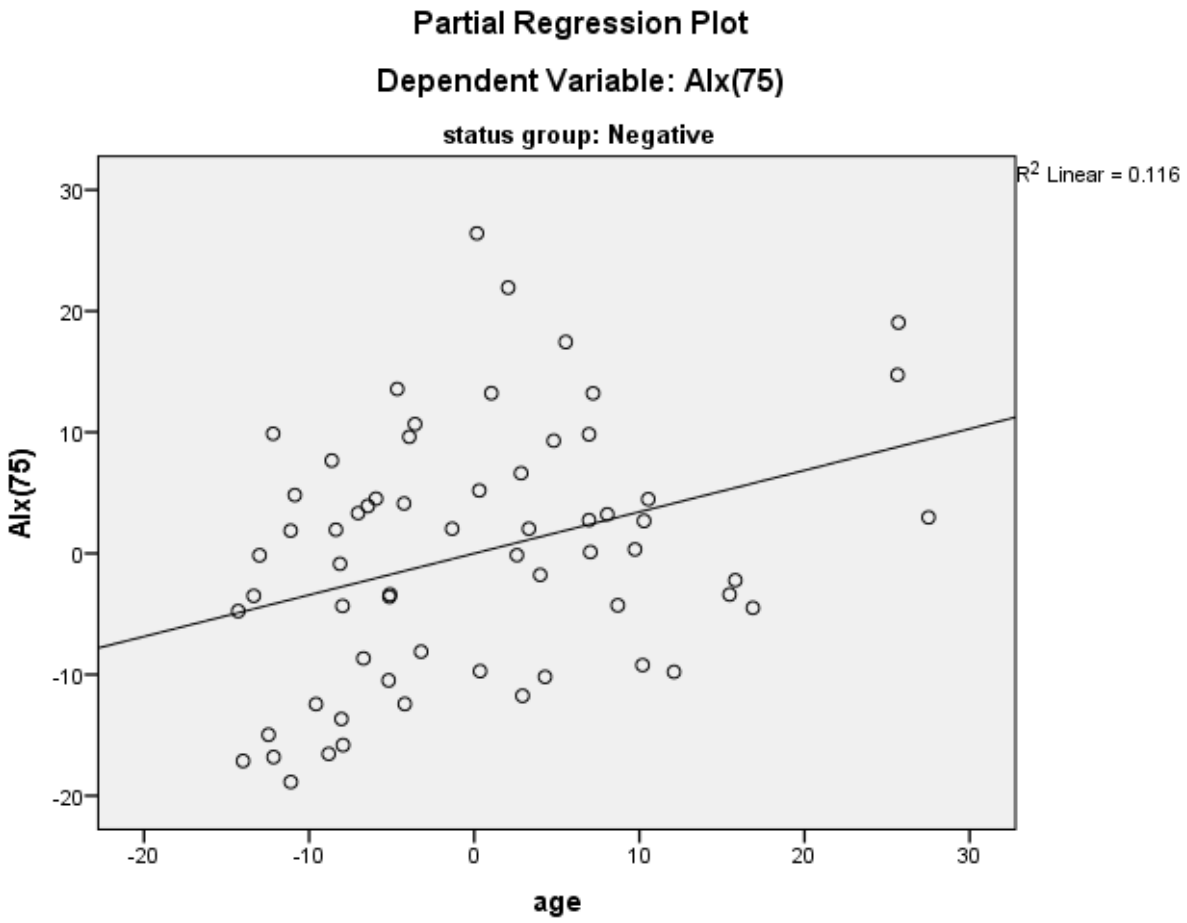

Figure 65. Partial Regression plot for AIx(75) as the dependent variable and age as the independent variable in HIV negative participants.

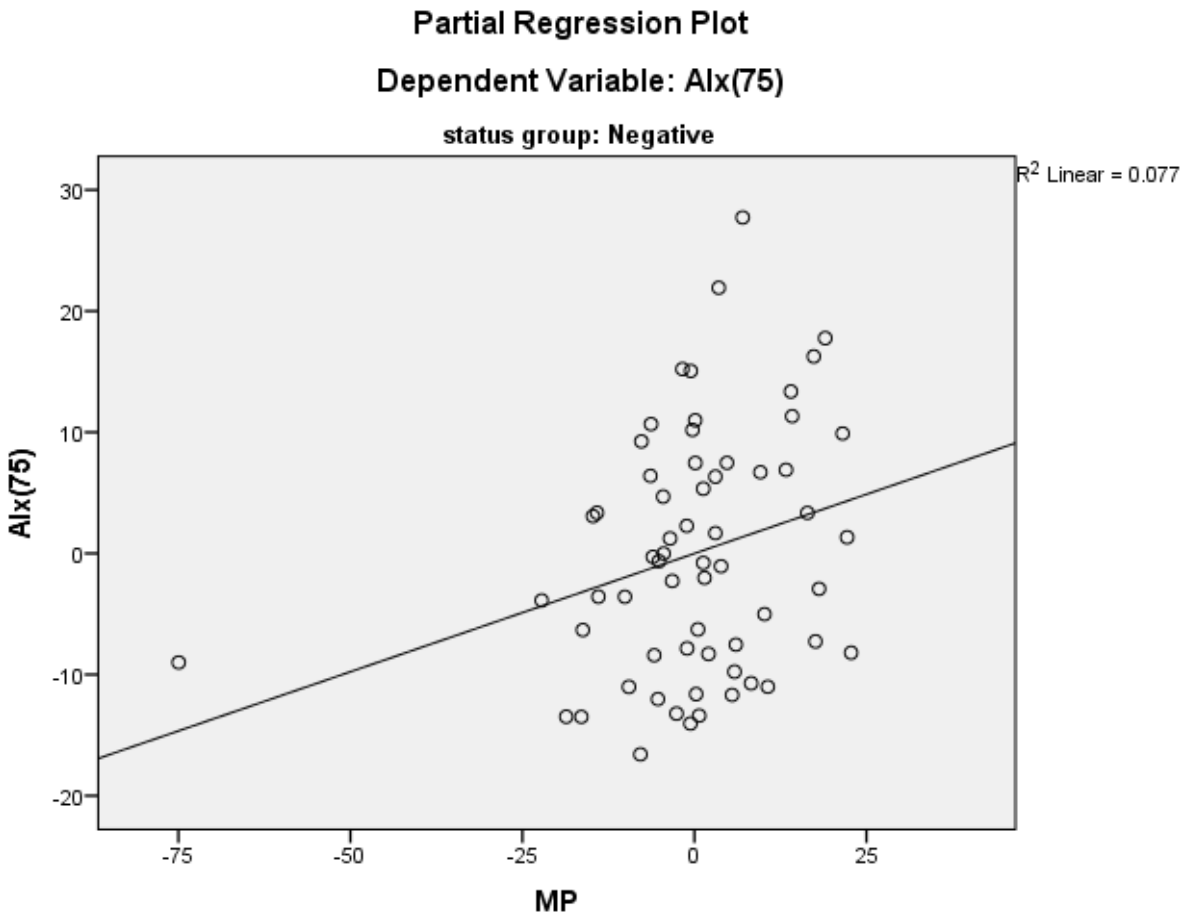

Figure 66. Partial Regression plot for AIx(75) as the dependent variable and MP as the independent variable in HIV negative participants.

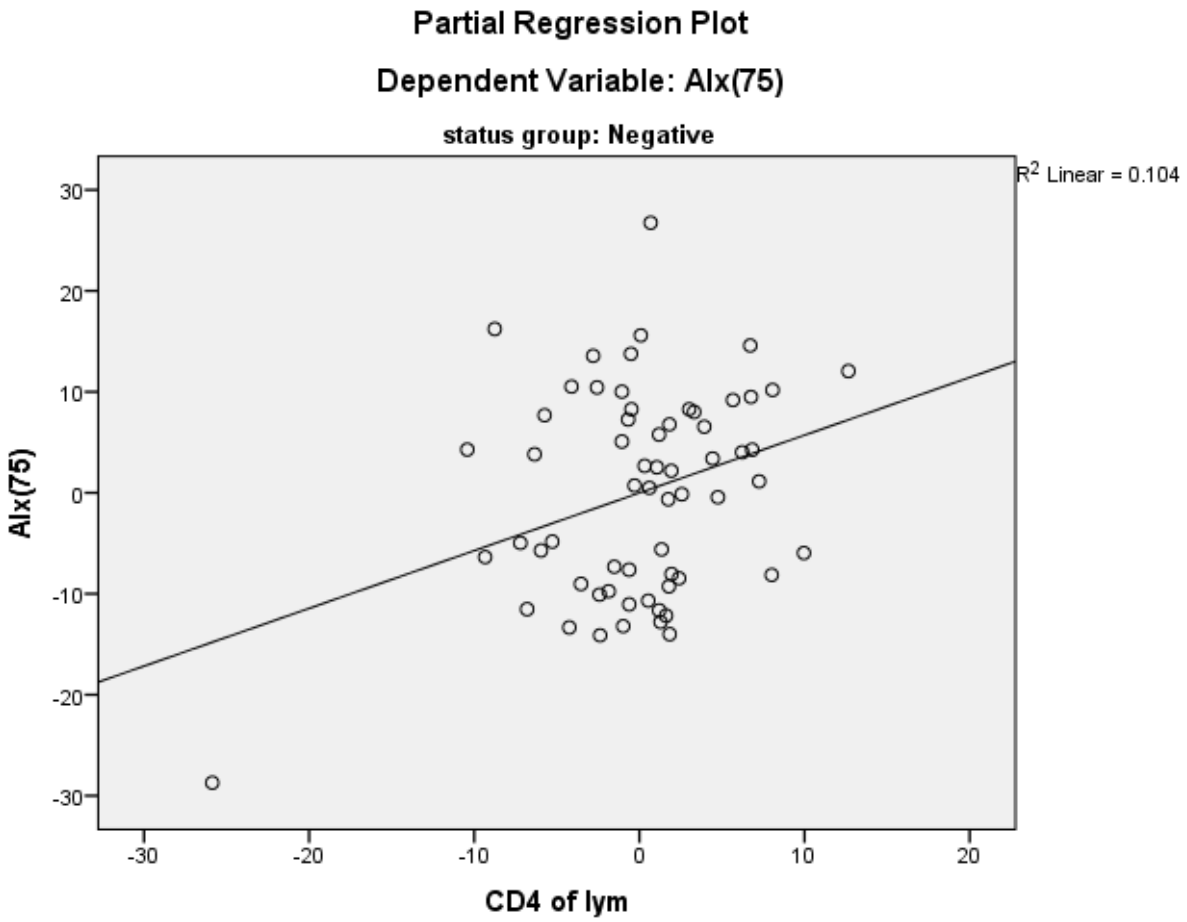

Figure 67. . Partial Regression plot for AIx(75) as the dependent variable and MP as the independent variable in HIV negative participants.

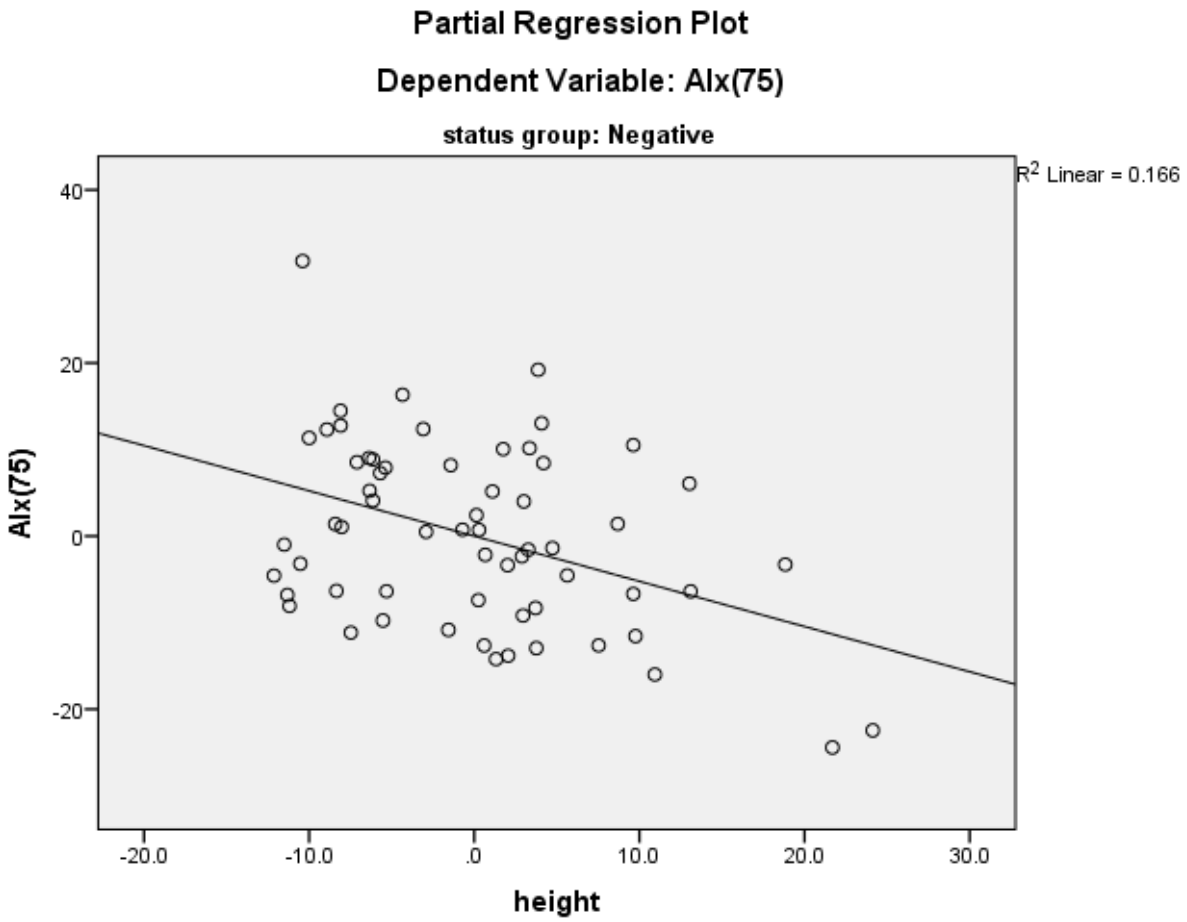

Figure 68. Partial Regression plot for AIx(75) as the dependent variable and Height as the independent variable in HIV negative participants.

4.5.2.2. HIV positive participants not on HAART

Table 34 shows that Spa and Age are independently and significantly associated with the variations of AIx(75) among HIV positives not on HAART. On the other hand height is negatively and significantly associated with AIx(75) amongst HIV positives not on HAART.

Table 34. Independent determinants of AIx(75) in HIV positive participants not on HAART

Variables	Beta	P-value
Spa	0.264	0.031
Height	-0.505	<0.001
Age	0.421	<0.001

R^2 =43.1% (adjusted R^2 =39.4%) Model adjusted for gender, smoking, alcohol intake, cytokines, weight, waist circumference, hip circumference, waist to hip ratio, cytokines, CD4, haematological data, lipid profiles, and other haemodynamic parameters.

Figures 69-71 highlight Age, Spa and Height correlations with PWV in HIV positives not on HAART.

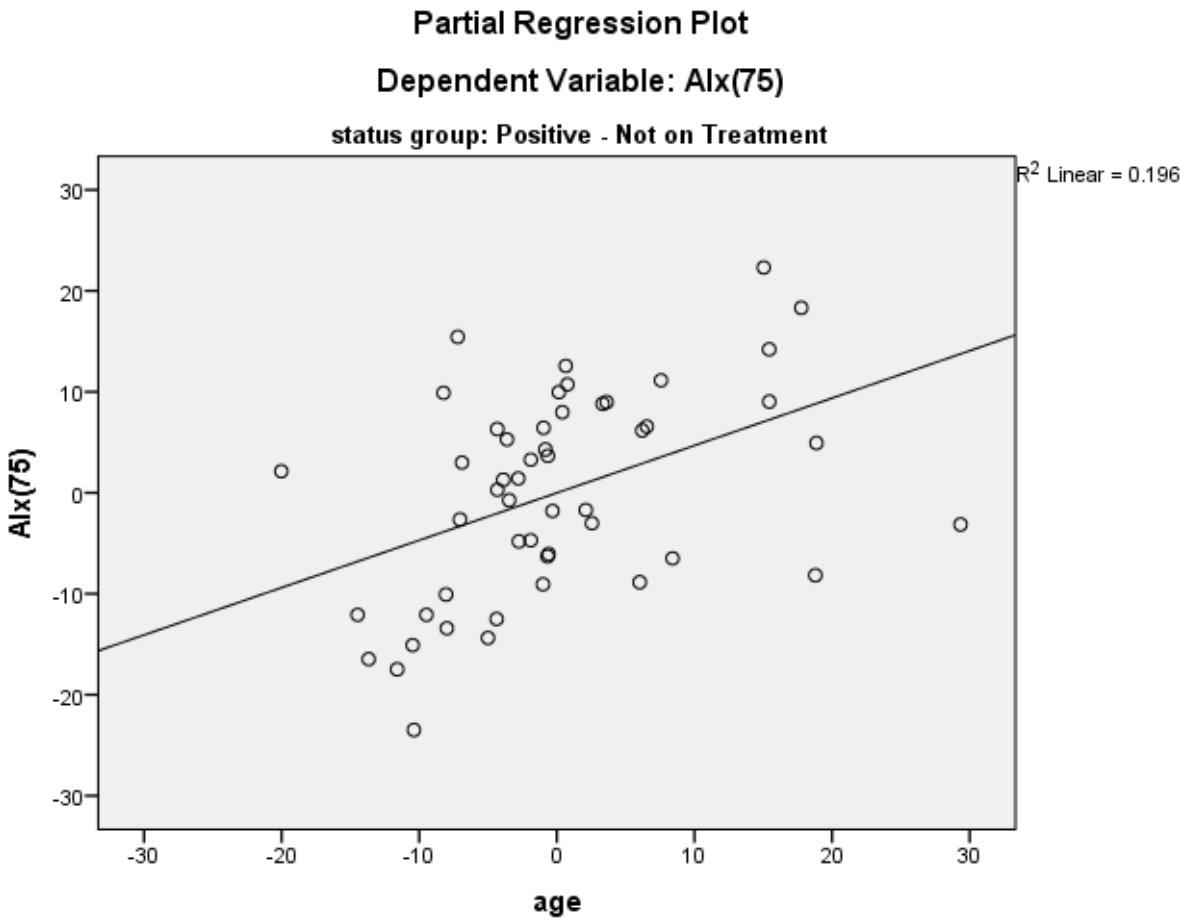

Figure 69. Partial Regression plot for AIx(75) as the dependent variable and age as the independent variable in HIV HAART naive participants.

