SUBCLINICAL CAROTID ATHEROSCLEROSIS IN HIV INFECTED PATIENTS AT MBAGATHI DISTRICT HOSPITAL COMPREHENSIVE CARE CLINIC

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DECLARATION

This dissertation is submitted as an extension of a previous study. The title of the parent study is **"Comparing Cardiometabolic Disease in HIV Negative and HIV Positive Individuals at Mbagathi District Hospital (P4/01/2015)"** approved on 18th May 2015, and whose investigators are:

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This study was conducted in the same group of patients and will add more information on cardiometabolic disease in HIV patients.

This dissertation is my own original work and has not been presented for a degree at any other university.

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CONTENTS

LIST OF ABBREVIATIONS

3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ATP	Adult Treatment Panel
AZT	Zidovudine
B-Mode	Brightness Mode
CAC	Coronary Artery Calcium
CCC	Comprehensive Care Clinic
CHD	Coronary Heart Disease
CIMT	Carotid Intima Media Thickness
CMV	Cytomegalovirus
CRP	C-Reactive Protein
CT	Computed Tomography
CVD	Cardiovascular disease
D4T	Stavudine
DBP	Diastolic Blood Pressure
EBCT	Electron Beam Computed Tomography
FPG	Fasting Plasma Glucose
HDL	High Density Lipoprotein
HIV	Human immunodeficiency Virus
IL6	Interleukin 6
IMT	Intima Media Thickness
IVUS	Intravascular Ultrasound
KNH	Kenyatta National Hospital
LDL	Low Density Lipoprotein
MCP	Monocyte Chemoattractant Protein
MDCT	Multidetector Computed Tomography
MDH	Mbagathi District Hospital
MI	Myocardial Infarction

MRI	Magnetic Resonance Imaging
NACC	National AIDS Control Council
NASCOP	National AIDS and STD Control Programme
NCEP	National Cholesterol Education Program
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
PAD	Peripheral Artery Disease
PI	Protease Inhibitor
PLWH	People Living With HIV
SBP	Systolic Blood Pressure
SPSS	Statistical Package for Social Sciences
STI	Sexually Transmitted Infection
TB	Tuberculosis
t-PA	Tissue Plasminogen Activator
VCAM	Vascular Cell Adhesion Molecule
vWF	Von Willebrand Factor
WHO	World Health Organization

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ABSTRACT

Background: With the advent of combination antiretroviral therapy (ART), the survival rate and life expectancy of HIV infected patients has increased, and AIDS related deaths have diminished significantly. However, cardiovascular disease (CVD) is common in persons living with HIV, and contribute to increased morbidity and mortality, with both traditional cardiovascular risk factors and HIV related factors playing a role. There is scarcity of data regarding the burden of atherosclerosis in HIV infected patients in our setting.

Objective: To determine the prevalence of subclinical carotid atherosclerosis using carotid intima media thickness, and explore the related clinical and cardiovascular risk factors in HIV infected patients at Mbagathi District Hospital Comprehensive Care Clinic (MDH-CCC).

Methods: This was a hospital based cross-sectional descriptive study carried out at Mbagathi District Hospital Comprehensive Care Clinic in HIV infected adolescents and adults aged >18 years. Study participants' medical records were examined to obtain socio-demographic and clinical data. Study subjects underwent a clinical assessment of their weight, height, body mass index (BMI), waist circumference, hip circumference, waist hip ratio and blood pressure. Their lowest CD4 count, lipid profiles and blood sugars were recorded. Measurement of the carotid intima media thickness (CIMT) and evaluation of carotid plaque was done using B-mode ultrasound.

Results: Between October & November 2016, 162 HIV infected patients were recruited into the study. 6 patients were ART naïve, 78 were on 1st line therapy and 78 on 2nd line therapy containing a protease inhibitor. Mean age of study subjects was 45.9 years (SD 9.0). 62.3% of the subjects were female. The mean duration since time of HIV diagnosis was 122.9 months (SD 55.9) and the mean duration of ART exposure was 115.5 months (SD 46.6). Mean nadir CD4 count was 142.8 (SD 128.0). 57 persons (35.2%) had hypertension, 20 (12.4%) were smokers, 30 (18.5%) had BMI >30, and 104 (64.2%) had dyslipidemia. 44 persons (27.2%) had evidence of subclinical atherosclerosis, of which 22 were on a 1st line ART regimen and 22 were on a 2nd line ART regimen. Persons with subclinical atherosclerosis as compared to those without, were older (mean age 51 years vs 43 years, $p < 0.001$), were more likely to have hypertension ($p = 0.002$), and had higher total and LDL cholesterol ($p < 0.001$).

Conclusion: In a fairly young HIV infected population, there was a high prevalence of subclinical atherosclerosis. This was largely driven by a high prevalence of traditional cardiovascular disease risk factors. Screening and management of these risk factors should be incorporated in the routine care of HIV infected patients.

BACKGROUND

Sub-Saharan Africa carries 70% of the global HIV burden. Approximately 1.6 million people in Kenya are infected with HIV, with prevalence rates of up to 6% in adults aged 15-49 years.[1]

Cardiovascular disease is the leading cause of morbidity and mortality globally.[2] HIV infection results in a state of chronic immune activation and inflammation. Use of ART has demonstrated impressive clinical efficacy and thus, AIDS related deaths have significantly diminished.[3–5] The increased life expectancy is, however, coupled with a rise in chronic non-communicable illnesses. New concerns have arisen with regards to emergence of cardiovascular disease in HIV, and it has been suggested that viral replication, and the use of ART results in metabolic derangements and dyslipidemias that contribute to increased risk of atherosclerotic vascular disease.[6–8]

Premature atherosclerosis is thought to occur in HIV infected patients according to data from previous published work.[9] Some studies have demonstrated that the atherosclerotic lesions tend to be worse with protease inhibitor therapy.[10,11] Both traditional CVD risk factors and HIV related factors have an implication in initiation and progression of cardiovascular disease. The inflammation and persistent immune activation, along with endothelial dysfunction in HIV can accelerate the process of atherosclerosis. The use of protease inhibitor therapy is associated with lipodystrophy, insulin resistance, type 2 diabetes mellitus and dyslipidemias, which can further contribute to vascular dysfunction.[12] Additionally, low CD4 count, high levels of viremia and opportunistic infections also have a bearing on progression of vascular disease.[13,14]

Nucleoside Reverse Transcriptase Inhibitors (NRTI's) are the backbone of 1st line ART regimens while protease inhibitors are used in 2nd line ART regimens in Kenya.[15] There is a growing number of HIV infected patients in Kenya being switched to 2nd line ART regimens in Kenya due to factors that include treatment failure and drug toxicity with 1st line ART regimens. Kenyan ART guidelines recommend screening for CVD in HIV patients on ART, including assessment of lifestyle, lipodystrophy, routine blood pressure monitoring, sugar monitoring and assessment for dyslipidemia.[15]

Due to the high morbidity and mortality associated with CVD, early detection and management of subclinical vascular disease is desirable to prevent future adverse cardiovascular outcomes.