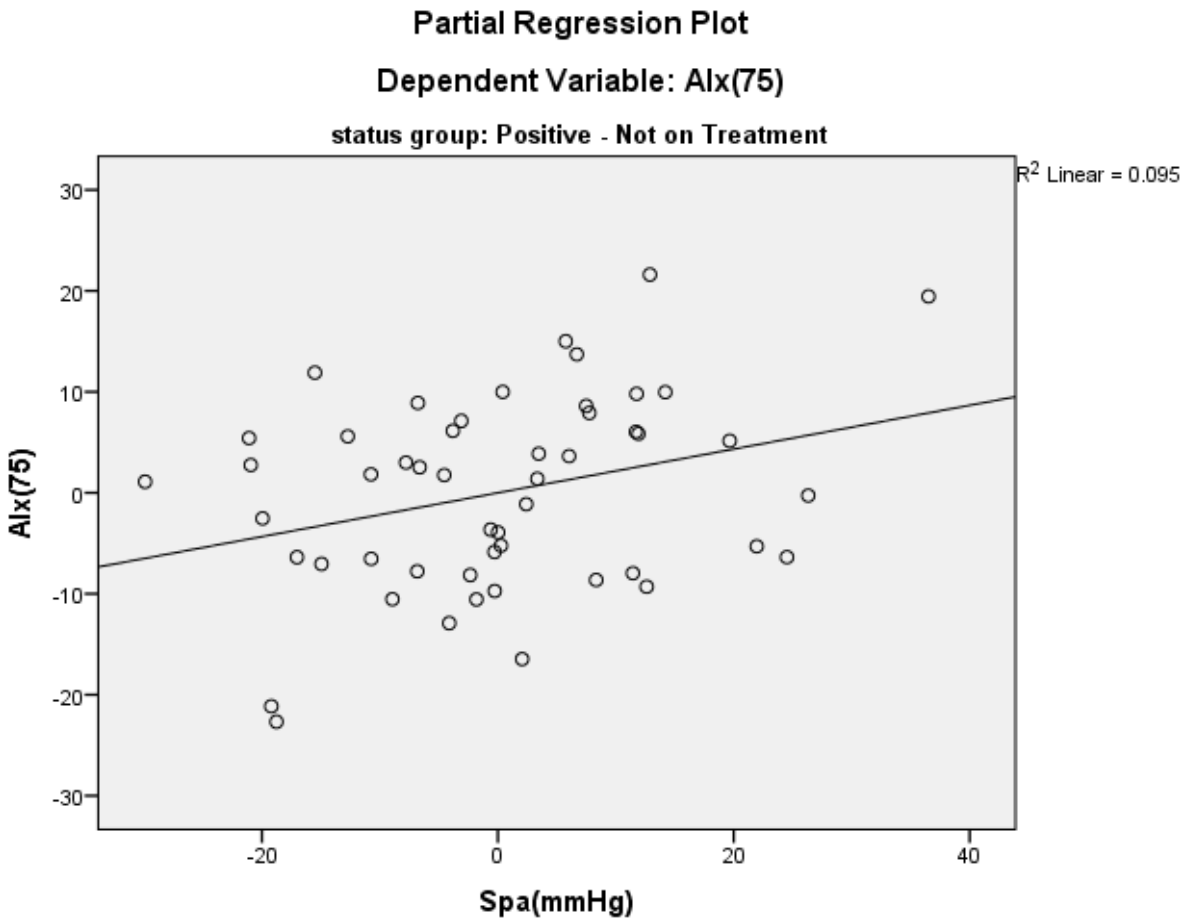

Figure 70. Partial Regression plot for AIx(75) as the dependent variable and Spa as the independent variable in HIV HAART naive participants.

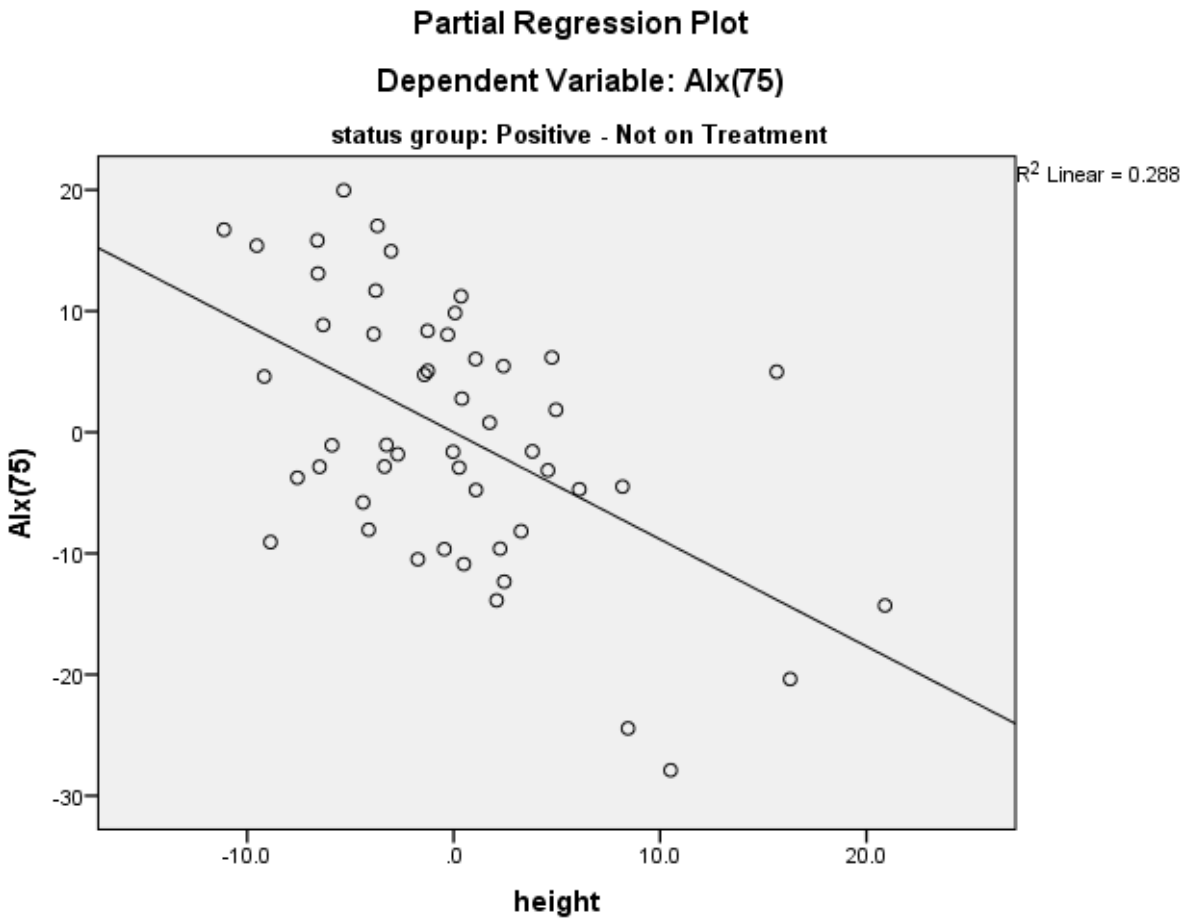

Figure 71. Partial Regression plot for AIx(75) as the dependent variable and height as the independent variable in HIV HAART naive participants.

4.5.2.3. HIV positive on HAART

After excluding confounding factors, height (negative correlation), age (positive correlation), MP (positive correlation) HDL-C (negative correlation), platelets (positive correlation) alcohol intake (excessive consummation associated with positive correlation) and TNFα (negative correlation) were identified as the independent and significant variables associated with increase in AIx(75) among HIV positive participants on HAART (Table 35).

Table 35. Independent determinants of AIx(75) in HIV positive participants on HAART

Variables	Beta	P-value
Height	-0.676	<0.0001
Age	0.460	<0.0001
MP	0.480	<0.0001
HDL-C	-0.212	0.015
Platelets	0.319	<0.001
Alcohol intake	-0.298	0.005
TNFα	-0.219	0.024

R^2 =68.1% (adjusted R^2 =63.3%). Model adjusted for gender, smoking, weight, waist circumference, hip circumference, waist to hip ratio, neutrophils, lymphocytes, monocytes,CD4 count, triglycerides, total cholesterol, LDL-C IL-6, E-Selectin, and other haemodynamic data.

The equation between these independent determinants and Aix(75) (dependent variables) in HIV positives on HAART are shown in Figures 72-78.

Figure 72. Partial Regression plot for AIx(75) as the dependent variable and age as the independent variable in HIV positive participants on HAART.

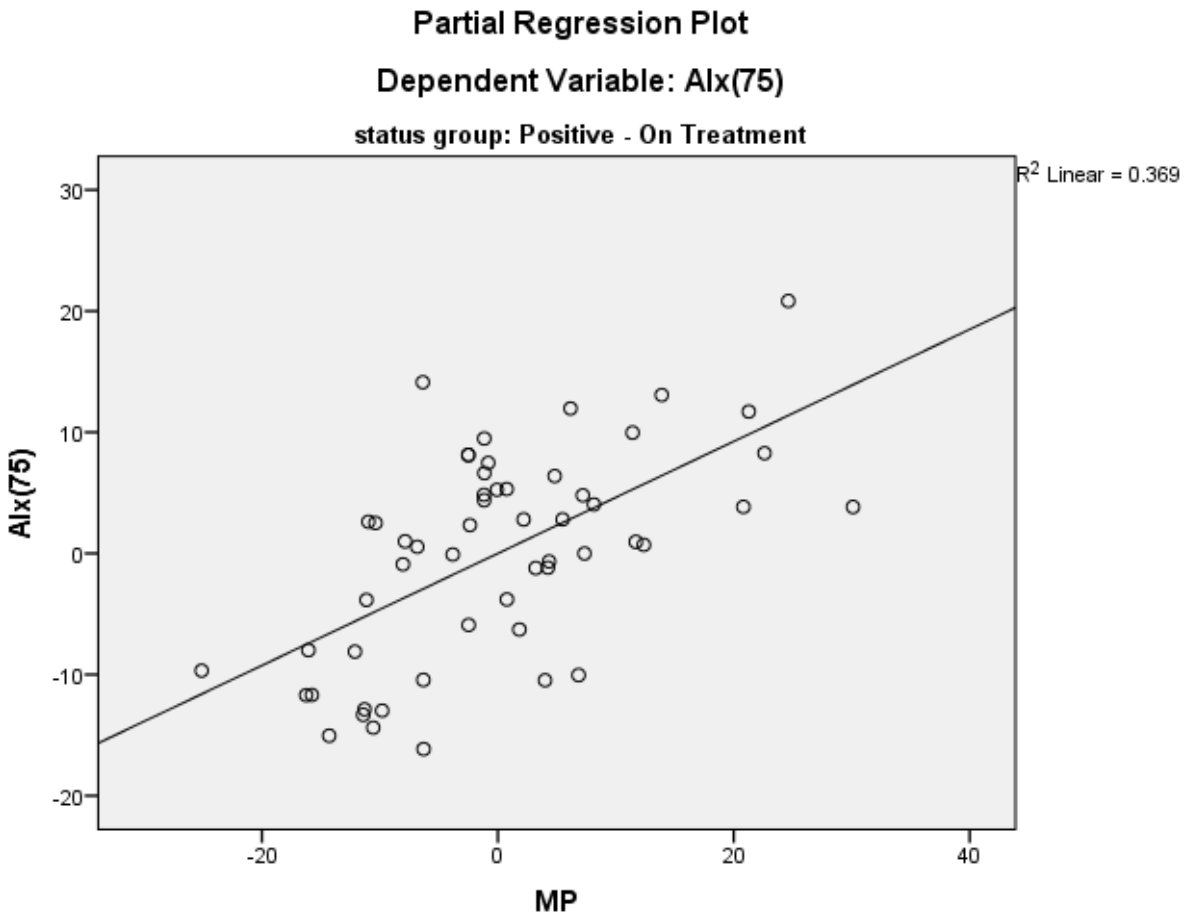

Figure 73. Partial Regression plot for AIx(75) as the dependent variable and MP as the independent variable in HIV positive participants on HAART.

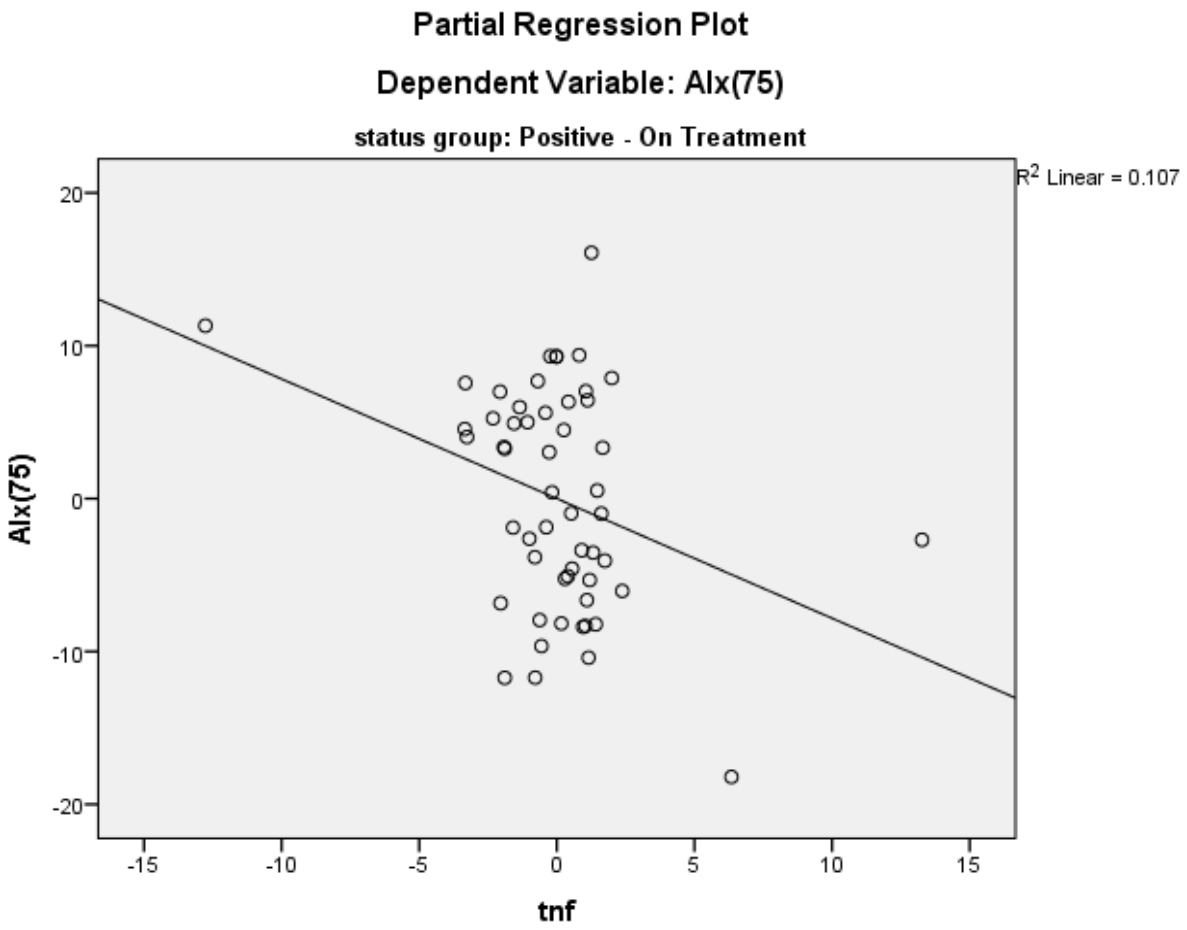

Figure 74. Partial Regression plot for AIx(75) as the dependent variable and TNFα as the independent variable in HIV positive participants on HAART.

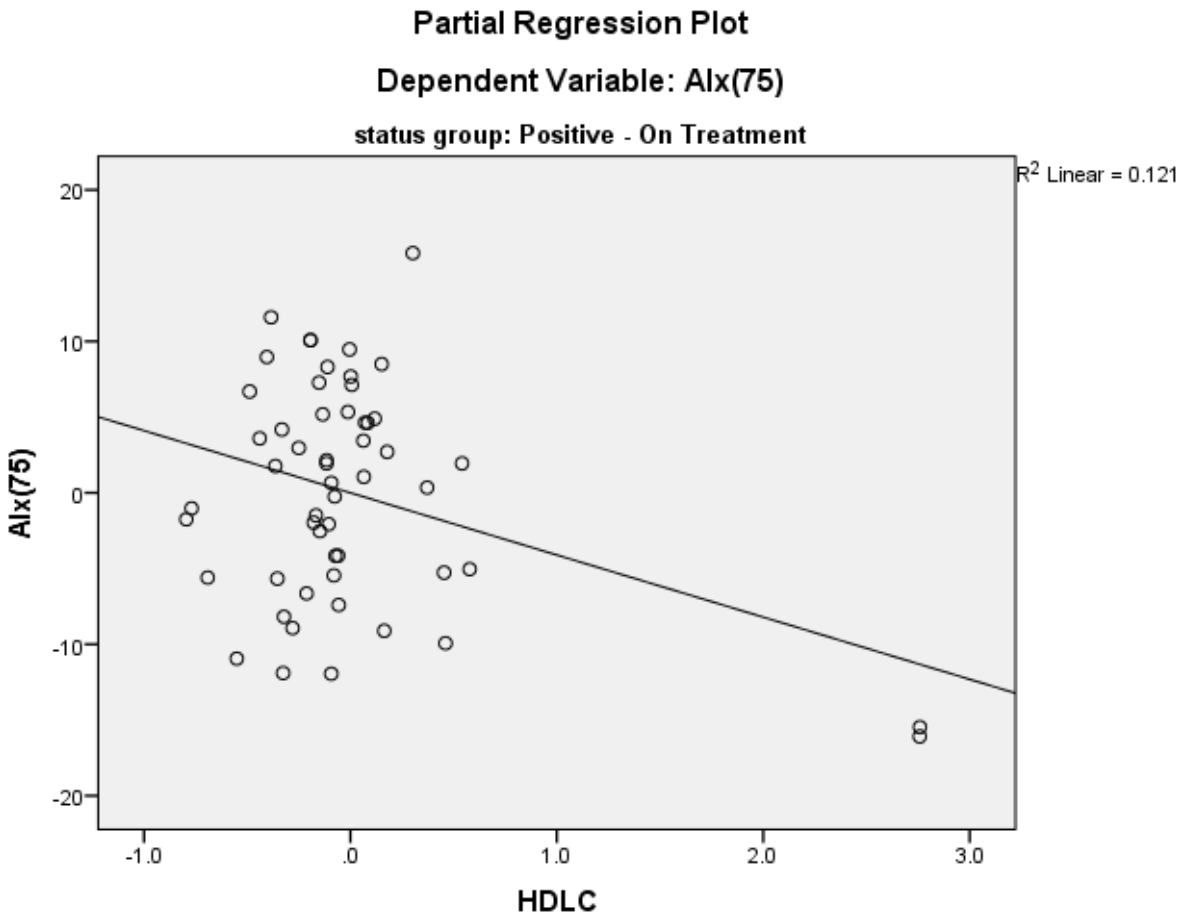

Figure 75. Partial Regression plot for AIx(75) as the dependent variable and HDL-C as the independent variable in HIV positive on HAART.

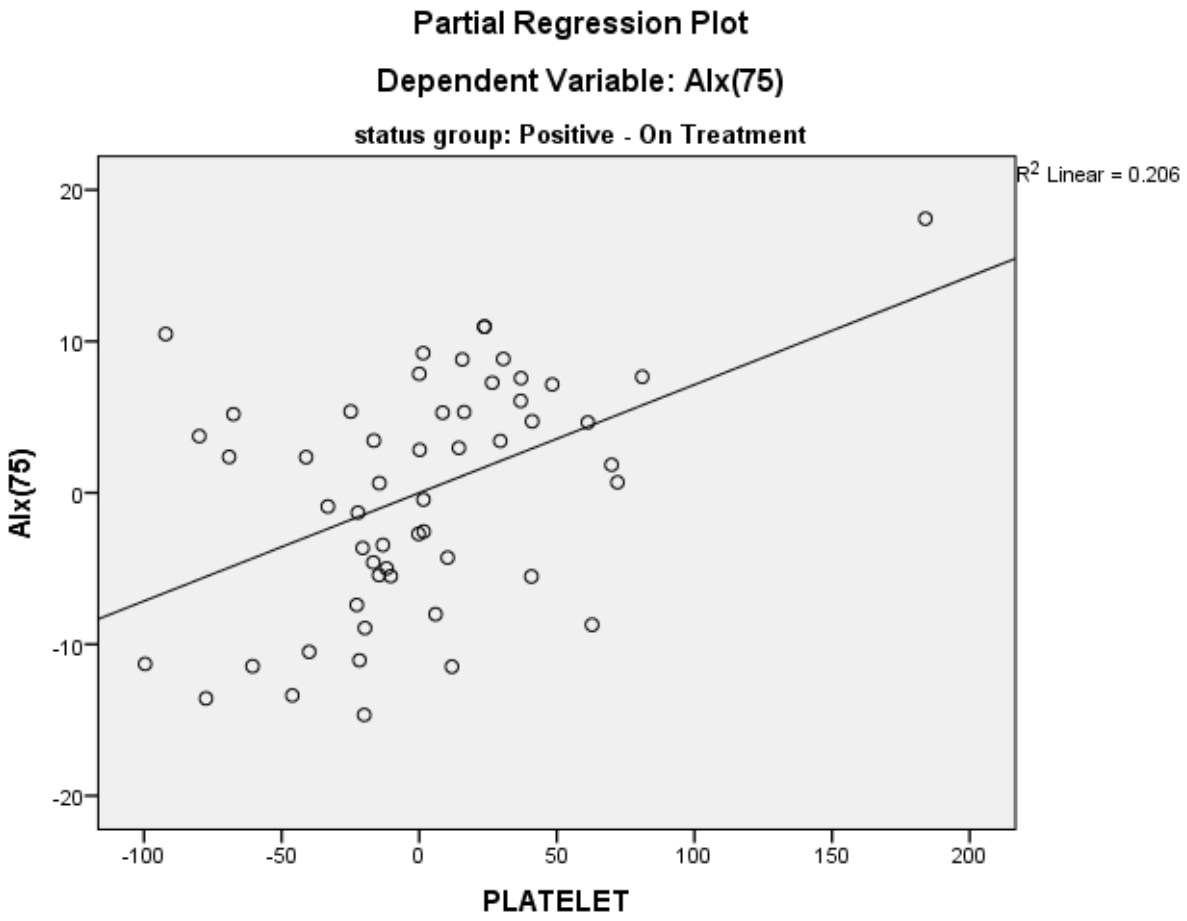

Figure 76. Partial Regression plot for AIx(75) as the dependent variable and platelets as the independent variable in HIV positive participants on HAART.

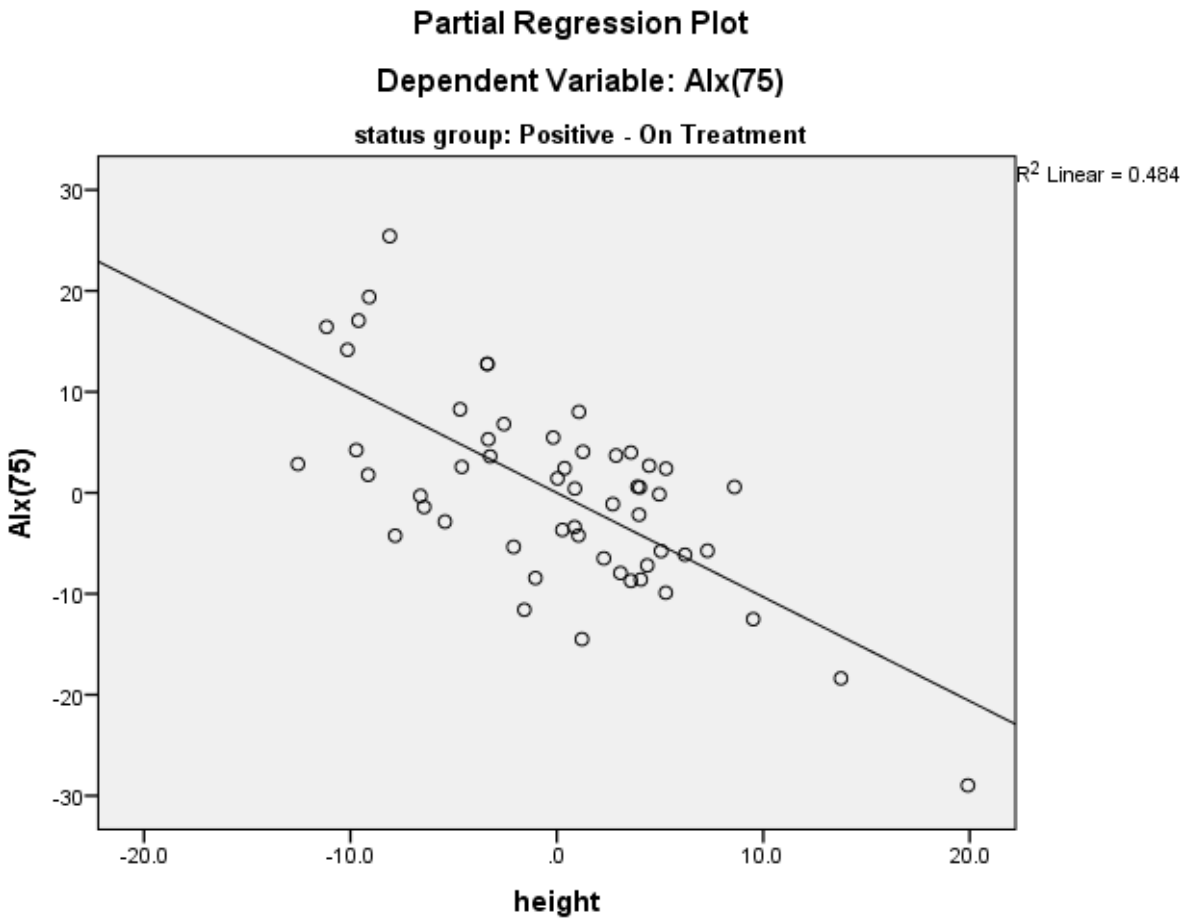

Figure 77. Partial Regression plot for AIx(75) as the dependent variable and height as the independent variable in HIV positive participants on HAART.

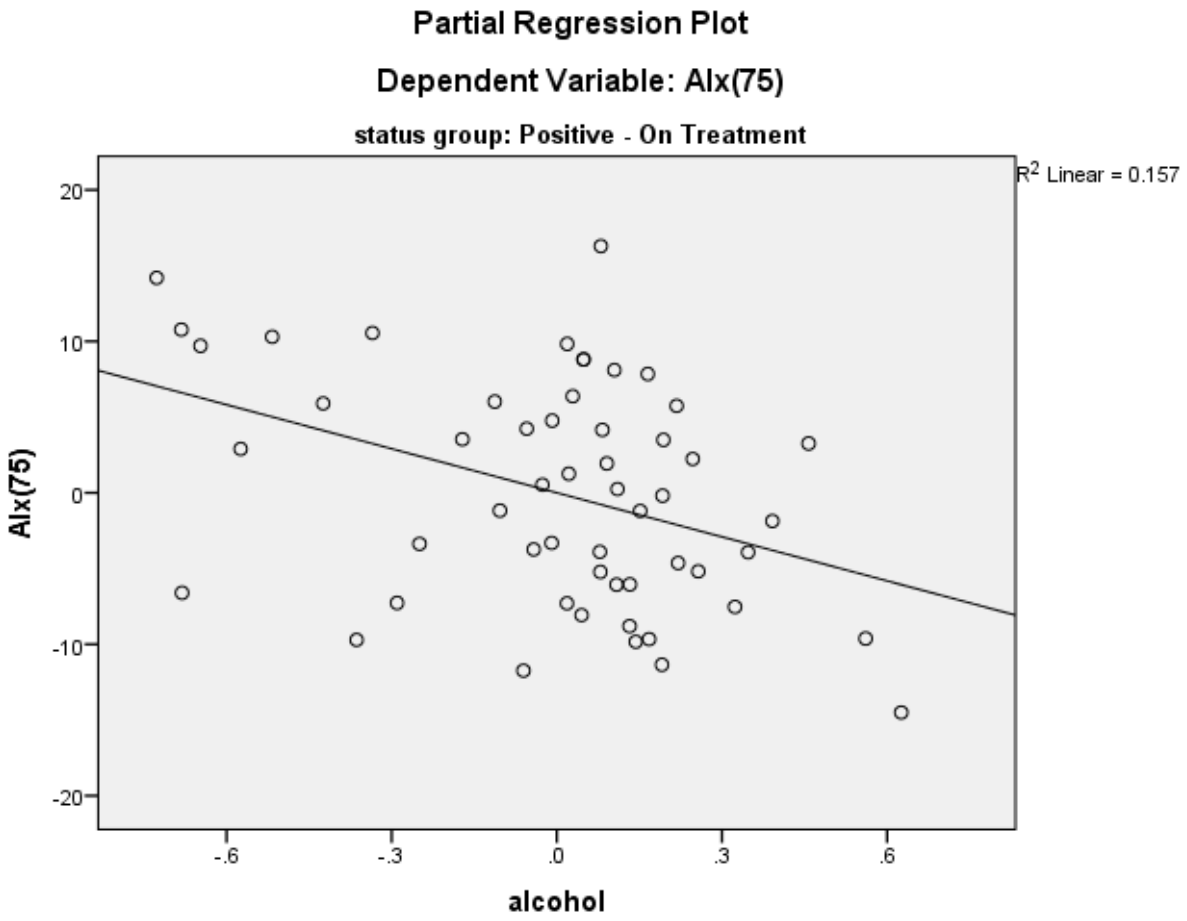

Figure 78. Partial Regression plot for AIx(75) as the dependent variable and alcohol as the independent variable in HIV participants on HAART.

CHAPTER 5

DISCUSSION

5.1. Compostion of the participants and main findings of the study

This study evaluated the functions of the vasculature which could lead to cardiovascular risks in male and female HIV positive and HIV seronegative participants of African ancestry. Some of the HIV participants who were positive and on treatment were slightly older than those participants who were not on treatment. This is not unexpected because most of the participants were diagnosed a long time ago and have been on treatment. Females were more than males in the two HIV positive groups. This may be because most attendees in our public hospitals were females and therefore constituted the majority of willing participants. Some of the participants in the study dropped out because of financial reasons, as they had to leave their homes to search for jobs making it difficult to contact them.

The main fidings of this study are that patients with HIV had increased arterial stiffness compared with HIV negative groups after adjustment for conventional cardiovascular risk factors. Within the HIV group, participants who are HAART naïve had increased arterial stiffness compared to participants exposed to HAART. Using multiple linear regresssion, individuals with HIV and on HAART had age, MP, HDL-C, and triglycerides as significant independent determinants of the variations of PWV. Using multiple linear regression with adjustment for gender, anthropometric data, smoking, alcohol, cytokines, adhesion molecules, total cholesterol, LDL-C, haematological data, CD4 count, and other haemodynamic parameters, only MP and waist circumference were identified as the most important and significant independent determinants of PWV in HIV positive participants not on treatment. Spa, age and triglycerides are the only independent and significant determinants of PWV among HIV negatives in a multivariate analysis. This study therefore demonstrates the importance of mean arterial pressure in the assessment of arterial stiffness as most previous studies focused on pulse pressure.

When participants were stratified by gender, there was no significant influence of gender on the variations in age, haemodynamic and anthropometric data, except that females were shorter. There were no significant differences in the levels of TNFα, IL-6, E-selectin and Crp in both males and females while ED % was higher in females ($p<0.000$). This might indicate that the diastolic period and coronary perfusion pressure and blood flow to the myocardium in the females were compromised. On the other hand, SEVR was higher in males ($p<0.000$). This shows that females are less tolerant to physical activity and more prone to develop sub-endocardial ischaemia. AIx(75) was also higher in females ($p<0.001$). AIx(75), a measure of systemic arterial stiffness might have increased in these female participants because of their short stature as corrobated by the study of Yasmin and Brown (1999). Using PWV there was no difference in arterial stiffness between males and females.

In HIV negatives, there was a significant positive correlation between age, number of cigarettes, weight and PWV. In HIV positive participants not on HAART there was a significant positive correlation between age, waist to hip ratio, height and PWV. Also in HIV positive participants on HAART there was a positive correlation between waist to hip ratio, height and PWV. This shows that there is a strong association of arterial stiffness with age in all groups and also waist to hip ratio in both HIV positive groups.

5.2. Carotid pulse wave velocity as the gold standard for measuring arterial stiffness in HIV negative, HAART naïve HIV positive participants and HIV participants on treatment

Carotid pulse wave velocity (PWV) was used to evaluate arterial stiffness in this study, and is recognised as the gold standard. The patients tolerated the procedure as it was not invasive and not time consuming if measured by an experienced investigator. Carotid-femoral PWV has been used in epidemiological studies demonstrating the predictive value of aortic stiffness for CV events. Pulse wave velocity (PWV) is considered a non-invasive diagnostic test for subclinical atherosclerosis (SA) or arterial stiffness, and is a predictor of cardiovascular risk and also a surrogate marker of vascular disease (London & Cohn 2002). Alterations in arterial stiffness are known to precede clinical hypertension by a substantial period of time (Willum-Hansen et al 2006). Increased arterial stiffness has been linked to a higher rate of future cardiovascular complications and death (Laurent et al. 2001). Premature atherosclerosis has also been reported in young adults with HIV infection in the pre- HAART era (Charakida et al. 2009).

Subclinical cardiovascular disease is an accurate reflection of the real prevalence of atherosclerosis at a potentially reversible stage, so that detecting it makes it possible to implement secondary preventive measures. Until now, there have been very few studies using PWV to evaluate SA or arterial stiffness in HIV patients, and these studies involved very few subjects with conflicting results (Ho et al. 2010; Schillaci et al. 2005; Schillaci et al. 2008).

5.3. Role of HIV infection in the development of arterial stiffness

In this study, participants who were HIV positive and HAART naïve had significantly increased pulse wave velocity than those who were on treatment and those who were seronegative. The results of this study are in agreement with a previous study that found

increased arterial stiffness in patients with HIV infection compared with healthy controls (Van Wijk et al. 2006).