Subclinical atherosclerosis can be detected via various non-invasive and invasive techniques.[16] B-mode ultrasound of the common and internal carotid arteries is a cheap and reproducible non-invasive technique used to assess thickness of the vascular intima-media complex.[17] This technique has been validated and is in widespread utilization to detect intima-media thickness and presence of plaque. An increased carotid intima media thickness (CIMT) is a surrogate marker for atherosclerosis and a predictor of future cardiovascular events.[18,19] CIMT measurement is thus a useful tool in screening for atherosclerosis, risk stratification for future CVD events[20] and assessment of drug efficacy such as statins and antihypertensive medications.[21,22]

In Sub-Saharan Africa, Ssinabulya *et al* in Uganda reported an 18% prevalence of subclinical atherosclerosis in HIV infected patients.[23] No previous study has evaluated the impact of HIV infection or ART on subclinical atherosclerosis in Kenya. Hence the need for this study to inform clinicians on early identification and prompt management of patients at high risk of CVD events.

1.0 LITERATURE REVIEW

1.1 BURDEN OF HUMAN IMMUNODEFICIENCY VIRUS (HIV)

HIV-1 is the cause of much of the global HIV pandemic. The HIV pandemic probably presents the greatest public health challenge in history. According to a WHO report, approximately 36.9 million people were infected with HIV globally at the end of 2014.[24] Of this, about 25.8 million people live in Sub-Saharan Africa, which represents about 70% of the global HIV burden.[24] In Kenya 1.6 million people are living with HIV according to the HIV and AIDS estimates report of 2014.[1] The prevalence rate of adults aged 15-49 years is 6%. About 33,000 people died of AIDS related illness in Kenya in 2014.[1]

1.2 HIV & CARDIOVASCULAR DISEASE (CVD)

1.2.1 Increased Risk of CVD in HIV

Among the comorbidities associated with HIV and ART, CVD is of special concern. CVD is one of the leading causes of mortality both in the general population as well as HIV infected patients.[25] CVD in HIV may manifest in several ways including pericardial disease, cardiomyopathy, vasculitis, infective endocarditis, pulmonary hypertension, carotid artery disease, peripheral artery disease, coronary heart disease and cerebrovascular disease. Findings from several retrospective studies suggest an increased incidence of cardiovascular end points in HIV-infected patients compared to uninfected individuals.[26–28]

Table 1: Increased risk of CVD hard end points in HIV infected patients

Study	Study Period	Study Design	Number	Major Findings
Obel *et al* [26] (Denmark)	1995-2004	Retrospective case-cohort	3953	Increased risk of hospitalization for CVD after initiation of cART in HIV-infected versus HIV-uninfected patients (RR: 1.4)
Triant *et al* [27] (USA)	1996-2001	Retrospective case-cohort	3851	Increased risk of MI in HIV-infected versus HIV-uninfected individuals (RR: 1.75)
Currier *et al* [28] (USA)	1994-2000	Retrospective case-cohort	28513	Increased risk of CVD in young HIV-infected versus HIV-uninfected individuals
Lang *et al* [29] (France)	2000-2006	Retrospective case-cohort	74958	Increased risk of MI in HIV-infected versus HIV-uninfected men and women with respective standardized morbidity ratios of 1.4 and 2.7
Durand *et al* [30] (Canada)	1985-2007	Retrospective case-cohort	7053	Increased risk of MI in HIV-infected versus HIV-uninfected individuals (RR: 1.72)

1.2.2 Mechanisms of Cardiovascular Disease in HIV

1.2.2.1 Inflammation

Inflammation plays a critical role in the initiation and progression of atherosclerosis, both in the general population and in HIV infection. Vascular endothelial cells may adversely be affected by ongoing active inflammation which results in a pro-thrombotic state. Infection with HIV has been linked to various inflammatory markers, key amongst which include C-Reactive Protein (CRP) and Interleukin 6 (IL6). These markers have been associated with an increased risk of cardiovascular events.

In men, infection with HIV has been associated with a rise in CRP levels when compared with HIV negative controls.[31] Furthermore, it has been documented that elevated CRP levels in HIV infected patients are independently associated with increased overall mortality.[32]

In advanced HIV infection, elevated levels of the pro-inflammatory cytokine IL6 have been observed. In one study, elevated IL6 was associated with unsuppressed viremia (viral load ≥ 500 copies/mL) or a CD4 cell count < 200 cells/mm^3.[33] Elevation of this cytokine has strongly been linked to development of coronary artery disease.[34,35]

1.3.2.2 Endothelial Dysfunction

Endothelial dysfunction plays a pivotal role in the early stages of atherosclerosis. HIV infection and ART may promote vascular endothelial dysfunction. HIV viral proteins including gp120 and the Tat protein are thought to be toxic to vascular endothelial cells rendering them dysfunctional.[36,37] Serum markers of endothelial dysfunction such as monocyte chemoattractant protein (MCP), P-selectin, vascular cell adhesion molecule (VCAM), and von Willebrand factor (vWF) have been found to be elevated in HIV infection.[38] Antiretroviral medications also contribute to abnormalities in levels of markers of endothelial function, such as soluble endothelial adhesion molecules such as P-selectin and tissue plasminogen activator (t-PA).[39–41]

1.3.2.3 Metabolic Derangements & Lipodystrophy

Several mechanisms that trigger the metabolic derangements seen in HIV have been postulated. These mechanisms may be related to HIV infection itself, or effects from exposure to ART.[42]

It has been reported that decreased adiponectin levels in humans is associated with insulin resistance and type 2 diabetes.[43] In HIV infected patients, adiponectin levels have been shown to be low especially in patients with lipoatrophy. The mechanism of this remains unclear, however it has been proposed that diminished adipocyte differentiation may lead to decreased adiponectin levels leading to insulin resistance.[44] Similarly, hypoleptinemia seen in HIV patients has been linked to insulin resistance and diabetes.[42]

ART exposure has also been suggested to result in lipodystrophy. Exposure to certain NRTI's is associated with lipoatrophy[45], while protease inhibitor exposure has been linked to lipohypertrophy.[46]

In Sub-Saharan Africa some studies have reported high prevalence of metabolic derangements and CVD risk parameters in HIV positive patients on both 1st and 2nd line regimens.[47–49]

In a study by Manuthu *et al* in Kenya, patients on 1st line ART regimen containing either AZT or d4T, plus a NNRTI had higher total cholesterol levels and low density cholesterol (LDL-c) compared to ART-naive patients.[47]