In the study by Van Wijk et al (2006), thirty-seven HIV-infected patients underwent assessment of flow-mediated vasodilation (FMD), aortic pulse-wave velocity (PWV), and carotid intima-media thickness (IMT). Age-matched type 2 diabetic patients (n = 13) and healthy controls (n = 14) served as reference groups. In the HIV infected patients there was an increased cardiovascular risk. The presence of the metabolic syndrome in some of the HIV patients was associated with even more advanced atherosclerotic changes. Presumably, HIV infection may promote atherosclerosis through mechanisms involving endothelial cells, either directly or indirectly via metabolic risk factors.

Van Vonderen et al (2009) in his study on HIV patients said the mechanism for the increase in pulse wave velocity with HIV patients is not completely understood, but the implication of classical cardiovascular risk factors, and of systemic chronic inflammation should not be under-estimated (Van Vonderen et al 2009). The study reported by Van Vonderen et al (2009) also did not agree with that of Monteiro (2012) in Brazil who found lack of association between arterial stiffness and HIV. Monteiro's study comprised of 261 HIV infected and 82 uninfected participants. He found no association between arterial stiffness and HIV but found association between age, gender and arterial stiffness.

A recent study compared measures of arterial stiffness using radial artery tonometry in 276 HIV-infected and 67 HIV-uninfected Rwandan women (Lazar 2009). They reported that HIV infection was associated with greater arterial wave reflection in women who are HAART naïve without other CVD risk factors. This is not very different from this study in which the PWV is highest in the HAART naïve participants. This could be due to the chronic

inflammation of the HI virus itself. HIV infection may lead to changes in the vascular endothelium by depressed immunity, sustained inflammation, and viral replication. Another explanation may be that treatment with HAART by reducing the inflammation due to active HIV infection may to some extent counteract any adverse effects of treatment. The balance between the beneficial effects of HAART may be essential to determine the net effect in an individual patient.

5.4. Role of antiretroviral therapy in the development of atherosclerosis

In this study, arterial stiffness was significantly higher in antiretroviral naïve HIV positive participants than in those who are on treatment and those who are seronegative implicating that the HIV virus causes more of arterial stiffness than HAART despite the older age of the participants who are on treatment. A study showed that endothelial dysfunction actually improved after starting HAART, despite the rapid onset of dyslipidemia (Torriani 2008). This might support the findings in this study that the arterial stiffness caused by HAART was not as much as that caused by that of the HIV infection. The study by Torriani (2008) included 82 treatment-naïve HIV-infected subjects who participated in a prospective, multicenter study of three class-sparing antiretroviral therapy regimens. Flow-mediated dilation of the brachial artery improved after 4 weeks (+0.74%, $p=0.003$) and at 24 weeks (+1.48%, $p< 0.001$), with similar observations in each arm ($p>0.600$). The improvement of endothelial function with treatment might be another reason why aortic stiffness decreased in HIV positive participants on HAART. Wolf et al in 2002 demonstrated that antiretroviral therapy reduced markers of endothelial and coagulation activation in HIV-infected patients (Wolf et al. 2002). These markers include soluble vascular

cell adhesion molecule (sVCAM-1) and soluble intercellular adhesion molecule (sICAM-1).

Another study by Torriani in 2005 also showed that cumulative PI use is associated with femoral artery stiffness, whereas no such association was found for cumulative NNRTI use. This may suggest that effects of HAART on arterial stiffness vary between different drug classes. Our participants were on NNRTI and not PI. Another controversy about the impact that administering HAART might have on increasing the stiffness of the arterial walls in HIV patients is that of Hulten et al (2009). The meta-analysis involving 5456 HIV-positive and 3600 HIV negative patients did not find an association between SA and HAART (Hulten et al. 2009) while other authors suggest that HAART has a possible protective effect on the development of early atherosclerosis (Ho et al. 2010). On the contrary, others report an increased rate of SA in HIV patients taking antiretroviral therapy administered over a longer period (Lekakis et al. 2009, Van et al. 2009).

Schillacci et al (2005) in their study consisting of 32 HIV-infected patients treated with protease inhibitors and 32 age, sex, and blood pressure–matched HIV-uninfected control subjects, obtained aortic pulse wave velocity and central aortic pressure waveform, from which aortic augmentation was calculated. HIV patients had a higher aortic pulse wave velocity (7.6±1.1 versus 6.8±1.2 $m \times s^{-1}$, P=0.015) and aortic augmentation (6.8±5 versus 4.6±4 mm Hg, p=0.037) than control subjects. In this study, the aortic pulse wave velocity was (6.84±1.17 vs 6.38±1.67,p=0.037) in HIV treated positives, although participants in this study were on first line antiretroviral therapy used in our public hospitals.

The relative contributions of conventional cardiovascular risk factors, metabolic side effects of antiretroviral drugs, and HIV infection itself on cardiovascular risk are difficult to identify (Phillips et al. 2008, El -Sadr et al. 2006). These cardiovascular risk factors include; genetic, smoking, lack of exercise and diet.

5.5. Impact of cardiovascular risk factors on the arterial wall

Smoking, lack of physical exercise, obesity were some of the factors considered in the questionnairres administered to the participants in this study as these are considered as traditional risk factors. 17.24% of the HAART naïve HIV positive participants smoked whilst only 2.8% of HIV participants who were on treatment smoked. This difference might be because the participants on treatment were always counselled not to smoke.

In a review by Safar in 2003 the prognostic importance of PWV has been attributed to it being an integrated measure of the impact of cardiovascular risk factors on the arterial wall and to adverse hemodynamic effects of aortic stiffening (Safar 2003). One of the reasons of the increased mortality in younger HIV infected patients on antiretrovirals may be the fact that they developed atherosclerosis early in life which increased the risk of cardiovascular disease (Triant et al. 2007). The HIV or the treatment might have gradually caused some degree of inflammation in the endothelium of the blood vessels as they grow in age. The HIV or the treatment might have gradually caused some degree of inflammation in the endothelium of the blood vessels as they grow in age since they were infected in childhood.

The study by Triant et al (2007) was performed using data from a Massachusetts administrative hospital database including 3851 HIV-infected patients and more than 1 million HIV uninfected patients from 1996 to 2004. The mean MI rates were 11.13 versus 6.98 per 1000 person-years, respectively. The MI rates were higher in HIV-infected patients

in all age groups, with very high rates in older patients. Haemodynamic effects of arterial stiffening include an increase in systolic blood pressure and pulse pressure with increased systolic load and decreased myocardial perfusion pressure (Najjar et al. 2008).

The prevalence of some risk factors may be higher in HIV-infected populations, however, in the D:A:D (Data Collection on Adverse Events of Anti-HIV Drugs) study involving 23,468 HIV-infected persons, at baseline, 11.4% had a family history of coronary disease, 1.4% had a prior history of coronary disease, 51.5% were current smokers, 3.5% had a body mass index greater than 30 kg/m2, 8.5% had hypertension, 2.5% had diabetes, 22.2% had elevated levels of total cholesterol, and 33.8% had elevated triglyceride levels (Friis-Moller et al. 2003). A recent analysis of modifiable risk factors and death in the D:A:D study showed that smoking (rate ratio, 1.20), hypertension (rate ratio, 1.53), and diabetes (rate ratio, 1.83) were independently associated with risk of death during treatment for HIV infection (Smith et al. 2009).

5.6. Arterial stiffness and Inflammation

Inflammation refers to the complex cascade of events that happen when the immune system recognizes a threat and goes into action, including migration and activation of various types of white blood cells (leukocytes) and release of chemical messengers known as cytokines. The word ‘inflammation’ often brings to mind the immune system’s immediate response to acute injury or infection. Inflammation can become chronic if the trigger persists, or if suppressive control mechanisms do not work properly.

In this study, IL-6 and E-selectin in HIV positives not on HAART were significantly and positively correlated with PWV ($P<0.05$). This means that these cytokines increase with increase in arterial stiffness in treatment naïve HIV positives.

With multiple linear regression excluding confounding factors, TNFα is one of the variables that was identified (negative correlation) as independent and significantly associated with increase in AIx(75) among HIV positive participants on HAART. One should bear in mind that Tnfα is both anti and proinflammatory. These observations were corroborated with increases in their values compared to controls and their significant values in univariate analysis. Although there were no differences in the inflammatory markers between the genders and the ages, infection with HIV might have resulted in dysregulation of the cytokine profile in vivo and in vitro. In an unpublished study by the author with over 300 participants it was found that E- selectin was significantly increased in HIV positive participants.

Cytokines play an important role in controlling the homoeostasis of the immune system (Krishnaswamy et al. 2000). During the course of HIV-1 infection, proinflammatory cytokines (IL-1, IL-6, IL-8) and tumour necrosis factor (TNF)-alpha, are increased. As stated earlier, cytokines were increased in this study but not significantly in the HIV positive participants. This abnormal cytokine production may contribute to the pathogenesis of the HIV disease by impairing cell-mediated immunity. A number of cytokines have been shown to modulate in vitro HIV-1 infection and replication in both CD^4 T lymphocytes and cells of macrophage lineage. HIV inductive cytokines include: TNF-alpha, and IL-6, which stimulate HIV-1 replication in T cells and monocyte-derived macrophages (Kedzierska et al. 2001).

It has also been shown that HIV infection induces increased production of TNFα by macrophages. The immuno-regulatory response of the host influences the pathogenesis of HIV-1 infection, triggering monocytes, macrophages, and natural killer cells to produce TNFα (Alfano & Poli 2005). In this study, the Tnfα is increased in HAART naïve positives compared to the other groups but there was a negative correlation with AIx(75). This may be because it is both antiinflammatory and proinflammatory. TNFα is an acute phase protein which initiates a cascade of cytokines and increases vascular permeability, thereby recruiting macrophage and neutrophils to a site of infection. TNFα secreted by the macrophage causes blood clotting which serves to contain infection, but high levels of TNFα correlate with increased risk of mortality (Rink & Kirchner 1996). Exogenous and endogenous factors from bacteria, viruses, and parasites stimulate production of Tnfα and other cytokines. Tnfα exhibits chronic effects as well as resulting in acute pathologies.This relationship suggests that reducing Tnfα levels may also reduce occurrence of HIV - 1 viraemia. In excess, Tnfα may cause severe inflammatory damage and toxicity, making control of its production and secretion highly important. Regulating its release serves as a potential means of therapy for HIV-1 and other diseases. Tnfα can also induce other pro-inflammatory cytokines such as IL-6 and IL-8 (Fernandez-Ortega et al. 2004). Studies have also shown the ability of Tnfα to stimulate production of anti-inflammatory cytokine IL-10, preventing further inflammation by causing Tnfα inhibition (Leghmari et al 2008). TNFα is secreted during the early phase of acute inflammatory diseases. Its pathogenic role in HIV-1 infection involves activation of nuclear factor ķB, stimulating apoptosis of T lymphocytes (Fernandez-Ortega et al 2004).

In this study there is a depletion of lymphocytes and absolute CD4 counts in HIV participants who are HIV positive. Tissue and plasma samples of hosts express high levels of TNFα contributing to fever, anorexia, and other symptoms of HIV/AIDS. In a pilot study of patients with untreated HIV infection, 8 weeks of treatment with the tumor necrosis factor inhibitor pentoxifylline resulted in improvements in the endothelial activation marker VCAM-1 and brachial artery flow-mediated dilation (Gupta et al, 2008). A study of women treated for cervical intraepithelial lesions showed that after treatment, there were increased levels of genital HIV, TNF-α, IL-6, and other activation markers in cervicovaginal lavage (Spear et al 2008). In univariate analysis, genital tract HIV RNA was significantly associated with plasma HIV RNA and several of the cytokines (Spear et al. 2008).

Many data sustain the role of a persistent low grade inflammation in the development of large arteries stiffness (McEniery & Wilkinson 2005, Loscalzo & Welch 1995, Moncada 1999). Chronic inflammation resulting from HIV infection may therefore explain the association between HIV infection and increased arterial stiffness, as has been shown in this study. In bivariate/univariate analysis Tnfα showed a negative association with PWV. Also in multiple linear regression Tnfα had a negative association with AIx(75).This could be because the TNFα may be proinflammatory or anti-inflammatory depending on the immune status of the HIV positive participants and duration of treatment. In this study none of the participants had detectable viral level on admission to the study.

A study by (Hsue et al. 2004) suggested that chronic inflammation may be a key contributor to the accelerated development of atherosclerosis in HIV patients. They compared carotid intima media thickness and levels of C-reactive protein (a marker of systemic inflammation)

in HIV-positive and HIV-negative patients. The carotid intima media thickness was greater in all groups of HIV patients, irrespective of level of viremia or exposure to antiretroviral therapy, than in healthy controls. In addition, C-reactive protein levels remained elevated in HIV-infected participants regardless of their level of viremia. These findings suggest not only that HIV-associated atherosclerosis is determined by advanced immunodeficiency, high-level viremia, and exposure to antiretroviral drugs, but also that persistent inflammation due to HIV infection may play an important role in accelerated atherosclerosis.

In this study, Crp level was highest in HIV participants on treatment although not significant than the other two groups. Crp is an acute phase reactant protein and could be proinflammatory. It probably helps in phagocytosis of invading organisms. Its increased level seen in participants on treatment might be a protective one. Another reason might be because the group may have other complications due to the treatment like; abnormal fat deposit which in itself is a source of inflammation which might increase the level of Crp.

Little is known about how different antiretroviral drugs affect Crp levels during successful antiretroviral therapy. Recent data from ACTG (AIDS Clinical Trials Group) study involving 5095 subjects demonstrated that Crp levels did not improve during 96 weeks of treatment with efavirenz. Shikuma et al (2009) in their study comprising of mainly women found out that the Crp level rose (Shikuma et al. 2009). Most of my participants were women and probably that is why there was an increase in the level of Crp with treatment.

Despite the potential association of inflammation with increased CVD risk, a number of small studies have not found a strong association between higher levels of high-sensitivity Crp (hsCRP) and IMT. In my study, there was a negative correlation with PWV. The negative

correlation of Crp with arterial stiffness also suggests that it could be protective. This may mean that Crp may be both inflammatory and anti-inflammatory thus decreasing arterial stiffness in HIV participants? Could TNFα and Crp be modulating HIV-1 infection? Hsue et al (2006) found no association of HSCRP or immune activation (CD38+, CD4+, CD8+ cell responses) with IMT, but they reported an association between IMT and cytomegalovirus-specific T-cell responses, suggesting that response to latent or persistent viral infection might be driving a proatherosclerotic response (Hsue et al. 2006).

Endothelial activation may also occur by cytokines secreted in response to leukocyte activation by HIV (Krishnaswamy et al. 2000). Cytokines play an important role in controlling the homoeostasis of the immune system. Abnormal cytokine production may contribute to the pathogenesis of the HIV disease by impairing cell-mediated immunity. A number of cytokines have been shown to modulate in vitro HIV-1 infection and replication in both CD4 T lymphocytes and cells of macrophage lineage. HIV-inductive cytokines include: TNFα, and IL-6, which stimulate HIV-1 replication in T cells and monocyte-derived macrophages (Kedzierska et al. 2001).

HIV infection per se has been attributed to have several pathways of instigating vascular complications. Similar to other chronic infections such as cytomegalovirus and Chlamydia (Zhu et al. 1999), HIV may contribute to the vascular endothelial damage and premature atherosclerosis via sustaining a low-degree inflammation. Secondly, both immunodeficiency and the immune reconstruction following the commencement of the antiretroviral therapy are associated with enhanced T-lymphocyte proliferation and activation that adds to the overall atherogenic pro-inflammatory state.

5.7. Arterial stiffness and aging

In this study participants who were older than 35 years had a greater pulse wave velocity and arterial stiffness than those who are less than 35 years ($p<0.001$). Pulse wave velocity had a positive correlation with age in the HIV negative group ($r =0.606$, $p<0.001$), and with HIV positive participants not on treatment ($r =0.647$, $p<0.001$).

Comparing participants who are over the age of 35 years and those who are older than 35 years all the indices with the exception of the lipid profile increased in participants over 35 years. The indices that increased are; the anthroprometric measurements, blood pressure, blood counts with the exception of CD4, the pulse wave velocity and the augmentation index. The cardiac functions such as as subendocardial viability ratio and ejection duration index were not affected by age.

With multiple linear regression, after excluding confounding factors; age (positive correlation), was amongst the variables identified as an independent and significant variable associated with increase in AIx(75) among HIV positive participants on HAART in this study. Since AIx(75) is an index of arterial stiffness, age is therefore an important factor in the development of atherosclerosis. This implies that as one gets older one is prone to developing atherosclerosis.

These results were not unexpected, as aging produces more collagen than elastin, hence the increase in PWV. An increased arterial stiffness and augmentation index (wave reflection) are now well accepted as the most important determinants of increasing systolic and pulse pressure in ageing societies, thus affording a major contribution to stroke and myocardial infarction (Guerin et al. 2008).

Vascular aging is an independent risk factor for cardiovascular disease, from atherosclerosis to target organ damage, including coronary artery disease, stroke and heart failure. Various strategies, especially controlling hypertension, show benefit in preventing, delaying or attenuating vascular aging (Lee 2010). In children, and in the youth, the arterial wall comprises an orderly arrangement of elastic laminae separated by interconnecting elastic fibers, smooth muscle, collagen fibers, and connective tissue. In older humans, this orderly structure becomes deranged, with thinning, splitting, fraying, and fracture of the elastic laminae and with increase in connective tissue and collagen fibers. This also supports the fact that arterial stiffness increases with aging as in this study.

Some other studies have also referred to arterial stiffness as being affected by age (Charadika et al. 2009, Avolio 1985). The present study showed positive correlations of arterial stiffness with age in the controls and HAART naïve participants. These findings are also in agreement with studies previously reported in HIV children by measuring their carotid arterial stiffness (Ananyeva et al. 1997 & Charadika et al. 2009). They studied 83 HIV-infected children with a mean age of 1.0 +/-3.1 years and 59 controls aged 12.2 +/-2.8 years. Among the HIV-infected children, 48 were receiving HAART (23 including a protease inhibitor). HIV-infected children had increased arterial stiffness compared with healthy children. These changes were more pronounced with increasing age in HIV-infected children particularly in those who were receiving HAART. This study differentiated between HIV positives on HAART and HAART naïve HIV positives. HAART naïve HIV positives had the greatest increase in arterial stiffness. Mitchell et al (2007) in the Framingham study reported that with advancing age, arterial stiffness and wave reflections increase and elevate systolic and pulse pressures.

A study by Monteiro (2012) showed that there was no statistically significant difference in mean PWV values between HIV-infected and uninfected individuals who did not agree with the findings of this study as indicated earlier. On the other hand Monteiro's study concluded that in both HIV infected and uninfected groups, PWV was significantly higher in individuals aged 40 years or older particularly in males, which agreed with this study.

Long-standing arterial pulsation which might be due to aging in the central artery might cause elastin fiber fatigue and fracture. Increased vascular calcification and endothelial dysfunction are also characteristic of arterial aging. These changes lead to increased pulse wave velocity, especially along central elastic arteries, and increases in systolic blood pressure and pulse pressure (Lee 2010). Vascular aging is accelerated by co-existing cardiovascular risk factors, such as hypertension, metabolic syndrome and diabetes. The major parameters to be taken into account, when evaluating the degree of arterial stiffness, are; age, blood pressure, and, to a lesser extent, gender and classical CV risk factors. This is in agreement with this study.

Aging is an important determinant of cardiovascular risk and is associated with a number of changes in the structure and function of the cardiovascular system including the large arteries (Lee 2010). Although the aorta is often thought to be an inert conduit vessel, it plays a vital role in buffering and smoothing the pulsatile nature of blood flow as it travels to the periphery. With age, the aorta stiffens (McEniery et al. 2005;2009), dilates, and becomes tortuous. Such changes lead to an increase in pulse pressure, which places an additional strain on the aorta and limits its buffering capacity.

The stability, resilience, and compliance of the vascular wall are dependent on the relative contribution of its 2 prominent scaffolding proteins: collagen and elastin. The relative content of these molecules is normally held stable by a slow, but dynamic, process of production and degradation. Histological examination of the intima of stiffened vessels reveals abnormal and disarrayed endothelial cells, increased collagen, frayed and broken elastin molecules, infiltration of vascular smooth muscle cells, macrophages and mononuclear cells, and increased matrix metalloproteinases, transforming growth factor (TGF)-β, intracellular cell adhesion molecules, and cytokines (Lakatta 2003). In addition to vessel wall thickening, aging is associated with a gradual increase in central artery lumen diameter; 9% per decade from 20 to 60 years in the ascending aorta (Watanabe et al. 1996), although some recent studies have suggested this does not occur (Mitchell et al. 2003).

Elderly people are predisposed to lose arterial elastic laminae and increase collagen depo. In a study carried out by Guerin et al (2008) stiffness of the whole aorta was greatest in older subjects, and the impact of age was most marked in those older than 50 years of age, in keeping with previous observations (Guerin et al. 2008). This is also in keeping with the observation in this study.

5.8. Arterial stiffness and other pathological conditions

A large number of publications and several reviews reported the various pathophysiological conditions associated with increased arterial stiffness and wave reflections (Oliver 2003). Apart from the dominant effect of ageing, they include physiological conditions such as; low birth weight (Lurbe 2003), menstrual cycle (Giannattasio 1999), CV risk factors such as obesity (Ferreira 2005), smoking (Kool 2005). Other factors that affect arterial stiffness

include; hypertension (Simon 1985), hypercholesterolemia (Wilkinson et al 2002), impaired glucose tolerance, metabolic syndrome, types 1 and 2 diabetes (Schram et al. 2005).

5.9. Blood pressure and arterial stiffness

In this study with HIV negative participants, using univariate analysis, there was a significant correlation between Ppa, MP, Spa, Dbp, and PWV. In HIV positive participants not on HAART and HIV positive participants on HAART, Ppa, MP and Spa were significantly and positively correlated with PWV. In both HIV positive groups, HR and Dbp were not significantly correlated with PWV. With multiple linear regression and the model adjusted for gender, anthropometric parameters, HDL-C, TC, LDL-C, haematologic data, haemodynamic data, cytokines, smoking, alcohol. Spa, age and triglycerides are the only independent and significant determinants of PWV among HIV negative participants.This also shows that systolic aortic pressure, triglycerides and age are strong determinants of PWV in HIV negatives.

Findings from this study confirm the well-established association of PWV with blood pressure. The aortic pulse pressure (Ppa) correlated positively with pulse wave velocity in all the three groups and so does the mean aortic pressure (MP). The systolic aortic pressure (Spa) correlated positively with both the HIV negative participants and HIV positive participants not on treatment in univariate/bivariate analysis. All the blood pressures correlated with pulse wave velocity in all the three groups of participants with the exception of the brachial diastolic blood pressure which correlated with the PWV in participants who are HIV negative.

An elevated central (aortic) pulse pressure as in the case of the HIV positive patients in this study is generally ascribed to increased wave reflection and portrays an unfavorable prognosis. With a higher aortic systolic pressure, and higher mean arterial pressure, these factors might be expected to cause fracture of elastic fibers leading to increased pulse wave velocty.

Using arterial tonometry, the Framingham study group evaluated central (carotid-femoral) and peripheral (carotid-brachial) pulse wave velocity, amplitudes of forward and reflected pressure waves, and augmentation index in 188 men and 333 women who were free of clinical cardiovascular disease, hypertension, diabetes, smoking within the past 12 months, dyslipidemia, and obesity. In their study using multivariable linear regression models, advancing age was the predominant correlate of higher carotid-femoral pulse wave velocity.

Their study is in agreement with this study in which age, blood pressure, triglycerides, heart rate and augmentation index correlated with carotid -aortic pulse wave velocity. With multivariate linear regression, this study also showed only MP and waist circumference as the most important and significant independent determinants of PWV in HIV positive participants not on treatment. Again the mean arterial pressure is being considered as significant in the determination of pulse wave velocity.

Other factors which were considered in this study were the environment, economic status, diet, smoking, and drinking habits. All these are factors that might contribute to arterial stiffness. In the district of Lugalawa (Tanzania), subjects who are used to eating fish show a lower BP and no increase in systolic BP with increasing age in comparison with those of the same genetic strain living some kilometers away and eating a more complex diet (Pavan et al.

1997). In Europe important differences were found in the prevalence of hypertension in a survey representative of the general population recruited from eight countries (Kuznetsova et al. 2002). Therefore, environmental factors play a role in allowing full expression of the genetic potential of individuals to become hypertensive with age. The increase in blood pressure with age should be seen as a sort of adaptation to the environment. In this study the participants come from very poor background eating mostly carborhydrate meals.

The diastolic aortic (central) blood pressure is the blood pressure when the heart is relaxed, measured at the level of the aorta. In normal arteries, when blood is pumped from the heart, the arteries expand (increase in diameter) due to elasticity, and "store" some blood and during diastole, the recoil of the arteries pushes the stored blood through the circulatory tree to give the diastolic pressure. When the compliance decreases, this capacity to "store" blood decreases and thus, the diastolic pressure decreases also.The diastolic brachial (peripheral) blood pressure was lower in the HIV negative participants. This is probably because the compliance of the blood vesel is affected by HIV and therefore the capacity of HIV negatives to store blood in diastole increases more than that of the HIV positive participants. Vessel compliance basically modulates blood pressure to keep it within a relatively narrow range, this is key given that the heart is an intermittent pump. Without compliance, blood pressure will peak and crash with every beat. Diseased aorta are less elastic/compliant, therefore they cannot modulate blood pressure quite well, hence systolic pressure increases and diastolic pressure decreases. Pulse pressure increases and this therefore enhanced arterial stiffness. The positive correlation of the blood pressure parameters with PWV were marked in all the three groups of participants in my study. Using multivariate linear regression in HIV positive participants on treatment, age, MP, HDL-C, and triglycerides were identified as the significant independent determinants of the variations of PWV.

The elevation of aortic (central) pulse pressure, aortic systolic pressure and aortic mean pressure in the HIV participants who are HAART naïve might be because the inflammatory state of the HIV has caused some structural abnormalities to the elastin and collagen fibres of the blood vessels. Arterial stiffening is the principal cause of increasing systolic pressure with advancing years. It is associated with progressive arterial dilation and is due to degeneration of the arterial wall, probably as a consequence of repetitive cyclic stress. It increases systolic pressure directly by increasing amplitude of the pressure wave generated by a given flow impulse from the heart and indirectly by increasing wave velocity so that wave reflection from the periphery occurs earlier, augmenting pressure in late systole (O'Rourke 1990).

Isolated systolic hypertension (defined as systolic blood pressure >140 and diastolic blood pressure <90 mm Hg) and elevated pulse pressure (PP=systolic blood pressure−diastolic blood pressure) are 2 clinical manifestations of decreased vascular distensibility (Dart & Kingwell 2001). The prevalence of hypertension increases with age such that >60% of people older than age 65 years are hypertensive with systolic blood pressure >140 mm Hg and/or a diastolic blood pressure >90 mm Hg; older blacks have a higher prevalence of hypertension than do whites in all age groups (Chobanian et al. 2003), However, unlike younger hypertensive subjects in whom systolic blood pressure, diastolic blood pressure, and MP are all risks for cardiovascular events, isolated systolic hypertension, elevated PP, and increased PWV pose more significance at risks for strokes, myocardial infarctions, heart failure, and overall mortality in older adults (Mitchell et al. 1997). This difference in risk implies a different pathophysiological mechanism for hypertension in younger versus older individuals and perhaps a different therapeutic approach (Darne et al. 1999). In fact, it is

reported that every 2-mm Hg increase in systolic blood pressure increases the risk of fatal stroke by 7% and fatal coronary heart disease event by 5% (Darne et al. 1999).

Chronic elevation of mean blood pressure also leads to thickening of arterial wall, mostly in the intima media. Elevation of peripheral vascular resistance combined with increased arterial stiffness in older subjects leads to development of isolated systolic hypertension. There is growing evidence that response of PP to therapy may also be relevant to outcomes. In post-hoc analysis of Systolic Hypertension in the Elderly Program (SHEP) trial data, widening of PP (>10 mm Hg) on active drug therapy was associated with increased risk of stroke (Vaccarino et al. 2001). Another analysis of the same study showed that the risk stemming from excessive diastolic blood pressure reduction is dose-dependent, with a threshold at ≈60 mm Hg (Somes et al. 2009).

It is important to underscore that a reduction in blood pressure and/or an increase in vascular compliance are associated with a reduction in cardiovascular risk. However, it is often difficult to separate the effects of pharmacological and lifestyle interventions on blood pressure reduction alone from their direct effects on the vascular wall properties. Changes in MP tend to correlate better with changes in arterial compliance than do changes in systolic blood pressure. As highlighted in these clinical and observational trials, interventions that lower blood pressure and that are associated with reduction in cardiovascular risk are associated with a decrease in measures of arterial stiffness (PWV, augmentation index, compliance); however, they may not necessarily have any direct effect on structural components of the vessel wall that contribute to stiffness.

The simple interpretation of systolic and diastolic pressures recorded with a sphygmomanometer as put forth by Mackenzie in 1926 is that "As regards the relative

importance of systolic and diastolic pressures, it may be said that the systolic pressure represents the maximum force of the heart while the diastolic pressure measures the resistance the heart has to overcome (Fisher 1985).

In this study, the diastolic blood pressures were greater in HIV positive participants on treatment. Diastolic blood pressure is more in keeping with arterial tone and happens during relaxation and during this time they would have transferred stress to the aorta. Thus arterial stiffness does not manifest itself much during diastole. Hence it can be said that the systolic pressure is more important in arterial stiffening than the diastolic pressure, so the participants who are antiretroviral naïve are more prone to arterial stiffness. The treatment might have caused the blood vessel to be less compliant and thereby increasing diastolic blood pressure.
The HIV participants on treatment might have had low or normal blood pressure at the beginning of treatment and as treatment advances the duration of treatment might affect the deposition on lipids on the arteries causing a decrease in the diameter of the lumen of the blood vessels. Thus the duration of treatment might have caused diabetes, insulin resistance or even the metabolic syndrome. (Awotedu et al 2010 & Awotedu et al. 2012).

It could be observed from these results that the aortic blood. pressures are better in assessing blood pressure than the brachial blood pressure. Aortic blood pressure is higher in the HAART naive HIV positive participants so also is the MP. The HI virus in these HAART naïve HIV positives might be causing structural deformities in the arterial wall.

It is inaccurate to use arterial stiffness brachial pulse pressure as a surrogate for aortic or carotid pulse pressure, particularly in young subjects because the amplitude of the pressure wave is higher in peripheral arteries than in central arteries (O'Rourke 1990). Pulse wave

velocity is directly related to brachial and aortic pulse pressure, mean arterial pressure and systolic blood pressure. According to Wilkinson (2001), pulse pressure over-estimates central systolic blood pressure and pulse pressure in young subjects.

One would say what matters most is the aortic or systolic blood pressure and pulse pressure but observations from this study has shown the mean arterial pressure is very important and should even take preference over the pulse pressure in the determination of arterial stiffness. Again, this study shows that the HI virus can cause raised systolic blood pressure.

A generally accepted mechanistic view is that an increase in arterial stiffness causes a premature return of reflected waves in late systole, increasing central pulse pressure, thus systolic blood pressure. Systolic blood pressure increases the load on the left ventricle, thus increasing myocardial oxygen demand. In addition, arterial stiffness is associated with left ventricular hypertrophy (Asmaar et al. 1988). The increase in central pulse pressure and the decrease in diastolic BP may directly cause sub-endocardial ischemia (Boutouyrie 1994).

Pressure wave reflection in the arterial system serves two beneficial purposes. When normally timed, the reflected wave returns to the central aorta in diastole and therefore enhances diastolic perfusion pressure in the coronary circulation (Mitchell et al. 2004). Partial wave reflection also returns a portion of the pulsatile energy content of the wave form to the central aorta where it is dissipated by viscous damping. Thus, wave reflection limits transmission of pulsatile energy into the periphery where it might otherwise damage the microcirculation (Woolam et al. 1962). Loss of this apparently protective function of wave reflection could contribute to the pathogenesis of a growing spectrum of cardiovascular and noncardiovascular accompaniments of aging that share a potential microvascular etiology

(Avolio et al. 1983) including white matter lesions of the brain and renal dysfunction (Sutton-Tyrrell et al. 2001).

In the normal arterial system, there is a steep gradient of increasing arterial stiffness moving outward from the heart. In a young adult, pulse wave velocity (PWV), a close correlate of arterial wall stiffness, is only 4 to 6 m/s in the highly compliant proximal aorta and increases to 8 to 10 m/s in the stiffer peripheral muscular arteries. This progressive increase in regional arterial stiffness, together with branching and narrowing of the lumen, creates an impedance mismatch and leads to a partial reflection of the advancing pressure wave (Kannel et al. 1979). Prior studies have shown that central arterial stiffness increases to a far greater extent than peripheral arterial stiffness with advancing age (Kelly & Fitchett 1992). As a result, aortic stiffness may equal or exceed peripheral arterial stiffness in the elderly. This reversal of the normal arterial stiffness gradient may diminish wave reflections and therefore increase transmission of pulsatile energy into the periphery and microcirculation.

5.10. Arterial stiffness and dyslipaedimia in HIV positive participants

In this study, both the means of HDL-C and Total choleterol levels in the HIV participants on treatment were significantly higher than the two other groups (p=.002 and p=.005 respectively). Non-nucleoside reverse transcriptase inhibitors have been associated with elevated levels of high-density lipoprotein cholesterol (Murphy et al. 2003). NNRTI were the antiretrovirals used in this study as in the study by Murphy.

Initiation of NNRTI-based HAART regimen has been shown to result in increases in HDL-C of approximately 40% depending on the agent used, with increases in total cholesterol (Haubrich et al. 2009). This is in agreement with this study in which NNRTI-based HAART

regimen was used and there was increase in HDL-C levels of participants on treatment. Use of NRTI is not free of dyslipidaemia.

Use of the NRTI stavudine has been associated with a worse lipid profile than the nucleotide reverse transcriptase inhibitor (NRTI) tenofovir, with significant increases in total cholesterol, LDL-C and triglycerides (Gallant et al. 2004). This study was started in 2009 and stavudine was amongst the drugs commonly used in the public clinics though at present tenofovir has been substituted for stavudine in cases of severe lipodystrophy. Although these data point to dyslipidaemia induced by HAART from all of the three commonly used drug classes, not all patients responded similarly to antiretroviral regimen.

Shor-Posner et al (1993) and Grunfeld et al (1992) in their study found out that HIV-infected, untreated patients (particularly those with more advanced disease) are more likely to have low total, LDL-C and HDL-C and elevated serum triglyceride (TGs) than HIV-negative controls. This is in agreement with this study which showed that HDL-C and TC were lower in the participants even in the HIV negatives. Lower HDL-C concentrations is associated with higher circulating HIV RNA levels and longer duration of HIV infection (Rose et al. 2006). These HAART naïve HIV positive participants may have the infection for a long time. Effective HAART in the majority of patients suppresses HIV RNA to undetectable levels thus allowing immune recovery, measured by increases in CD4 cell counts. This is usually accompanied by some increases in total cholesterol and LDL-C (Ridler 2003) which some have suggested may be a return to normal.

Many infections favour or are directly implicated with lipid metabolism changes and/or increased risk of coronary heart disease. HIV itself has been shown to increase lipogenesis in

the liver and to alter lipid profile (Melzi et al. 2010). HIV-1 infection causes a specific pattern of dyslipidemia, resulting from a combination of increased production and decreased clearance of lipoproteins.