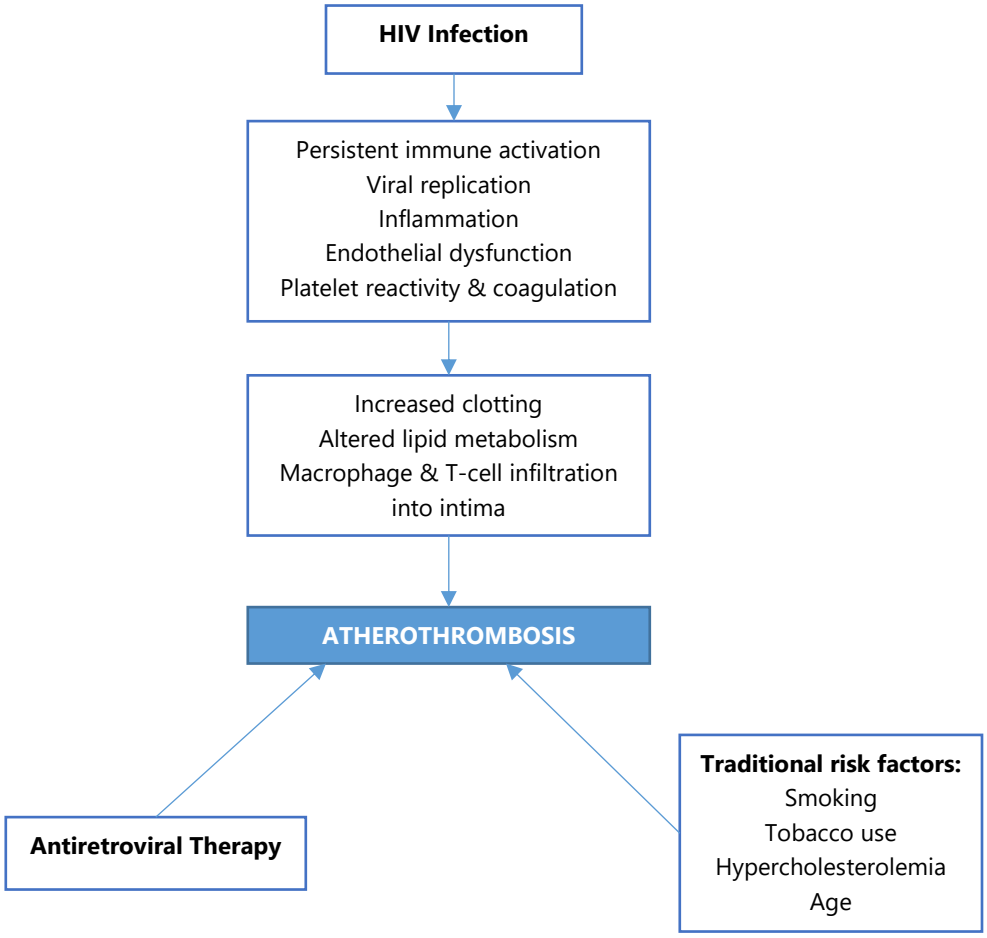
1.3.2.4 Coagulation Abnormalities

Coagulation abnormalities, especially vascular thrombosis, is common in the setting of HIV infection. HIV infection, particularly in advanced stages, is associated with aberrant coagulation resulting in a hypercoagulable state. Low CD4 counts and increasing levels of viremia are correlated with elevated plasma levels of endothelial cell products, including vWF, soluble thrombomodulin, D-dimer and prothrombin in those living with HIV.[33,50–52] These findings suggest that increasing levels of viremia may increase the risk for thrombosis.[52]

Elevated D-dimer levels in HIV is associated with an increased risk of CVD events.[53] Interruption of ART results in elevated fibrinogen levels, which has been associated with increased mortality in HIV-infected patients.[32,54] This suggests that continuing viral replication plays a key role in elevation of the pro-thrombotic molecule fibrinogen.

The impact of ART on coagulation is not clear. In a study by Madden *et al*, it was recorded that PI use was associated with rising fibrinogen levels, whereas the use of NNRTI was associated with lower fibrinogen levels.[55]

Figure 1: Mechanisms and Pathogenesis of Atherosclerosis in HIV



1.3 SUBCLINICAL ATHEROSCLEROSIS

Atherosclerosis is a chronic pathologic process that causes systemic vaso-occlusive disease. It is an intimal disease characterized by the presence of plaques and occlusive lesions. Occlusive lesions of the coronary, cerebral, and peripheral arteries can have deleterious effects and may manifest as ischemic heart disease, stroke and critical limb ischemia or gangrene, respectively. Atherosclerosis is often first detected when patients experience a major cardiovascular event.

Subclinical atherosclerosis is disease that is not severe enough to present with readily observable symptoms. However, several techniques have demonstrated an ability to recognize early atherosclerosis, when still in its preclinical phases. It has been previously recorded from studies

in the USA that approximately 50% of men and 64% of women who die suddenly from CHD had no prior manifestation of atherosclerotic disease, and the majority of these individuals were not classified to be at high risk according to Framingham risk stratification.[16]

5000 adults aged 65 years and above who participated in the Cardiovascular Health Study were assessed for subclinical atherosclerosis. The reported prevalence of subclinical atherosclerotic disease from this study was 36% in women and 38.7% in men. The prevalence was also noted to increase with age.[56] Jaffer *et al* randomly selected 318 asymptomatic subjects from the Framingham Offspring Study cohort and used cardiovascular magnetic resonance imaging (MRI) to evaluate subclinical aortic atherosclerosis and reported a 38% and 41% prevalence in women and men respectively.[57]

The presence of subclinical vascular disease may therefore provide an improved method for quantifying the atherosclerotic burden and predicting risk for acute cardiovascular events.

1.4 DIAGNOSIS OF SUBCLINICAL ATHEROSCLEROSIS

Measurement of atherosclerosis and subclinical atherosclerosis involves a range of invasive and non-invasive techniques.[16] The available techniques can assess various parameters such as luminal diameter, luminal stenosis, vessel wall thickness, plaque size/volume, and the specific localization and distribution of plaques and occlusive atherosclerotic lesions.[16]

Table 2: Characteristics of techniques used for identification of subclinical atherosclerosis

	Technique				
Characteristic	**B-mode ultrasound**	**Intravascular Ultrasound**	**Coronary Angiography**	**MRI**	**Electron Beam CT**
Invasive	No	Yes	Yes	No	No
Primary Measure	Intima media thickness (IMT)	Plaque volume and composition	Stenosis	Plaque volume and composition	Coronary artery calcification (CAC)
Plaque Composition	No	Yes	No	Yes	No
Plaque Burden	No	Yes	No	Yes	Yes
Plaque Vulnerability	No	Yes	No	Yes	No

1.4.1 Intima Media Thickness (IMT)

Intima media thickness (IMT) measurements of the carotid artery with B-mode ultrasound can accurately reflect arterial wall alterations occurring due to atherosclerosis. IMT measurements can also provide information on apparently healthy and at-risk populations, and is an acceptable and valid surrogate for atherosclerosis and vascular disease risk.[18,19,58] Serial IMT measurements can also provide data on the efficacy of therapy such as antihypertensive and lipid lowering agents.[21,22,59,60]

High resolution, B-mode ultrasonography is useful in determining the combined thickness of the arterial intimal and medial layers, usually measured in the common carotid artery.[17] The CIMT reflects the atherosclerotic disease burden and has been validated as a measure of the risk for cardiovascular events.[18]

1.4.2 Arterial Stiffness

Large artery stiffness may contribute to pathogenesis of cardiovascular disease. Evidence of preclinical atherosclerosis may be provided by non-invasive measurements of the compliance and distensibility of the large vessels, which can be measured by pulse wave velocity or ultrasound.

Alterations in arterial distensibility, may provide evidence of subclinical atherosclerotic disease.[61]

1.4.3 High Resolution MRI

High resolution MRI is a non-invasive technique that can evaluate atherosclerotic plaque burden by determining the plaque volume, composition and integrity of fibrous cap. Consequently, it also provides a measure of susceptibility to rupture.[62,63]

1.4.4 Coronary Artery Calcium (CAC) Score

Coronary artery calcification (CAC) also reflects coronary plaque burden, and is an independent cardiovascular risk factor.[64] The CAC score is incremental to traditional risk factors, and can add valuable prognostic information when considered in conjunction with traditional CVD risk

factors.[65] The CAC can be detected and quantified by electrocardiogram-gated electron-beam computed tomography (EBCT) or multidetector computed tomography (MDCT).[66,67] The MDCT is generally more sensitive than EBCT and can therefore also be used for detailed studies of the coronary anatomy.

1.4.5 Coronary Angiography and Intravascular Ultrasound (IVUS)

Coronary angiography and intravascular ultrasound (IVUS) are invasive techniques. Angiography is useful in localizing plaques and can estimate degree of luminal stenosis, thus identifies the impact of atherosclerosis on the vessel lumen.[68] As an added advantage, IVUS can reveal composition of plaques in addition to quantifying their size, along the entire thickness of the vessel wall. Consequently, IVUS can provide information about the scale of plaque burden, location and composition of lesions.[69]

1.5 CAROTID INTIMA MEDIA THICKNESS IN DETECTION OF SUBCLINICAL ATHEROSCLEROSIS

Carotid intima-media thickness (CIMT) is a simple, reproducible and economical tool used to assess preclinical vascular disease. It is also an independent predictor of future cardiovascular risk, and can identify vulnerable patients who would benefit from aggressive interventions to prevent CVD events.[18,19,59]

CIMT is a measure of the thickness of the intima and media layer of the carotid artery most commonly assessed by B-mode (brightness mode) ultrasound to detect early atherosclerosis. The use of B-mode ultrasound using linear array transducers at frequency range of 5-15 MHz can be used to visualize the large superficial arteries, particularly the carotids. Longitudinal images are obtained from the near and far walls of the right and left distal common carotid arteries, the carotid bifurcation, and the proximal internal carotid arterial segments. Using standardized ultrasound equipment and protocols and dedicated software for image analysis, the inter-scan and inter-observer reproducibility of CIMT measurement is excellent.(62)

CIMT measurement thus has useful indications including screening for atherosclerosis, risk stratification for future CVD events[20] and assessment of drug efficacy such as statins and antihypertensive medications.[21,22]

1.6 INCREASED CIMT & CVD RISK

Vaso-occlusive cardiovascular events such as myocardial infarction, sudden cardiac death, and stroke are common manifestations of atherosclerotic vascular disease. Thus, it is desirable to accurately identify individuals at risk of such events. Timely management of subclinical disease is therefore necessary before complications develop.

An increased CIMT can be regarded as a surrogate marker of atherosclerosis and therefore, an increased cardiovascular risk. Furthermore, thickening of the intima-media complex is modestly related to coronary atherosclerosis. Studies among the general population have shown a gradual graded increase in CVD risk with increased CIMT.[18,19]

Data from prospective epidemiological studies have shown that an increase in IMT due to cardiovascular risk factors is associated with an increase in relative risk for myocardial infarction and stroke.

The incidence of CVD end points associated with subclinical atherosclerosis has been analyzed in 2 systematic reviews of prospective epidemiological studies. Simon *et al* found a yearly incidence of coronary events to be <1% in the absence of subclinical atherosclerosis. However this figure rose to up to 3% in patients in whom subclinical atherosclerosis was detected.[19] Similarly Lorenz *et al* in his meta-analysis of 8 studies of CIMT and vascular events, reported an increased yearly incidence of both myocardial infarction and stroke in patients with subclinical atherosclerosis.[18] Both authors concluded that early detection of asymptomatic disease may be a valuable screening test in prediction of future CVD events.

It has also been demonstrated that a decrease in IMT following drug therapy is associated with a decline in CVD event rates. In the REGRESS trial, it was observed that statin therapy has a positive impact on the coronary lumen, decreased the intima-media thickness of both the carotid and femoral arteries, and resulted in a lower incidence of clinical cardiovascular events.[59,60]

1.7 SUBCLINICAL ATHEROSCLEROSIS IN HIV

HIV-associated immune activation and inflammation are suggested to contribute to accelerated atherosclerosis and subsequently increased CVD risk among adults living with HIV.[71–73] Generally HIV patients are of a younger age group. Essentially, their short-term cardiovascular

risk is thus low. The burden of CVD in HIV is therefore indirectly assessed using surrogate markers. Several studies have established an increased occurrence of subclinical atherosclerosis in HIV-infected patients. In the general population, thickening of intima media complex, presence of intraluminal arterial plaque and assessment of coronary artery calcification have been associated with coronary atherosclerosis and increased risk of future CHD and CVD events.[74]

Both traditional and HIV related factors have an implication on progression of vascular disease in PLWHIV. These factors are summarized in the table below:

Table 3: Traditional & HIV related CVD risk factors in HIV infection

Traditional risk factors	HIV related risk factors
Older age	Chronic inflammation
Hypertension	Endothelial dysfunction
Dyslipidemia	Low CD4 count
Tobacco use	High levels of viremia
Family history of CVD	Co-infection with other viruses e.g. CMV
Diabetes/IFG	Opportunistic infections
	Metabolic derangements from ART

In HIV infected asymptomatic adults, most studies of IMT of the carotid artery have found an increase in measurement.[9,75–82] In a systematic review of observational studies evaluating subclinical atherosclerosis in HIV patients, Hulten *et al* reported a higher CIMT with HIV compared with HIV negative patients in 11 out of 13 studies.[75]

In a cross-sectional study by Grunfeld *et al*, the mean difference in the CIMT between HIV infected patients and HIV negative controls was 0.148mm.[76] Hsue *et al* also showed a comparable increase in the CIMT and also recorded a higher prevalence of carotid artery plaque with HIV infection.[78]

In Sub-Saharan Africa, Ssinabulya *et al*, recorded an 18% prevalence of subclinical atherosclerosis amongst HIV infected patients in Uganda, by measuring the CIMT. In this study,

traditional CVD risk factors were shown to be associated with subclinical atherosclerosis in the course of HIV disease.[23]