Molecular mechanisms responsible for the numerous lipid-related disorders in HIV-infected individuals are not well understood. Adipose tissue hosts multiple cell types including monocytes, macrophages, endothelial and vascular smooth muscle cells. These immune cells are functionally active in the adipose tissue and produce numerous cytokines and other regulatory factors that influence lipid homeostasis, regulation of steroid hormones, prostaglandin, and fat-soluble vitamins. These factors also control storage of excess lipids and triglycerides (either normal and abnormal fatty acids) present in the circulation.

Many infectious agents including HIV-1 have profound impact on adipocytes which become dysfunctional and cannot store most lipids that is, triglycerides properly (Melzi et al. 2010). The above factors might also explain the elevated levels of triglycerides and cholesterol found in the HIV positive participants in this study by controlling storage of excess lipids in the circulation.

Riddler et al (2003) evaluated changes in serum cholesterol associated with HIV infection and subsequent antiretroviral therapy. They tested saved blood samples from 50 of 517 male seroconverters from the Multicenter AIDS Cohort Study. The outcome measures were; changes in total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). These parameters were evaluated at six time points over a period of 12 years. After HIV seroconversion, they noted significant declines in TC, HDL-C, and LDL-C. Following the initiation of HAART, increases in TC and LDL-C to slightly above pre-seroconversion levels were seen. The authors concluded that HIV infection

alone results in substantial decrease in TC, HDL-C, and LDL-. They postulated that the post treatment elevations in TC and LDL-C probably represent a return to pre-infection lipid levels. This is in agreement with our study that shows decreased level of HDL-C in HAART naïve HIV positive participants compared to the other two groups (p=0.002) and decreased level of TC also (p=0.05). The decreased level of the lipid profile with the exception of the LDL-C seen in HAART naive participants might be because of the HIV itself or because of wasting as a result of chronic inflammation.

The triglyceride level of HIV participants on treatment correlated positively with pulse wave velocity (r= 0.490; p=.002) so was the total cholesterol level (r=0.370;p=0.019). In a study by Yao et al 2012 they found that elevated levels of circulating triglycerides and increased arterial stiffness are associated with cardiovascular disease. Numerous studies have reported an association between levels of circulating triglycerides and arterial stiffness. They investigated the association between circulating triglyceride levels, the apolipoprotein A-V (ApoA5) -1131T>C single nucleotide polymorphism and brachial-ankle pulse wave velocity (baPWV) by examining data from 4421 subjects aged 18-74 years who were recruited from the Chinese population. baPWV was significantly associated with the levels of circulating triglycerides after adjusting for age, sex, body mass index (BMI), systolic blood pressure, heart rate, waist-to-hip ratio, antihypertensive treatment and diabetes mellitus status. This is in agreement with this study in which with the use of multiple linear reggression Spa, age and triglycerides were the only independent and significant determinants of PWV among HIV negatives. My model was adjusted for gender, anthropometric parameters, HDL-C, TC, LDL-C, haematologic data, haemodynamic data, cytokines, smoking, alcohol.

With HIV positive participants on HAART, age, MP, HDL-C, and triglycerides were identified as the significant independent determinants of the variations of PWV whilst

adjusting for gender, anthropometric data, smoking, alcohol, cytokines, adhesion molecules, total cholesterol, LDL-C, haematological data, CD4 count, and other haemodynamic parameters. Aznaouridis et al (2007) in their study found out that in healthy men, serum triglyceride levels are associated with indices of arterial stiffness and wave reflections, which are important determinants of cardiovascular function and risk (Aznaouridis et al 2007). The importance of HDL-C as a determinant of PWV is emphasized in this study.

HDL-C levels in the HIV positive participants on treament was significantly higher than the other two groups. It could be that the treatment has helped in alleviating arterial stiffness in those on treatment. The cholesterol level in those on treatment was also the highest in the three groups and this is also significant. With HDL-C there was a slight negative correlation with PWV in HIV positive partiipants on treatment. This shows that with increase in HDL there was decrease in arterial stiffness in the HIV positives who are on treatment. This is in agreement with a study by Wang et al (2011) in which HDL-C was independently inversely associated with aortic stiffness and peripheral stiffness (Wang et al 2011).). According to Velagaleti et al (2009) decreased HDL-cholesterol levels are associated with increased left ventricular mass, decreased diastolic function, and a lower ejection fraction in individuals with normal and narrowed arteries, which inevitably leads to increased arterial stiffness.

In my study, triglyceride correlated with aortic stiffness in HIV participants who are on treatment. The result of this study is also in agreement with that of Grunfeld at al (1999) in which he found that HIV infection was associated with elevated triglyceride levels that worsened with progression of HIV-related disease. Serum triglyceride levels are associated with indices of arterial stiffness and wave reflections, which are important determinants of cardiovascular function and risk. (Aznaouridis et al 2007). HIV has also been associated with

dyslipidemia independent of antiretroviral therapy (Riddler et al 2007). This is contrary to the result from this study in which there was no significant association of arterial stiffness with lipids in HAART naïve HIV positive participants.

Patients with hypercholesterolaemia have a higher central pulse pressure and stiffer blood vessels than matched controls, despite similar peripheral blood pressures (Wilkinson et al 2007). These haemodynamic changes may contribute to the increased risk of cardiovascular disease associated with hypercholesterolaemia and their assessment may improve risk stratification. Lipid-lowering therapy, particularly with statins, generally leads to a reduction in arterial stiffness, re-enforcing the concept that stiffness is a modifiable parameter and risk factor. There are a number of potential mechanisms linking arterial stiffness and plasma lipids, including atherosclerosis, changes in the elastic elements of the arterial wall, endothelial dysfunction and inflammation (Wilkinson et al. 2007).

In this study, the group of HIV participants on antiretrovirals had the highest level of cholesterol. Young subjects with isolated hypercholesterolemia have normal or even increased arterial compliance (Lehmann 1992). With progressing age, the relationship between arterial compliance and low-density lipoprotein (LDL) cholesterol becomes negative, as a result of more pronounced endothelial dysfunction (Giannattasio 1996).

Antiretroviral therapy can also contribute to dyslipidemia. Dyslipidemia has been described as being more common and more severe in HIV patients receiving antiretroviral therapy than in patients not on therapy (Aznaouridis et al. 2007). It remains unclear whether the deposition of lipids in the vascular wall and development of atherosclerotic lesions alone contribute to vessel stiffness.

Initial concerns of increased rates of myocardial infarction arising as a result of dyslipidaemia in HIV-infected patients on antiretrovirals have been confirmed by studies such as the D:A:D study (Carr et al. 1998 & Holmberg et al. 2002). D:A:D study is a large, prospective, multi-cohort study that showed associations between exposure to antiretroviral therapy and an increased risk of myocardial infarction (Friis-Moller et al. 2003). In multivariate analyses, for every mmol/L increase in total cholesterol, the relative risk of myocardial infarction increased by a factor of 1.26 (Friis-Moller et al. 2007). In another analysis from D:A:D which included stroke, acute myocardial infarction and invasive cardiovascular procedures there was a similar effect of hypercholesterolemia, with every mmol/L increase in total cholesterol associated with a relative risk of 1.11 of the combined endpoint (d'Arminio et al. 2004).

Although the triglyceride level in HAART naïve HIV positive participant was higher than those on treatment, in this study it was not significant. This is probably because hypertriglyceridemia in untreated HIV-infected patients may be a response to a systemic inflammatory response against persistent viral infection. Triglyceride concentrations, and TG clearance time in untreated HIV-infected patients have been shown to correlate with serum interferon-alpha (IFN-) which is overproduced in HIV infection (Grunfeld 1991). In these untreated patients, the activity of lipoprotein lipase (LPL) and hepatic lipase, which are both involved in TG clearance from the circulation, are decreased compared to controls.

5.11. Heart rate and arterial stiffness

Heart rate was lower in the HIV negative participants in this study. Heart rate did not show any significant changes in both the genders. There was also no significant difference in the

heart rate between the ages below 35 years and above 35 years. Increased heart rate may be an indicator of increased sympathetic tone, which may increase the stiffness of large arteries directly (Michelle et al. 2004). Alternatively, increased large artery stiffness is associated with reduced baroreceptor sensitivity, which could alter sympathetic tone and heart rate (Michelle et al. 2004).

As discussed earlier, elevated heart rate was observed in HIV positive participants compared to HIV negative participants. This chronically elevated heart rate may increase large artery stiffness by accelerating elastin breakdown in the arterial wall. In paced animal models, increased heart rate was shown to increase stiffness of large elastic arteries while having a variable effect on muscular arteries. It could also be that the HIV participants had increased heart rate because the inflammation has caused some arterial stiffness which could alter the sympathetic tone. HIV positive participants on the other hand might have increased heart rate because of their underlying anxiety and the increased heart rate might accelerate elastin breakdown leading to arterial stiffness.

5.12. Augmentation index in the estimation of arterial stiffness

Pulse wave velocity (PWV) and the augmentation index (AIx (75)) are the two major noninvasive methods of assessing arterial stiffness. Some studies suggest that the predictive value of aortic stiffness may be slightly better than carotid artery stiffness, and because of the ease of measurement, aortic (carotid-femoral) PWV has been proposed as the gold standard for arterial stiffness measurement. The fact that PWV is dependent on arterial stiffness is one of the reasons that the augmentation index, which has been correlated with PWV (Yasmin & Brown 1999), has been proposed as a marker for arterial stiffness (Wilkinson et al. 1988).

However, the relationship between PWV and the augmentation index may not be as clear cut as initially believed.

Wilkinson (1998) reported that PWA is a simple and reproducible technique with which to measure PWV and AIx(75). Reproducibility accords with that reported by other workers using different methodologies. Augmentation index using PWA may, therefore, be suitable for large-scale population and intervention studies investigating the clinical relevance of vascular stiffness. The central aortic pressure within the larger arteries - including the brachial and femoral arteries - consists of a forward wave generated during ventricular systole, followed by a reflected wave from the periphery. With increasing arterial stiffness, this reflected wave arrives earlier, augmenting pressure during late systole (Nichols 2002). This can be measured as the ratio of the reflected wave to the pulse pressure ("augmentation index", or AIx(75).

AIx is the most widely researched index of PWA, with several studies indicating that AIx is independently predictive of adverse cardiac events. Since AIx (75) varies with heart rate it is commonly adjusted to a 'standard heart rate of 75 beats per minute' (AIx (75)).

This highly reproducible technique of PWA is based on processing of the pulse wave contour depicting local changes in arterial pressure occurring during one cardiac cycle (Wilkinson 1998). The stiffer the conducting vessels, the faster the pressure wave travels and the earlier it is reflected back. Consequently, it superimposes on the forward-travelling wave of the following cardiac cycle, causing an increase, or augmentation in central systolic pressure and a decrease in diastolic pressure. The augmentation index has also been identified as an independent risk marker of the severity of premature coronary artery disease among men

undergoing coronary angiography (Weber et al. 2004) and as a predictor of cardiovascular disease among healthy individuals (Nurnberger et al. 2002).

In this study the augmentation index was significantly higher in HIV positive partcipants on treatment which also confirms that arterial stiffness is more in this group of HIV positives ($p=0.024$) but the augmentation index correllated positively with PWV in participants who are HIV negative and those who are HAART naïve. With the augmentation index in HIV negatives the multiple linear regression model identified age (positive correlation), height (negative correlation), CD4 (positive correlation) and MP (positive correlation) as the independent and significant determinants of AIx(75), with the model adjusted for gender, smoking, alcohol, weight, waist circumference, hip circumference, waist to hip ratio, lipid profiles, cytokines, adhesion molecules, haematological data, and other haemodynamic parameters.

In HAART naïve HIV positive participants Spa and age were independently and significantly associated with the variations of AIx(75) among HIV positives not on HAART and height was negatively and significantly associated with AIx(75). This implies that the shorter one is the greater the AIx(75). The model in this study was adjusted for gender, smoking, alcohol intake, cytokines, weight, waist circumference, hip circumference, waist to hip ratio, cytokines, CD4, haematological data, lipid profiles, and other haemodynamic parameters.

In HIV positive participants on HAART after excluding confounding factors, height (negative correlation), age (positive correlation), MP (positive correlation) HDL-C (negative correlation), platelets (positive correlation) alcohol intake (excessive consummation associated with positive correlation) and TNFα (negative correlation) were identified as the

independent and significant variables associated with increase in AIx(75) among HIV positive participants on HAART.

The study found an inverse association with multiple linear regression using augmentation index with height in HIV negative participants. Also in women the augmentaion index was greater than that of men. This is probably because of the shorter height of women in the study as the augmentaion index increases as one becomes shorter. This means that shorter people are more prone to arterial stiffness.

Yasmin & Brown (1999) investigated whether there was a correlation between the simultaneous assessments of augmentation index (AIx(75)) and pulse wave velocity (PWV), undertaken by the SphygmoCor system, in 105 offspring (41 men, 64 women) aged 19–71 years, of patients with familial hypertension. Arterial stiffness was measured using the SphygmoCor pulse wave analysis system. AIx and PWV correlated significantly and positively ($r=0.29$, $p<0.005$) and the strength of the correlation was greater when each gender was examined separately. In my study AIx(75) correlated significantly and positively with PWV in the participants using their status and gender.

Yasmin & Brown (1999) observed several-fold higher AIx(75) in women (22.04±12) than in men (8.59±13) ($p<0.001$); the difference could be explained only in part by an inverse regression correlation between AIx(75) and height ($r=-0.45$; $p<0.001$), but not PWV. This is an agreement with this study. The AIx(75) in women in this study was (21.15 ±12.81) compared to that of men which was (14.64± 11.78) ($p<0.001$). The finding of increased AIx(75) in women is also consistent with the published literature by London et al (1996). In the study they showed that multiple regression showed that the effect of the increased

augmentation index is partly explained bydecreased height in women.

The inverse correlation found between AIx(75) and body height in the HIV negative participant and HAART naive HIV positive participants have also been noted in a previous study by Marchais et al (1993). They concluded that it is probably due to earlier reflection of the aortic wave in short people. AIx(75) was also more influenced than PWV by heart rate and blood pressure. It is probable that separate normal ranges should be established for men and women, while further studies determine what parameters other than height are responsible for the gender difference.

Women had larger reflected waves than men in this study hence increased augmentation index. This is in part due to shorter height and closer physical proximity between heart and reflecting sites (Mitchell et al. 2004). However, height was not sufficient to fully explain higher reflected wave pressure in women which is consistent with findings of a prior study of elderly men and women who were matched for height (Gatzka et al. 2001). Thus, there are unexplained differences in arterial structure or function between men and women that lead to increased wave reflection in women hence increased augmentation index.

In this study, there was also a positive correlation between PWV and AIx(75) in both the HIV negative group and HIV positive group not on treatment (r=0.850, p=0.004., r=0.027, p=0.27 respectively). Augmentation index was also significantly higher in participants who are more than 35 years.

Kelly et al (2001) found that the augmentation index correlated with PWV, age and blood pressure in univariate analysis. However, in multivariate analysis it was related only to age.

The investigators suggested that the relationship between the augmentation index and PWV may be stronger when the aorta becomes stiffer, as PWV may not change greatly whilst the aorta remains elastic. In this present study with multiple linear regression, AIx(75) was associated with age, MP, height and excessive consumption of alcohol, and HDL which are significant determinants of augmentation index in HIV positive participants on HAART. All these variables which are associated with augmentation index in HIV positive participants on HAART might have caused increase in the time of reflection. Thus, in an elastic aorta, the AIx(75) is more likely to be related to the intensity of the reflected wave rather than to its velocity.

Overall, HIV participants had greater PWV and augmentation index than those who are HIV negative. This is in agreement by the study reported by Falasca, et al (2012) who compared HIV patients and non infected subjects. Compared with uninfected subjects, HIV-infected subjects in their study had higher, PWV and AIx(75) values.

Sevastianova et al (2005) reported that the duration of antiretroviral treatment affected the augmentation index. The increased augmentation index observed in this study might be due to the duration of antiretroviral therapy to which the participants were exposed.

According to McEniery et al (2005), the AIx(75) might be a more sensitive marker of arterial alteration and cardiovascular risk in younger individuals, whereas the aortic PWV is likely to be a better measure in older individuals.

5.13. Relationship of some cardiac functions to arterial stiffness in HIV

The duration of left ventricular systolic ejection (systolic time interval in milliseconds) can be

measured using PWA. The ratio of the duration of systolic ejection to the total duration of a cardiac cycle is the ejection duration index (ED %). Patients with systolic dysfunction have been found to have a higher ED% than those with diastolic dysfunction (Nichols 2004). The HIV positive participants in this study have higher ejection duration index ED%. The ED% was lowest in the HIV negative participants and highest in those that are not on treatment.($p<0.001$). This might indicate that the HIV positive participants not on treatment are at a higher risk of developing cardiovascular problems such as systolic dysfunction.

Sub-endocardial viability ratio (SEVR), calculated through pulse wave analysis, is an index of myocardial oxygen supply and demand (Tsiachris et al 2010). The SEVR% is highest in the HIV negative participants and lowest in HAART naïve participants. The tonometric sub-endocardial viability ratio (SEVR) is a non-invasive estimate of myocardial perfusion relative to cardiac workload (Chemla et al 2008). It is impaired in the HIV positive participants. In HIV positive participants there was also significant arterial stiffness compared to HIV negative partcipants. This shows that increased aortic stiffness and decreased subendocardial viability ratio predisposes to myocardial ischaemia by increasing the systolic tension–time index and by decreasing aortic pressure throughout diastole. Thus the HIV positive participants are prone to having myocardial infarction or stroke because of the impaired oxygen supply to the heart. From this study, there was also no significant correlation between ejection duration index and subendocardial viabilty ratio with arterial stiffness.

5.14. Arterial stiffness and anthropometry

There is a positive correlation of age, waist, waist to hip ratio, and weight in HIV negative participants with PWV. With HAART naïve participants there is a positive correlation with age ,waist to hip ratio and height. There is also a positive correlation of waist to hip ratio, height, and PWV in participants on treatment.