Table 4: CIMT in HIV infected patients

Author	Year	Country	Study Design	No	Major Findings
Hulten *et al*	2009		Meta-analysis	13 published studies	HIV positivity slightly increases CIMT
Grunfeld *et al*	2007	USA	Cross-sectional	827	HIV infection was accompanied by more extensive atherosclerosis measured by IMT
Hsue *et al*	2003	USA	Cross-sectional	211	Carotid IMT is higher in HIV patients than in age-matched control subjects
Mercie *et al*	2002-2003	France	Prospective-cohort	346	Moderate increase in the common carotid artery median IMT by 0.02mm
Currier *et al*	2003-2006	USA	Prospective-cohort	133	Slight increase in CIMT, nadir CD4 count and ritonavir use were predictors of CIMT progression
Ssinabulya *et al*	2012	Uganda	Cross-sectional	245	18% prevalence of subclinical atherosclerosis in HIV infected patients

1.8 SUBCLINICAL ATHEROSCLEROSIS WITH PROTEASE INHIBITOR THERAPY

Most PI's, particularly the older ones such as lopinavir and ritonavir, have unfavorable effects on the lipid profile, resulting in hypercholesterolemia and hypertriglyceridemia.[83] The

dyslipidemias, together with other metabolic derangements associated with PI use has been proposed to result in vascular dysfunction, ultimately leading to atherosclerotic disease.

Findings from several studies suggests increased risk of atherosclerotic vascular disease in HIV infected patients taking a protease inhibitor regimen. In a Swiss study by Depairon *et al*, PI treated patients had increased carotid plaques demonstrated.[10] Maggi *et al* also demonstrated premature carotid lesions in PI treated patients.[11] Johnsen *et al* studied CIMT in HIV infected women and found that PI therapy was associated with an increased CIMT compared to therapy with non PI based regimens.[84]

Conversely, other studies did not demonstrate any significant association between PI use and an increased CIMT. One study concluded that the presence of traditional CVD risk factors in HIV patients overshadowed the impact of PI use.[85] In a systematic review, 12 studies that compared CIMT in HIV infected patients on a PI regimen versus those on a PI sparing regimen yielded no significant difference in the intima-media thickness.[75] Thus, there is need for further prospective studies to determine whether PI therapy is associated with increased CIMT in the long term.

2.0 STUDY JUSTIFICATION

HIV infected patients are at an increased risk of atherosclerosis. There are only limited studies done in Sub-Saharan Africa on CVD risk profiles in HIV infection. These studies suggest increased cardiovascular morbidity and mortality in HIV infection. There is an emerging need to understand the burden and determinants of CVD among people living with HIV (PLWH) in Sub-Saharan Africa.

Prior to this study, no local data existed regarding the burden of atherosclerosis in our HIV infected population. Information from this study, and previous studies that assessed cardio-metabolic disease in HIV infected patients, will guide the need for screening and early diagnosis, with the aim of providing a basis for interventions to reduce cardiovascular morbidity and mortality.

3.0 RESEARCH QUESTION

What is the burden of subclinical carotid atherosclerosis in HIV infected patients at MDH-CCC?

4.0 OBJECTIVES

4.1 Broad Objective

To determine the prevalence of subclinical carotid atherosclerosis and assess selected clinical and CVD risk factors in HIV infected patients at MDH-CCC.

4.2 Specific Objectives

4.2.1 Primary Objectives

1. To determine the prevalence of subclinical carotid atherosclerosis using CIMT in HIV infected patients at MDH-CCC.

2. To determine selected clinical characteristics of HIV infected patients with subclinical carotid atherosclerosis at MDH-CCC:
    a. Lowest CD4 count
    b. Duration of exposure to ART

4.2.2 Secondary Objectives

1. To compare the proportion of HIV infected patients with subclinical carotid atherosclerosis who are ART naïve, on 1st line and 2nd line ART regimens.

2. To compare prevalence of selected traditional CVD risk factors in HIV infected patients who have subclinical carotid atherosclerosis and those without subclinical carotid atherosclerosis.
    a. Dyslipidemia
    b. Diabetes mellitus
    c. Hypertension
    d. Obesity
    e. Smoking

5.0 METHODOLOGY

5.1 Study Design

This study was an extension of a previous study whose title is "Cardiometabolic Disease in HIV Negative and HIV Positive Individuals at Mbagathi District Hospital." This sub-study was carried out on the same cohort of patients of the parent study, in a hospital based cross-sectional descriptive study.

The primary study was a cross-sectional descriptive study carried out at MDH from October 2016 to February 2017, and compared cardiometabolic disease in a cohort of HIV positive and HIV negative individuals, by measuring the FBS, serum creatinine and lipid profile. The sample size of the parent study was 200 HIV negative subjects and 300 HIV positive subjects. Of the 300 HIV positive subjects, 100 were ART naïve, 100 were on 1st line ART regimen and 100 were on 2nd line ART regimen.

5.2 Study Site

The study was carried out at Mbagathi District Hospital Comprehensive Care Clinic, a donor funded clinic with a cumulative number of HIV infected patients as at November 2016 of

approximately 12,500. The clinic runs daily from Monday to Friday, 8am to 5pm. The patients are attended to by trained nurses, clinical officers and a medical officer. The patients also receive nutrition and general counselling. The laboratory is run by a laboratory technologist. Medications are dispensed by pharmacists. The medical records are handled by qualified data clerks and records officers. The average number of patients seen per day is 80. The average number of patients seen on second line ART per day is 5.

5.3 Case Definition

Active case: any HIV infected patient has been on follow-up at the CCC for at least 3 months, and participated in the parent study.

ART naïve patient: an HIV infected patient who has never been exposed to ART.

1st line ART regimen: a regimen which is initiated on a previously ART naïve HIV infected patient.

2nd line ART regimen: a regimen which is administered to a patient after either failure of 1st line therapy, drug toxicity from 1st line regimen or comorbid states that preclude the use of 1st line ART regimens.

5.4 Patient Selection

5.4.1 Inclusion Criteria

- HIV infected adolescents and adults aged >18 years.
- Signed informed consent to participate in the study.

5.4.2 Exclusion Criteria

- Patients with confirmed atherosclerotic vascular disease i.e. those with previous stroke, myocardial infarction or peripheral artery disease.

5.5 Sample Size

Minimum sample size to determine the prevalence of subclinical atherosclerosis in HIV infected patients was calculated using the Fishers formula, as shown below:

$$n = \frac{Z^2 * P(1-P)}{d^2}$$

where:

- n – sample size
- Z – upper limit of normal distribution = 1.96 (95% confidence interval)
- P – estimated prevalence. An assumed prevalence of 18% was used based on a previous study in Uganda by Ssinabulya *et al* [23]
- d – precision (margin of error) = 6%

Therefore n = **158**

5.6 Study Period

Patient recruitment for the study was done over a 2-month period, from October to November 2016.

5.7 Sampling Technique

For adequate representation, study subjects were sampled in 3 groups i.e. ART naïve patients, patients on 1st line ART and patients on 2nd line ART. A minimum of 53 participants were required in each of the 3 groups, to attain the minimum sample size of 158. Study participants were recruited from the cohort of the parent study, which had 100 study subjects in each group. Simple random sampling technique was used to recruit 53 subjects from the 100 in each group. However, since the introduction of the new Kenyan ART guidelines of 2016 [15] which recommend that all HIV infected patients be initiated on ART regardless of WHO stage or CD4 count, it was noted that majority of the patients who were ART naïve at the time the parent study was done had already been initiated on ART. As such only 6 patients of the parent study who were still ART naïve were recruited, and to attain the required sample size of 158, 78 subjects on 1st line ART and 78 subjects on 2nd line ART had to be recruited.

5.8 Recruitment Procedure

Study subjects were recruited from MDH CCC by the principal investigator and research assistant. Records of the parent study were used to obtain contacts of study participants. Participants of the parent study were contacted by phone and briefed about the new study, and invited to participate in it. A screening proforma (Appendix 1) was used to obtain demographic data and assess eligibility of the subjects to participate in the study.

Patients who fulfilled the inclusion criteria, and provided informed consent were recruited into the study. A questionnaire (study proforma) was administered to the recruited subjects by the principal investigator and research assistant. The principal investigator performed a physical examination on the participants, who were then subjected to CIMT measurement using B-mode carotid ultrasound imaging by a radiologist.

6.0 DATA COLLECTION

6.1 Clinical Methods

A study proforma (Appendix 4) was used to obtain demographic data, and a complete medical history from the enrolled patients was obtained to clinically characterize the patient with regards to HIV infection and CVD risk.

Relevant medical history to assess CVD risk i.e. diabetes, hypertension, dyslipidemia, lifestyle characteristics (e.g. cigarette smoking and alcohol use) and family history of CVD was obtained. The most recent lipid profiles and blood sugar testing done on the study subjects were recorded.

Patients' medical records were studied to obtain data on duration of HIV infection since diagnosis, duration of exposure to ART, previous and current ART regimens and CD4 count trends. Lowest recorded CD4 count were noted. The principal investigator undertook a comprehensive physical examination including anthropometric measurements and blood pressure, using standard procedures:

6.1.1 Height

Standing height was measured once to the nearest 0.5cm barefoot, the back square against the wall tape, eyes looking straight ahead, with a set square resting on the scalp and against the wall.[86]

6.1.2 Weight

Weight was measured once to the nearest 100 grams using a lever balance, barefoot, in light garments.[86]

6.1.3 Body Mass Index

The body mass index (BMI) was calculated using the World Health Organization (WHO) criteria as weight (in kilograms) divided by height (in meters) squared.[86]

6.1.4 Waist Circumference

The waist circumference was measured using a standard tape measure half way between the lowest rib and the iliac crest.[86]

6.1.5 Hip Circumference

Hip circumference was measured as the broadest circumference below the waist using a non-extensible tape.[86]

6.1.6 Waist Hip Ratio

Waist hip ratio is the proportion of the circumference of the waist to that of the hips. This was calculated as waist circumference (in centimeters) divided by the hip circumference (in centimeters).

6.1.7 Blood Pressure

Blood pressure was measured as per WHO recommendations, using a standard adult blood pressure cuff and a manual sphygmomanometer with the subject sitting upright with the back and arm supported.[87]

6.2 CIMT Measurement

All patients meeting the inclusion criteria were subjected to CIMT measurement using standard protocol. Imaging of the carotids was done bilaterally by a consultant radiologist with experience in vascular imaging (assisted by a team of other consultant radiologists) at the Department of Diagnostic Imaging & radiation medicine at Kenyatta National Hospital, using a linear array transducer of 6-15 MHz (General Electric Logiq S7 ultrasound equipment). The carotid arteries were studied with the patient lying supine, the head being in the midline position and neck extended upwards. Adjustment of the probe was done in the longitudinal plane to obtain the

maximal luminal diameter. Images were recorded from the near and far walls of the distal 1 cm of the common carotid and the carotid bulb bilaterally from 3 angles; anterior, lateral and posterior, in accordance with the American Society of Echo guidelines.[88] Images were recorded digitally and the CIMT measurements were recorded using automated software at all the segments. The CIMT was reported as the average of all measurements recorded at the different segments.

6.3 Quality Assurance

All carotid ultrasound scans were performed using a single machine, the General Electric Logiq S7 ultrasound machine. The principal investigator and the radiologists undertook an orientation session from a technician at General Electric Inc. (Nairobi), prior to the onset of the study. The orientation included utilization of the machine and acquisition of images for interpretation and analysis. All ultrasound scans were performed by a radiologist experienced in vascular imaging. CIMT measurements were obtained electronically using automated software.

Every seventh patient was evaluated separately by two radiologists to assess for interobserver variability. 2 different radiologists repeated imaging that they had done on particular patients at earlier dates and the findings were used to determine the intra-observer variability. The role of chance in inter-observer and intra-observer agreement was determined using kappa statistic during analysis.

6.4 Definition of Study Variables

6.4.1 Dependent Study Variables

Subclinical carotid atherosclerosis

Abnormal CIMT and/or the presence of carotid plaque as established on carotid ultrasonography.

- CIMT >0.8 mm
- presence of carotid plaque defined as presence of focal wall thickening i.e. CIMT that is >50% compared to the surrounding vessel wall or IMT of >1.5 mm.[89,90]

6.4.2 Independent Study Variables

6.4.2.1 Hypertension

Study subjects were considered hypertensive if they had a SBP ≥140 mmHg or a DBP ≥90mmHg or if they were on treatment with antihypertensive medications.[91]

6.4.2.2 Diabetes & Impaired Fasting Glucose

Subjects were considered to be diabetic if any of the following was present:[92]

- reported by self as diabetic
- use of hypoglycemic medication
- fasting plasma glucose (FPG) ≥7.0 mmol/L

Impaired fasting glucose (IFG) was defined as FPG ranging from 6.1 mmol/L to 6.9 mmol/L.[92]

6.4.2.3 Dyslipidemia

The NCEP/ATP III guidelines were used to classify study subjects according to lipid profile status:[93]

Total Cholesterol

- <5.17 mmol/L - Desirable
- 5.17 - 6.18 mmol/L - Borderline high
- >6.21 mmol/L - High

LDL Cholesterol

- <2.59 mmol/L - Optimal
- 2.59 - 3.34 mmol/L - Near Optimal
- 3.36 - 4.11 mmol/L - Borderline high
- 4.14 - 4.89 mmol/L - High
- >4.91 mmol/L - Very high

HDL Cholesterol

- <1.03 mmol/L - Low
- <1.29 mmol/L - Optimal
- >1.55 mmol/L - High

Triglycerides

- <1.69 mmol/L - Normal
- 1.69 - 2.25 mmol/L - Borderline high
- 2.26 - 5.64 mmol/L - High
- >5.65 mmol/L - Very high

Dyslipidemia was defined if any one of the following was present:

- Total cholesterol ≥5.17 mmol/l
- LDL cholesterol ≥2.6 mmol/l
- HDL cholesterol ≤1.03 mmol/I
- Triglycerides ≥2.26 mmol/l.