Although, BP remains the major determinant of arterial stiffness and endothelial dysfunction, waist circumference independently predicts arterial stiffness (Lilitkarntakul 2012). With waist circumference, there was also an association with PWV using multivariate linear regression with the the HAART naive HIV positive groups.

Results of the present study support the concept that central obesity, represented by anthropometric measurement, such as BMI, percentage of body fat, waist circumference, and waist/hip ratio, play an important role in the association between obesity and arterial stiffness. Interestingly, a study showed that BMI, waist circumference, and waist/hip ratio were significant and inverse predictors of arterial stiffness in women, (352 healthy patients, including 200 premenopausal women), whereas in men, only BMI inversely predicted arterial stiffness. The authors suggested that the relationship of arterial stiffness and obesity may be sex specific; however, they indicate the need for further studies to validate their findings (Budimir et al. 2012).

Although a definite mechanism is yet to be elucidated, several factors associated with central obesity may play a role, such as the increased lipolytic activity of visceral adipocytes,increased insulin and pro-inflammatory cytokine levels, greater sympathetic nervous system activity, or even an imbalance between vasodilatory and vasoconstricting substances, all of which have been shown to play key roles in the process of arterial stiffening (Eckel et al. 2002).

For the genders, there was also no significant difference in other anthropometric measurements except for hip circumference and height ($p<0.001$ and $p<0.001$) respectively.

There was a correlation between the waist to hip ratio and arterial stiffness in HIV negative participants, HAART naïve group of participants and HIV positive participants on treatment (r=0.479;p=0.015,r=0.319;p=0.037,r=0.319 p=0.037 respectively). In a study by Recio-Rodriguez et al (2012), measurement of the waist circumference correlated with arterial stiffness relatively to BMI. This was evaluated by PWV and C-IMT respectively.

The waist circumference and the waist to hip ratio of participants on treatment were more than that of the other two groups of the participants. This might imply some degree of lipodystrophy in those particiats who are on HAART. Waist to hip ratio is positively associated with pulse wave velocity in all the groups.

With multiple linear regression waist circumference and MP were identified as the most important and significant independent determinants of PWV in HIV positive participants not on treatment, whist the model was adjusted for gender, weight, height, hip circumference, waist to hip ratio, lipid profiles, haematological data, cytokines adhesion molecules, smoking, alcohol, other haemodynamic parameters and CD4 count. The significant waist circumference seen in this group of participants might be due to metabolic syndrome due to their increased waist circumference and consequently insulin resistance. Fat distribution affects metabolism and cardiovascular risk. Truncal obesity is associated with insulin resistance, dyslipidemia, and hypertension (Awotedu et al 2010). The women generally are often obese in the Transkei region and this may be because of genetic reasons. A study by Sevastianova et al (2005) stated that time since HIV diagnosis, severity of immunodeficiency or presence of HAART-associated lipodystrophy bore no relationship to augmentation index which is contrary to this study.

Zheng et al (2011) reported that obesity was an independent risk factor of PWV, which was an early marker of cardiovascular and renal diseases, among community population in Beijing area. Arterial stiffness and wave reflections are markers of cardiovascular health and outcomes. Corrigan et al (2012) reported that although arterial health is influenced by weight, it is not known whether fat distribution differentially modulates arterial function (Corrigan et al 2012).

5.15. Association of arterial stiffness with CD4 count, macrophages, monocytes, platelets, neutrophils in HIV positive participants

The monocyte levels of the HIV positive participants on treatment correlated positively with pulse wave velocity even though the monocyte levels of the HAART naïve positives were higher and it did not correlate with PWV. Monocytes are the precursors of the lipid-laden foam cells within the atherosclerotic plaque and produce high levels of proinflammatory cytokines such as IL-6. The minor CD14+/CD16+ 'proinflammatory' monocyte subpopulation is preferentially susceptible to HIV infection and may play a critical role in the pathogenesis of HIV-related CAD (Ziegler-Heitbrock, et al 2007).

Monocytes play a critical role in atherogenesis and HIV disease. Monocytes develop initially in the bone marrow, emigrate into peripheral blood, from where they provide routine immunosurveillance or respond to infection/inflammation, and are subsequently delivered to tissues, where they differentiate into macrophages (Auffray et al 2009). In humans, peripheral blood monocytes may be divided into at least two major subsets: a minority subset of monocytes expresses CD16 and has variable expression of CD14, produces more inflammatory cytokines, and expresses higher levels of TLR-4 compared with the majority CD14+/CD16– monocytes population *(*Ziegler-Heitbrock, et al 2007). Monocytes are

precursors of the macrophages within atherosclerotic lesions, including lipid-laden foam cells *(*Ziegler-Heitbrock, et al 2009) , and lesion-associated macrophages represent a major source of a number of cytokines and chemokines that direct monocytes into vascular lesions, thus creating a positive-feedback loop, although the roles of the two monocyte subsets are poorly characterized. Changes that occur in monocytes and macrophages during HIV-1 infection are likely to impact on atherogenic processes.

During HIV infection, monocytes display an activated phenotype, although correlates of monocyte phenotype and cardiovascular disease are poorly characterized. Additionally, HIV interferes with the ability of macrophages to handle excessive cholesterol by inhibiting cholesterol efflux (Mujawar et al. 2006)*;* both of these mechanisms may impact on initiation and progression of atherosclerotic plaques. Hence the association of monocytes in participants who are on treatment, with PWV in this study. The monocytes develop into macrophages which are the precursors of foam cells which now progress to atherosclerotic plaque formation.

There was also a significant positive correlation between the neutrophil count and pulse wave velocity in this study. This could be because the inflammation produced by the HI virus causes increased migration of leucocytes to the site. This was more evident in those on treatment in this study. Consequently the migration of the leucocytes contributed to the arterial stiffness seen in these HIV positive participants. Monocytes are implicated as a viral reservoir based on the detection of infectious virus from monocytes isolated from HIV-positive individuals on antiretroviral therapy (Naif et al. 1998). It appears that CD16- positive monocytes (5% of monocyte population) are both more susceptible to infection and preferentially harbour the virus long-term (Arfi et al. 2008). Blood monocytes and resident

macrophages are important in vivo cell targets for HIV infection and their role in AIDS pathogenesis are well documented (Alfano et al. 2005). HIV infects immune cells of the macrophage and T-cell lineage. It is therefore not surprising that there are increases in the levels of the monocytes and neutrophils in HIV positive participants. These leucocytes also had positive correlation with PWV in HIV positive participants. This means that the increased monocyte count also contributed to arterial stiffness in HIV positive participants.

Kedzierska et al (2005), in their study explained that monocytes, macrophages and dendritic cells play important roles in the initial infection of HIV and contribute to its pathogenesis throughout the course of infection (Kedzierska et al 2005). After excluding confounding factors, platelets (positive correlation) was also amongst the variables identified as independent and significant variable associated with increase in AIx(75) among HIV positive participants on HAART in this study.

With multivariate linear regression in HIV negative participants, CD4 was a significant determinant of AIx (75), whist the model in this study was adjusted for gender, smoking, excessive alcohol intake, weight, waist circumference, hip circumference, waist to hip ratio, lipid profiles, cytokines, adhesion molecules, haematological data, and other haemodynamic parameters.

To identify risk factors for augmentation index normalized for a heart rate of 75 beats/min, Hsue et al (2004) devised a multivariate model that weighed systolic blood pressure, diabetes mellitus, hyperlipidemia, family history of heart disease, injection drug use, high-sensitivity C-reactive protein (hsCRP), estimated glomerular filtration rate (eGFR), current CD4 count, duration of HIV infection, antiretroviral therapy, and PI therapy. In that model a nadir CD4

count below 350 was independently associated with 7.2% higher normalized augmentation index, and four traditional risk factors were also independently associated with a higher normalized augmentation index. Hsue and colleagues believe their findings 'suggest that cardiovascular risk among HIV-infected individuals could be reduced through early initiation of antiretroviral therapy, before CD4 T-cell counts are depressed'. They called for prospective studies to test this hypothesis.

Kaplan et al (2011) among a cohort of HIV infected women studied the association of carotid artery stiffness with expression of markers of T cell activation. They suggested that activation of CD4+ T cells is associated with increased vascular stiffness among HIV infected women (Kaplan et al. 2011). Progressive depletion in numbers of circulating CD4+ T cells occurs in almost all cases of untreated HIV infection. The number of circulating CD4+ T cells is widely used as a measure of global 'immune competence' and provides a predictor of the immediate risk for opportunistic illnesses (Masur et al. 1989). CD4 levels are reduced in the HIV positive participants taking part in this study with level being lowest in those not on treament. There was negative correlation of CD4 count with pulse wave velocity. This means that as the CD4 count decreases arterial stiffness increases.

Another recent study showed that arterial stiffness was associated with a low CD4 count in HIV infected indivividuals concluding that an early ARV treatment may be of benefit in reducing arterial modifications (Ho et al. 2010). Literature data regarding the effect of HAART on arterial stiffness are conflicting. Several studies have reported an association between HAART and increased arterial stiffness in HIV infected patients (Boccara et al 2006). Other studies found no influence of HIV specific antiretroviral therapy on arterial stiffness (Bonnet et al 2004), or a possible favourable interaction related to its anti-

inflammatory effect and to the reduction of immunosupression (Ho et al 2010).

Other studies showing conflicting results are those that reported an association between a lower nadir CD4+ T cell count and arterial stiffness (Schillaci 2005) while other reported no association (Sevastianova 2004). The results of a more recent trial showed that the nadir CD4+ T-cell count, as represented by a nadir CD4+ T-cell count below 350 cells/mm^3, is a predictor of PWV (Ho et al. 2010). The association appeared to be independent of other factors.

In a study by Omoregie et al (2009) in Benin City, they found a significant reduction in CD4 count in HIV seropositive patients compared with their seronegative counterparts ($p < 0.001$). They concluded that HIV attacks and destroys cells with the CD4 antigen and this explains why HIV-positive patients had lower CD4 counts than HIV-negative individuals.

Like the CD4 count, the platelet count was significantly lower in HIV seropositive patients ($p=0.001$). Impaired thrombopoiesis and production of anti-platelet antibodies have been suggested as possible mechanisms. Impaired thrombopoiesis can result from infection of megakaryocytes by HI virus because megakaryocytes possess CD4 and CXCR4, which are known receptors for HIV, and various megakaryocyte lines are infectable with HIV. Also in this study, there may be endothelial activation by the cytokines in response to the leucocyte activation by HIV since the monocyte, neutrophils are higher in HIV positive participants with depletion of their correponding lymphocytes. Thus the increase in the cytokine levels in the HIV positives might be because of their response to leucocytes activation by HIV.

5.16. Proposed mechanism of arterial stiffness in HIV positive participants

The mechanism of increased arterial stiffness in HIV participants that took part in this study is proposed as follows. Injury to endothelial cells by the HI virus perpetuated a local inflammatory response that promoted thrombosis, impaired vessel responsiveness. HIV replication may activate endothelial surfaces directly or via up-regulation of pro-inflammatory cytokines (Ren et al. 2002)

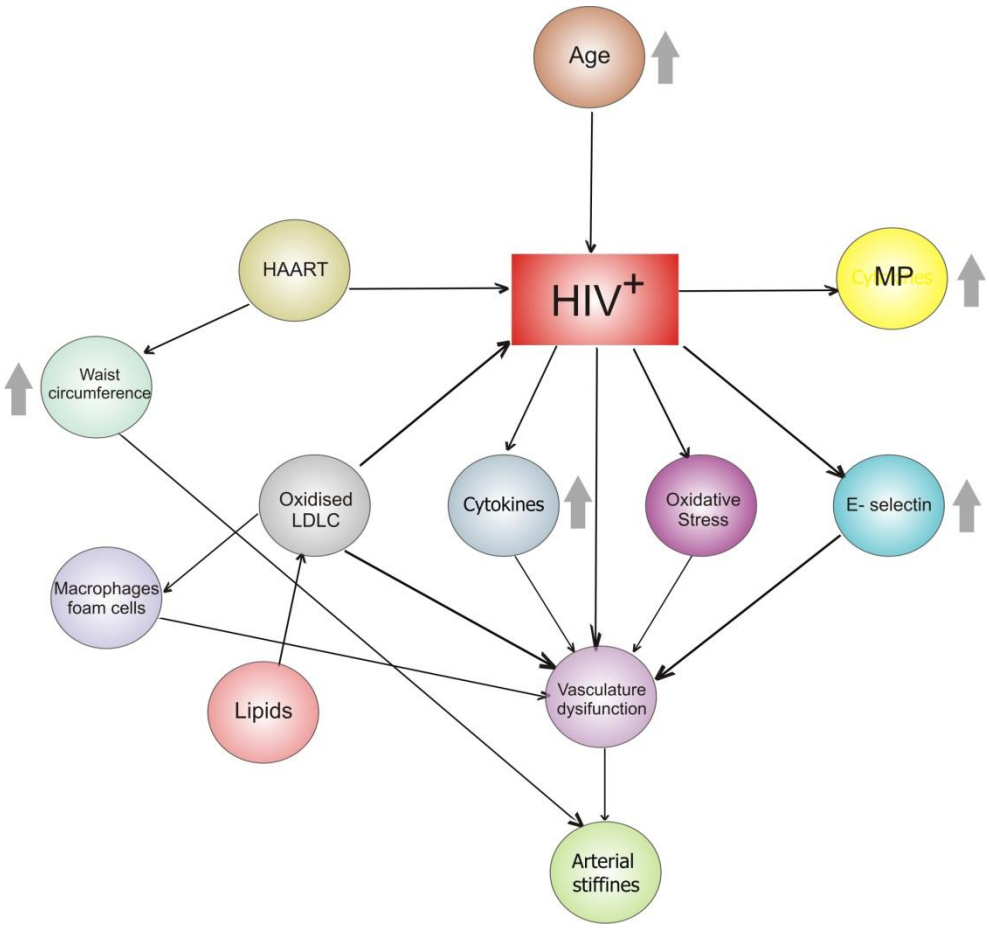

Figure 79. Integrated summary of some cardiovascular risk factors affecting the vasculature

Endothelial cell adhesion molecules (intercellular and vascular cell adhesion molecules; ICAM and VCAM respectively) such as E-selectin are expressed by endothelial cells in response to leukocytes and cytokines which were also elevated in HIV positive participants

when compared to HIV negative controls although not significantly. E-selectin was elevated in treatment naïve HIV positive paricipants in a yet to be published work by the author. This study involved over 400 participants and funded by the medical research council of South Africa . This is in agreement with previous work by Leeuwenberg (1999). Endothelial cell activation, though a normal physiological response to inflammation, can lead to vessel damage and dysfunction with chronic stimulation (Blann &Taberner 2000). Studies using a murine AIDS model have demonstrated that vascular dysfunction was correlated to oxidative stress and endothelial activation (Balga et al. 2005). Hence the oxidative stress seen in the map. HIV has been reported to infect smooth muscle cells in vitro and in vivo and increases secretion of a monocytes chemoattractant (CCL2, or MCP-1), which facilitates development of foam cells (Eugenin et al, 2008). Macrophages, which play a pivotal role in atherosclerosis, are also hosts for HIV. The HIV Nef protein impairs the adenosine triphosphate binding cassette (ABCA-1) transporter in macrophages, which is important to reverse cholesterol transport. This inhibition may lead to conversion of macrophages into foam cells and initiate plaque formation in vessel walls (Mujawar et al. 2006). HIV may also directly impair HDL metabolism, thus enhancing transfer of HDL to atherogenic apolipoprotein B lipoproteins (Rose et al. 2008). Collectively, these findings suggest that untreated HIV infection could contribute to the development of dyslipidemia hence atherosclerosis.

Another mechanism could be that the normal healthy endothelium regulates vascular tone and structure and exerts anticoagulant, antiplatelet, and fibrinolytic properties. The maintenance of vascular tone is accomplished by the release of numerous dilator and constrictor substances. A major vasodilative substance released by the endothelium is nitric oxide (NO), originally identified as endothelium-derived relaxing factor (EDRF).

Other endothelium-derived vasodilators include prostacyclin and bradykinin. Prostacyclin acts synergistically with NO to inhibit platelet aggregation. Bradykinin stimulates release of NO, prostacyclin, and endothelium-derived hyperpolarizing factor, another vasodilator, which contributes to inhibition of platelet aggregation. Bradykinin also stimulates production of tissue plasminogen activator (t-PA), and thus may play an important role in fibrinolysis.

The endothelium also produces vasoconstrictor substances, such as endothelin (the most potent endogenous vasoconstrictor identified to date) and angiotensin II. Angiotensin II not only acts as a vasoconstrictor but is also pro-oxidant and stimulates production of endothelin. Endothelin and angiotensin II promote proliferation of smooth muscle cells and thereby contribute to the formation of plaque. Activated macrophages and vascular smooth muscle cells, characteristic cellular components of atherosclerotic plaque, produce large amounts of endothelin.

Damage to the endothelium upsets the balance between vasoconstriction and vasodilation and initiates a number of events/processes that promote or exacerbate atherosclerosis; these include increased endothelial permeability, platelet aggregation, leukocyte adhesion, and generation of cytokines. Decreased production or activity of NO, manifested as impaired vasodilation, may be one of the earliest signs of atherosclerosis. Nitric oxide is a pivotal endothelium-derived substance. The hallmark of endothelial dysfunction is impaired endothelium-dependent vasodilation, which is mediated by NO. A defect in NO production or activity has been proposed as a major mechanism of endothelial dysfunction and a contributor to atherosclerosis. There might be a problem with the production of NO due to HI virus disrupting the endothelium as seen in concept map. Nitric oxide mediates endothelium-dependent vasodilation by opposing the effects of endothelium-derived vasoconstrictors such as angiotensin II and endothelin. It also inhibits platelet adherence and aggregation, leukocyte

adhesion/infiltration, and proliferation of vascular smooth muscle cells. Nitric oxide prevents oxidative modification of low-density lipoprotein (LDL) cholesterol.