6.4.2.4 Smoking

Study subjects were classified according to their smoking status:[94]

- Current smokers were defined as those who had smoked at least 100 cigarettes in their lifetime and were still smoking or would have quit smoking within the preceding year.
- Former smokers were those who had smoked at least 100 cigarettes in their lifetime but would have quit smoking more than one year earlier.
- Non-smokers were those who had smoked less than 100 cigarettes in their lifetime or who had never smoked.

6.4.2.5 Obesity

Overweight and obesity were defined as a BMI 25-29.9kg/m^2 and >30 kg/m^2 respectively.[86]

6.4.2.6 Age

As a CVD risk factor, age was defined as:[93]

- Male ≥45 years
- Female ≥55 years

6.4.2.8 CD4 Count

The lowest CD4 count was recorded.

6.4.2.9 Duration of ART Exposure

The duration of exposure to ART was indicated in months from the date of initiation of ART. For patients on 2nd line ART, the duration of ART was the cumulative period on 1st and 2nd line ART.

6.5 Data Management & Statistical Analysis

Data was entered, managed and analyzed in SPSS version 21.0 software for Windows. Descriptive characteristics of the study population was presented using summaries of socio-demographic and clinical variables. Categorical variables were summarized into proportions and continuous data was summarized into means or medians and compared between ART naïve patients, patients on 1st line ART and patients on 2nd line ART.

Prevalence of subclinical atherosclerosis was analyzed as a percentage with 95% confidence interval and compared between the 3 groups. Patients with subclinical atherosclerosis were described using age, gender, lowest recorded CD4 count and duration of exposure to ART, and then compared with patients with normal CIMT.

Prevalence of selected CVD risk factors including dyslipidemia, diabetes, hypertension, obesity and smoking was determined in patients with an increased CIMT and compared to those with a normal CIMT. Categorical data was compared between groups using Chi square test or Fisher's exact test in case of small numbers. Means were compared using Student's t test and medians using Mann Whitney U test. Multivariate analysis using logistic regression model was done to determine the factors independently associated with an increased CIMT while controlling for confounders. All statistical tests were performed at 5% level of significance.

7.0 ETHICAL CONSIDERATIONS

The study was conducted after approval by Department of Clinical Medicine and Therapeutics, University of Nairobi, Kenyatta National Hospital/University of Nairobi Ethics and Research Review Committee and Mbagathi District Hospital.

Participants were recruited on a voluntary basis. A consent explanation (Appendix 2) was given to study participants, and eligible subjects who agreed to participate signed an informed consent form (Appendix 3).

Transport reimbursement was provided to the patients. The cost of the carotid doppler ultrasound for all the study subjects was borne by the principal investigator.

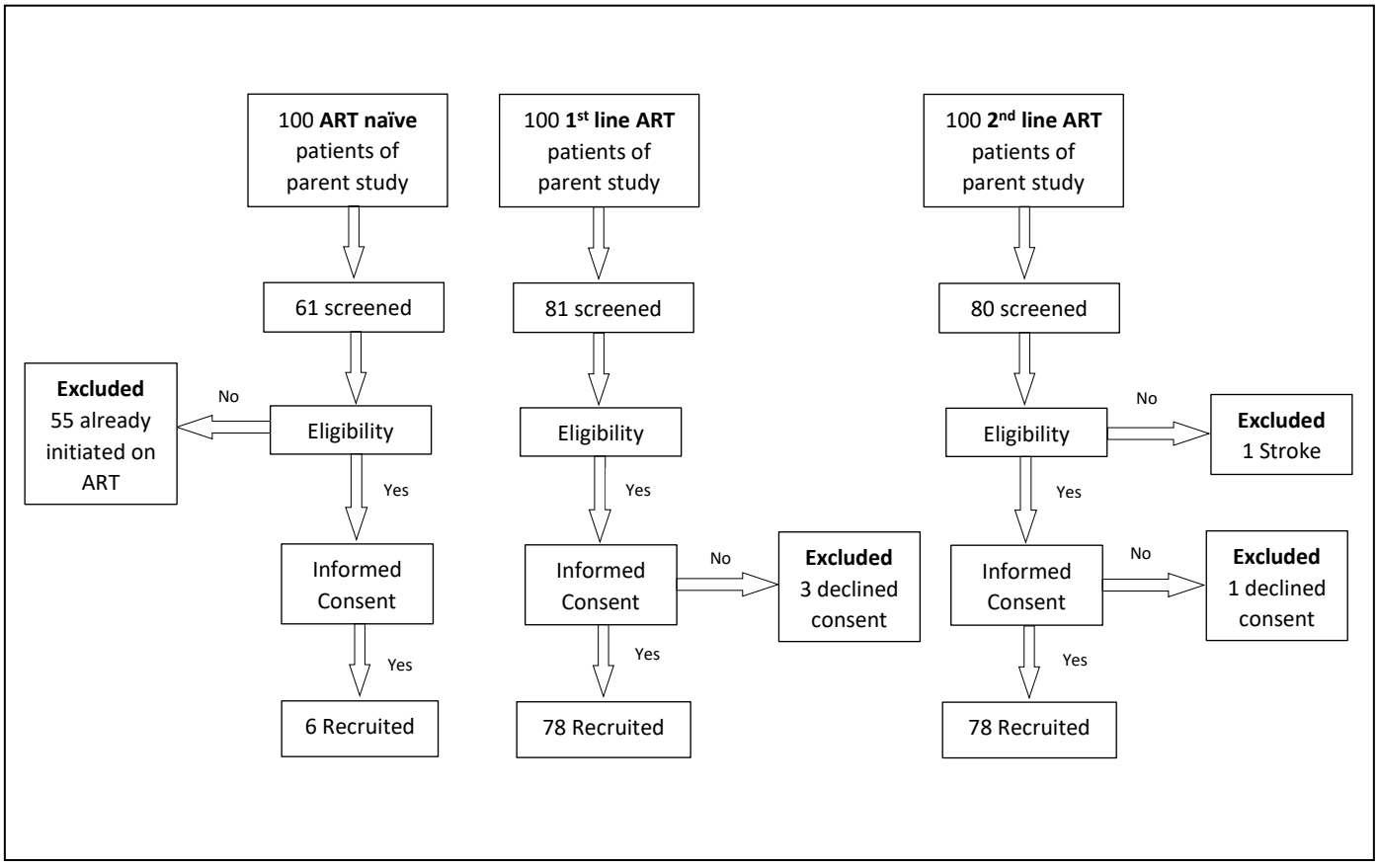
The principal investigator ensured that patient confidentiality was strictly observed. All data obtained from this study was used for the sole purpose of meeting the objectives stated in this proposal. The study results were communicated to the patients and the clinicians attending to them.