Oxidation of LDL has been proposed as a major mechanism of the atherosclerotic process. Furthermore, plasma and macrophage content of oxidized LDL in coronary plaques correlate with severity of acute coronary syndrome (Rubbo et al. 2002). Conversely, impaired production or activity of NO such as may be the case of the HI virus leads to events or actions that promote atherosclerosis, such as vasoconstriction, platelet aggregation, smooth muscle cell proliferation and migration, leukocyte adhesion, and oxidative stress. Oxidized LDL cholesterol increases synthesis of caveolin-1, which inhibits production of NO by inactivating eNOS (Endres et al. 1998) . Oxidative stress in this case which migh be the HI virus can also interfere with the production and activity of NO by a number of mechanisms that are independent of LDL. For example, the free radical superoxide anion rapidly inactivates NO and destroys tetrahydrobiopterin, a cofactor required for NO synthesis.

Nitric oxide expression may itself be reduced (d'Alessio et al. 2004). Increased expression of a natural nitric oxide synthase (NOS) inhibitor, asymmetrical dimethylarginine, has been linked to vascular stiffening (Miyazaki et al. 1999). The level of nitric oxide expression may be reduced in HIV positive participants. Bioavailability of nitric oxide is also reduced by activation of reactive oxygen species caused by stress, hormones, and likely AGEs (Taddei et al. 2001) and probably by the HI virus. The formation of peroxynitrite and other highly reactive species results in abnormal vascular tone (Peng et al. 2003).

Although many studies have established a role of endothelial dysfunction in vascular stiffening, recent studies have suggested the opposite holds as well ie, that structural

stiffening could alter endothelial function and thereby worsen stiffening.

It has been shown that HIV infection induces increased production of free radicals by macrophages. Free radical formation occurs as a by-product of oxidative stress. Oxidative stress occurs when there is a disproportion between the reactive oxygen elements in the body versus the ability of the body to properly eliminate these reactive species. The presence of free radicals has been implicated in disturbing and damaging a number of biological processes (Karthikeyan et al 2010). With regards to HIV infection the increase of oxidative stress has been seen to influence components in antioxidant defense in physiological antioxidants such as glutathione which are seen to decrease dramatically in HIV patients (Pace and Leaf, 1995).

In addition to glutathione, vitamin A, C, and E in high doses while improving low levels of selenium were also associated with the prevention of HIV infection progression by working as antioxidants to remove free radicals (Garland and Fawzi, 1999). The progression of HIV is correlated with a decreased immunity. One way in which this decreased immunity progresses is by free radical overload of monocytes and granulocytes which leads to deficiency of antioxidant mechanisms which may lead to the loss of CD4 cells often seen in the progression of HIV (Dobmeyer et al 1997). The decreased immunity may also be related to the reactive oxygen species and free radical presence which is higher in HIV infected patients.

With HIV infection progression there is an increased production of reactive oxygen species which leads to the theory of free radical mediated apoptosis of lymphocytes which reduces the ability for immune response to progressive HIV infections (Dobmeyer et al 1997). In this

study there was depletion of lymphocytes and reduced absolute counts of CD4 cells. With regards to CD4 cell counts the apoptosis of lymphocytes by free radicals leads to progression of immunodeficiency and makes for a quicker transition from HIV infection to AIDS (Bautisita, 2001).

It has been published that during HIV-1 infection, haematopoietic cells are exposed to high amounts of free radicals. Subsequently there is a reduction of leukocytopoiesis and increase susceptibility to further infections (Masutani, 2000). Furthermore, there is a link between lipid peroxidation observed in patients with HIV or AIDS and a deficiency of antioxidants which leads to free radical proliferation (Favieret al 1994). There is no significant difference in the LDL levels in the groups of participants. This could be that some of the LDL particles have undergone oxidation.

It has been suggested for 20 years that oxidative stress, and particularly LDL oxidation, could induce atherosclerosis (Toshima et al 2000), and that markers of LDL oxidation in plasma (circulating oxidized LDL, autoantibodies against oxidized LDL) could be used to assess the development of atherosclerosis in patients.

The HIV positive participants had increased levels of cholesterol, triglycerides, hyperlipidaemia. Hypertension, diabetes and smoking are associated with overproduction of reactive oxygen species or increased oxidative stress. The HIV participants in the study had increased blood pressure.

The body's immune system responds to the damage to the arterial wall caused by oxidized LDL by sending specialized white blood cells (macrophages and T-lymphocytes) to absorb the oxidized-LDL forming specialized foam cells (Lowe et al 2003) . These white blood cells

are not able to process the oxidized-LDL, and ultimately grow, and then rupture, depositing a greater amount of oxidized cholesterol into the artery wall. This triggers more white blood cells, continuing the cycle. Eventually, the artery becomes inflamed. The cholesterol plaque causes the muscle cells to enlarge and form a hard cover over the affected area. This hard cover is what causes a narrowing of the artery, reduces the blood flow and increases blood pressure.

During the course of inflammation, E-selectin plays an important part in recruiting leukocytes to the site of injury. E- Selectin is increased in HIV positive partcipants in this study but not significantly. The local release of cytokines TNF-α by damaged cells as in this study induces the over-expression of E-selectin on endothelial cells of nearby blood vessels according to (Collins et al 1991). Leukocytes in the blood expressing the correct ligand will bind with low affinity to E-selectin, also under the shear stress of blood flow, causing the leukocytes to "roll" along the internal surface of the blood vessel as temporary interactions are made and broken. As the inflammatory response progresses, chemokines released by injured tissue enter the blood vessels and activate the rolling leukocytes, which are now able to tightly bind to the endothelial surface and begin making their way into the tissue. This will now cause an increased expression of adhesion molecules and inflammatory cytokines, namely, of tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), monocyte chemo attractant protein-1 (MCP-1) and von Willebrand factor (VWF), which are all molecules involved in the recruitment and adhesion of leukocytes at sites of atheroma initiation (Deanfield et al 2007). This will now initiate the formation of plaques.

HIV positive patients who take combination antiretroviral therapy (cART) to combat HIV

infection could see the numbers of CD4 cells in their immune system rise to concentrations found in HIV negative individuals, if they remain on the therapy for long enough and their HIV viral load load is suppressed to below 50 copies per ml (Science Daily, 2007). HAART may also lessen the effect on endothelial dysfunction due to the cardiovascular risk factors.

An increase of approximately 26% of the risk for myocardial infarction has been reported in patients on HAART (Fris Moller et al 2003). The detrimental effect of HAART on the arterial wall properties (Charadika et al 2009(b)) has been proposed as an underlying mechanism, while it has been documented that HIV infection per se may promote atherosclerosis through immunodeficiency, chronic inflammation progress, viral load, and endothelial cell dysfunction, and either directly or indirectly via metabolic risk factors (Coll et al 2007). The exact pathophysiological events underlying the development of metabolic changes in HIV-infected patients are still under investigation.

Recently, in a study by the National Institute of Allergy and Infectious Diseases (NIAID 2011) which is part of the National Institutes of Health, men and women infected with HIV reduced the risk of transmitting the virus to their sexual partners by taking oral antiretroviral medicines when their immune systems were relatively healthy. The results are the first from a major randomized clinical trial to indicate that treating an HIV-infected individual can reduce the risk of sexual transmission of HIV to an uninfected partner.

The WHO (July 2012) has issued its first guidance to nations considering providing ARVs (antiretrovirals) to HIV-negative, high risk people. WHO says its recommendations were based on human trials which showed that administering PrEP (pre-exposure prophylaxis) once daily to HIV-negative individuals who are at high risk of becoming infected, is both safe

and effective in helping prevent HIV infection

5.17. Limitations of the study

This study has some limitations. First, the cross-sectional design did not allow the investigation of some abnormalities in arterial properties. Longitudinal studies are needed to clarify this. Also matching for age and gender and other potential risk factors was not successful in all cases. The study started with 300 enrolled participants only 169 finished although this was anticipated. Although the duration of antiretrovirals uses were asked in the questionnaire because many of them were not given exact date it was not used in the study as some of the dates were not recorded. Exercising habit was recorded but was not included although all potential cofounders in the analysis were then considered.

5.18. Perspectives

The results of the present study provide evidence that aortic stiffness is increased in HIV treatment-naive patients free from cardiovascular disease. These findings suggest HIV infection as a potentially relevant contributor to arterial stiffness and provide a conceptual background for the increased cardiovascular risk observed among HIV-infected individuals regardless of antiretroviral treatment. Close, noninvasive evaluation of preclinical atherosclerotic disease should be considered for HIV patients, especially those with additional risk factors for cardiovascular diseases, with the aim of addressing intensive lifestyle and pharmacological interventions aimed at reducing cardiovascular risk. Statins and aspirin should be added to those with dylipaedimia.

Liver function test and haematological tests including blood cytokines should be evaluated to detect those at risk. Blood pressure, especially MP should be monitored regulaly. More

research should be done on the lipids and cytokines in case one can find an inhibitor to the cytokines which could help in modelling the endothelium.

HIV may also directly impair HDL metabolism, thus enhancing transfer of HDL to atherogenic apolipoprotein B lipoproteins (Rose et al, 2008). Strategies to increase
HDL cholesterol levels in HIV-infected individuals should be investigated In this study, multivariate analysis shows HDL-C as a determinant of arterial stiffness. This potential mechanism is consistent with findings in the recent SMART trial analysis indicating an association of total HDL particles with risk of CVD in patients in the drug-conservation group (Duprez et al, 2009). In particular, risk was elevated in patients with declining HDL cholesterol levels after stopping non-nucleoside reverse transcriptase inhibitor (NNRTI) treatment. MP was also a determinant of arterial stiffness even in those who are HIV negative. More studies on these will be needed in confirming the fact that MP is more important than pulse pressure which used to be a main index of arterial stiffness. HDL-C can also be regarded as anti inflammatory with further research done to confirm this.

CHAPTER 6.

6.1. Conclusion

This study show that HIV infection and HAART increase arterial stiffness. The use of NNRTI and NRTI to treat the HIV patients may increase the risk of cardiovascular disease as indicated by the increases triglyceride levels and waist circumference. On the other hand, from these results, antiretroviral drugs might be protective as their use was associated with reduction of endothelial dysfunction and thereby reducing arterial stiffness. HIV infection increases arterial stiffness, as evident from these results that anti-retroviral naïve HIV positive participants had increased arterial stiffness, increase adhesion molecules, increase cytokine levels. Cytokines levels which were increased in HIV positive participants might explain the fact that cytokines are key modulators of inflammation. blood pressure, cytokine levels.

Shorter people had increased Augmentation index (Arterial stiffness). Arterial stiffness decreases with increased HDL. Also using Augmentation index, arterial stiffness decreased with TNFα increase. This shows that TNFα is both anti inflammatory and proinflammatory.

The management of cardiovascular risk factors among the HIV-1 infected participants needs to be improved. There is continued need for developing less harmful, better tolerated, and

effective treatments for HIV-1 infection. This should be with a better knowledge of pharmaceutical and non pharmaceutical measures directed at reducing cadiovascular risks by physicians involved in the management of HIV pateints.

The sphygmocor is an accurate, non invassive and useful tool in the evaluation of arterial stiffness and its use in clinical practice should be encouraged. PWV and the augmentation index (AIx(75) are the two major non-invasive methods of assessing arterial stiffness. Because some studies suggest that the predictive value of aortic stiffness may be slightly better than carotid artery stiffness, and because of the ease of measurement, aortic (carotid-femoral) PWV has been proposed as the gold standard for arterial stiffness measurement.

Life style modification should be incorporated into the management of HIV patients so as the continuous monitoring of their haematological and lipid profile.

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Questionnaire

Cardiovascular risk factors in HIV positive patients and HIV naïve patients

a) Name/Igama

.....

b) Sex/Isini

Female/ umfazi	
Male/indoda	

c) Date/umhla

.....

d) Where do you live/uhlala phi?

.....

e) Phone number----------

f) Are you employed? Uyasebenza?

yes	
no	

g) How old are you? mingaphi iminyaka yakho?

.....

h) Approximately how much do you earn? Urhola malini?

.....

i) What is your favorite food? Kukuphi ukutya okuthandayo?

Junk food/izinto ezimnandi	Meat/inyama	Fried food/ukutya okuqhotsiweyo	Vegetables/imifuno	Millie-samp/umnquso	umvubo

j) How many teaspoonfuls of sugar do you consume pr day/ugalela iteaspoon ezingaphi zeswekile?

.....

k) Do you consume alcohol? Uyabusela utywala?

yes	
no	

l) If yes which one of the following drinks do you consume? Kokuphi utywala obuselayo?

Whisky	Brandy/ibranti	Beer /ibhiya	Seders /isiphuzo esibandayo	Wine/iwayini	Chibuku/umqomboti

How many bottles or glasses/usela iglasi zibengaphi okanye ibhotile ezingaphi?

m) Do you smoke? / Uyatshaya?

Yes	
No	

n) What do you smoke? Utshaya ntoni?

Cigarettes /icuba	Cigar /	Pipe /inqawa	Boxery/iboxari

o) How many loose cigarettes or packets of cigarettes do you smoke a day/ utshaya amacuba amangaphi ngemini?

……..

p) Do you exercise? Uyazilolonga?

yes	
no	

q) What type of exercising do you do? Uzilolonga njani?

Walking /ukuhamba	Running /ukubaleka	Cycling /ibhaysekile	Dancing /umdaniso	Sport

r) How often do you take exercise? Uzilolonga kangaphi?

Everyday /qho ngemini	Three times a week/kathathu ngeveki	Once a week/kanye ngeveki

s) How many hours do you sleep in a day? Ulala iyure ezingaphi ngemini?

1-3 hours	4-6 hours	7-9 hours	10 and above

t) What are your hobbies? Ziziphi izinto ozithandayo?

Watching TV/ukubukela umabonakude	Listening to music/ukumamela umculo	Reading/ukufunda	Traveling/ukuhamba	sport	Dancing/umdaniso	Performing/ukuperfoma

u) Are you sexually active? Uyabelana ngesondo?

yes	
no	

v) How many partners do you have? Mangaphi amaqabane wakho?
.....

w) Do you have any health problems that you know? Ingaba kukhon ukuhkathazeka kwempilo apha emzimbeni wakho okwaziyo?

Yes	
No	

x) What is the health problem that you have? Ikuhlupha njani impilo?

Hypertension/i-hayhay	Diabetes/iswekila	Cardiovascular disease/isifo sentlizi yo	Tuberculosis/isifo sepheph a	Epilepsy/ ukuxhuzul a	Allergies	Asthma/isifuba	Chest infections/isifo sesifub a	Others/eziny e izifo

y) Do you have any chronic infection recently/Ingaba sikhona isigulo esinganyangekiyo onaso?

z) Do you know your HIV status? Uyasazi isimo sakho segciwane le aids?

yes	
no	

i) If positive, are you on antiretroviral drugs? Uba unalo, zikhona ipilisi ozityayo?

yes	
no	

ii) When were you diagnosed as having HIV/ Ufamanise nini ukuba unayo lentsholongwane ka gawulayo?

iii)) If yes, which type of drug(s) are you using? Ziziphi ipilisi ozityayo?

……

iv) For how long have you been on antiretroviral drugs? Unexesha elingakanani usitye ipilisi?

……

v) When was your last menstrual period? Ugqibele nini ukuya exesheni? ……..

vi) Are you breast feeding? Uyancancisa?.....

vii) Which traditional drugs do you take…….

viii)Anthropometric measurements? Ngawaphi amayeza esintu owasebenzisayo

APPENDIX K

WALTER SISULU UNIVERSITY

DIRECTORATE OF POSTGRADUATE STUDIES

MANDATORY CONSENT FORM: ELECTRONIC THESES & DISSERTATIONS (ETD) AND PLAGIARISM REQUIREMENT (For postgraduate research outputs from 2009 September)

TEMPLATE FOR THE STUDENT AND SUPERVISOR CONSENT FOR PUBLICATION OF ELECTRONIC RESEARCH OUTPUT ON INTERNET AND WSU INTRANET

FACULTY: _____

QUALIFICATION NAME: _____ ABBREVIATION: _____ YEAR: _____

STUDENT'S FULL NAME: _____ STUDENT NUMBER _____

TYPE OF RESEARCH OUTPUT: RESEARCH PAPER/MINI-DISSERTATION/DISSERTATION/THESIS (TICK ONE)

TITLE OF THE RESEARCH OUTPUT: _____

_____

CONSENT: I HEREBY GIVE MY CONSENT TO WALTER SUSULU UNIVERSITY TO PUBLISH MY RESEARCH OUTPUT FOR THE QUALIFICATION ABOVE ON THE WSU INTRANET AND INTERNET. I CERTIFY THAT TO THE BEST OF MY KNOWLEDGE, THERE IS NO PLAIGARISM IN THE RESEARCH OUTPUT AS SUBMITTED. I HAVE TAKEN REASONABLE CARE TO ENSURE THAT THE RESEARCH OUTPUT MEETS THE QUALITY LEVEL EXPECTED FOR THE PRESENT QUALIFICATION LEVEL BOTH IN TERMS OF CONTENT AND TECHNICAL REQUIREMENTS. I FULLY UNDERSTAND THE CONTENTS OF THIS DECLARATION.

_____ _____

SIGNATURE OF STUDENT DATE

ENDORSEMENTS BY:

SUPERVISOR:

FULL NAME: _____ SIGNATURE: _____ DATE: _____

CO-SUPERVISOR(S):

1 FULL NAME: _____ SIGNATURE: _____ DATE: _____

2. FULL NAME: _____ SIGNATURE: _____ DATE: _____