8.0 RESULTS

8.1 Characteristics of study patients

Between October and November 2016, 222 patients who participated in the parent study were screened for eligibility at Mbagathi District Hospital CCC; 61 ART naïve, 81 on 1st line ART & 80 on 2nd line ART. 55 patients who were ART naïve in the parent study had already been initiated on ART and were therefore excluded. A further 5 patients were excluded for the following reasons: 1 had previously suffered from stroke and was therefore ineligible, and 4 declined consent. 162 patients were enrolled for carotid ultrasonography and final analysis of which 6 (3.7%) were ART naïve, 78 (48.1%) were on 1st line ART regimen and 78 (48.1%) were on a protease inhibitor containing 2nd line ART regimen.

Figure 2: Flow Chart of Screening & Recruitment



8.1.1 Sociodemographic Characteristics

The mean age of study patients was 45.9 years (SD 9.0) with a range of 20-74 years. 101 participants (62.3%) were female. 56% of the study patients were married. Approximately three-quarter of our patients (73.5%) had at-least secondary education. Majority of the study participants (73.5%) had some source of income as they were either self-employed or employed.

Table 5: Sociodemographic Characteristics

Summary of characteristics

	Frequency	Proportion
Variable	**n = 162**	**%**
Age Category		
<30	4	2.5
30-39	36	22.2
40-49	65	40.1
50-59	43	26.5
60-69	13	8.0
≥70	1	0.6
Gender		
Female	101	62.3
Male	61	37.7
Marital Status		
Single	26	16
Married	91	56.2
Divorced	3	1.9
Widowed	31	19.1
Separated	11	6.8
Education Level		
None	6	3.7
Primary	46	28.4
Secondary	73	45.1
Tertiary	37	22.8
Employment Status		
Unemployed	39	24.1
Self Employed	58	35.8
Employed	61	37.7
Retired	3	1.9
Student	1	0.6

8.1.2 Clinical Characteristics

6 patients had missing records of CD4 counts. Mean nadir CD4 count of the remaining 156 study patients was 140.4 (SD 124.7). The mean duration of HIV infection since date of diagnosis was 122.9 (SD 55.9) months and the mean duration of exposure to ART was 115.5 (SD 56.6) months.

Table 6: HIV Related Clinical Characteristics

Summary of clinical characteristics

Study Variable	All study subjects (n = 162)	Male (n = 61)	Female (n = 101)
Age (years), mean (SD)	45.9 (9.0)	48.9 (9.85)	44.1 (8.0)
Duration of HIV (months), mean (SD)	122.9 (55.9)	123.7 (63.6)	122.4 (51.0)
Duration of ART (months), mean (SD)	115.5 (46.6)	114.9 (49.5)	115.9 (45.1)
1st line ART (%)	78 (48.2)	28 (45.9)	50 (49.5)
2nd line ART (%)	78 (48.2)	30 (49.2)	48 (47.5)
Lowest CD4 count, mean (SD)	142.8 (127.9)	139.5 (134.5)	144.7 (124.6)
CD4 count <200 (%)	122 (78.2)	49 (80.3)	73 (72.3)

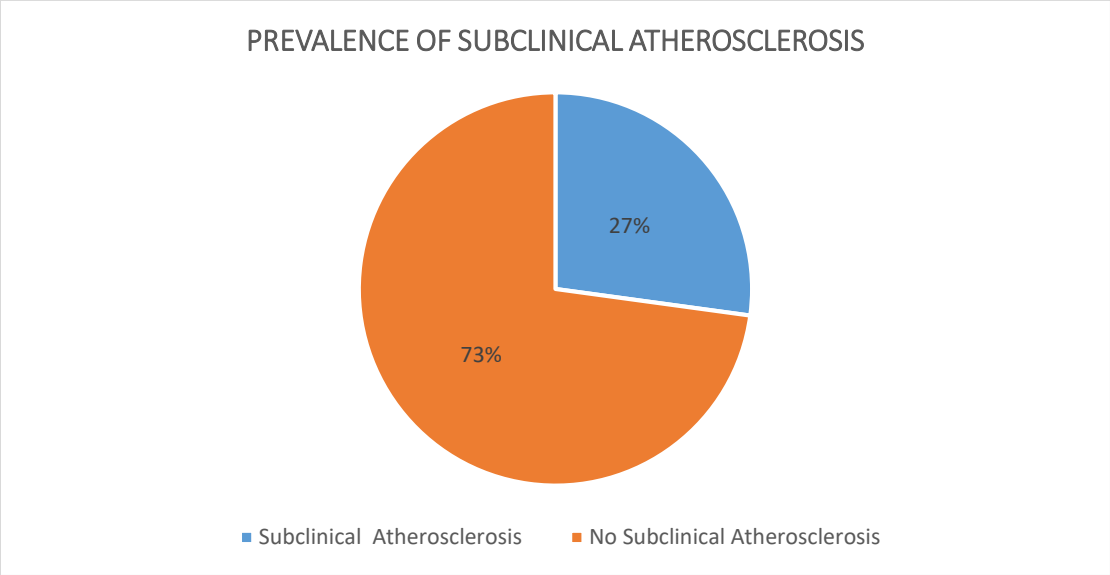
8.2 CIMT Results

8.2.1 CIMT Measurement

All study participants had carotid ultrasound imaging performed. 44 patients (27.2%) had evidence of subclinical carotid atherosclerosis (figure 3). 7 (4.3%) patients had an abnormal CIMT, carotid plaque was observed in 21 (13%) patients, and 16 (9.9%) patients had both an abnormal CIMT and carotid plaque. Out of the 44 patients with subclinical atherosclerosis, 22 (50%) patients were on 1st line ART regimen and 22 (50%) were receiving a protease inhibitor containing 2nd line ART regimen. Due to the small number of ART naïve patients who underwent carotid imaging, this group was excluded in the comparison analysis. The mean age

of patients with subclinical carotid atherosclerosis was significantly higher than patients without subclinical atherosclerosis (51.8 versus 43.7 years; $p < 0.001$).

Figure 3: Prevalence of Subclinical Atherosclerosis



8.2.2 HIV Related Characteristics in Patients with Subclinical Atherosclerosis

17 males (27.9%) and 27 females (26.7%) had subclinical carotid atherosclerosis. Subclinical atherosclerosis was commonest in the CD4 < 200 category, occurring in 84.1% of patients. Patients with subclinical atherosclerosis had a mean duration of HIV infection of 127.7 months, compared to 121 months in those with a normal CIMT. Mean duration of ART exposure was 117.9 months & 115 months in those with subclinical atherosclerosis and normal CIMT respectively. The differences in durations of HIV and ART exposure did not reach statistical significance ($p = 0.501$ & 0.698 respectively) as illustrated in table 7.

Table 7: Comparison of HIV Related Characteristics in Patients with Subclinical Atherosclerosis vs those with Normal CIMT

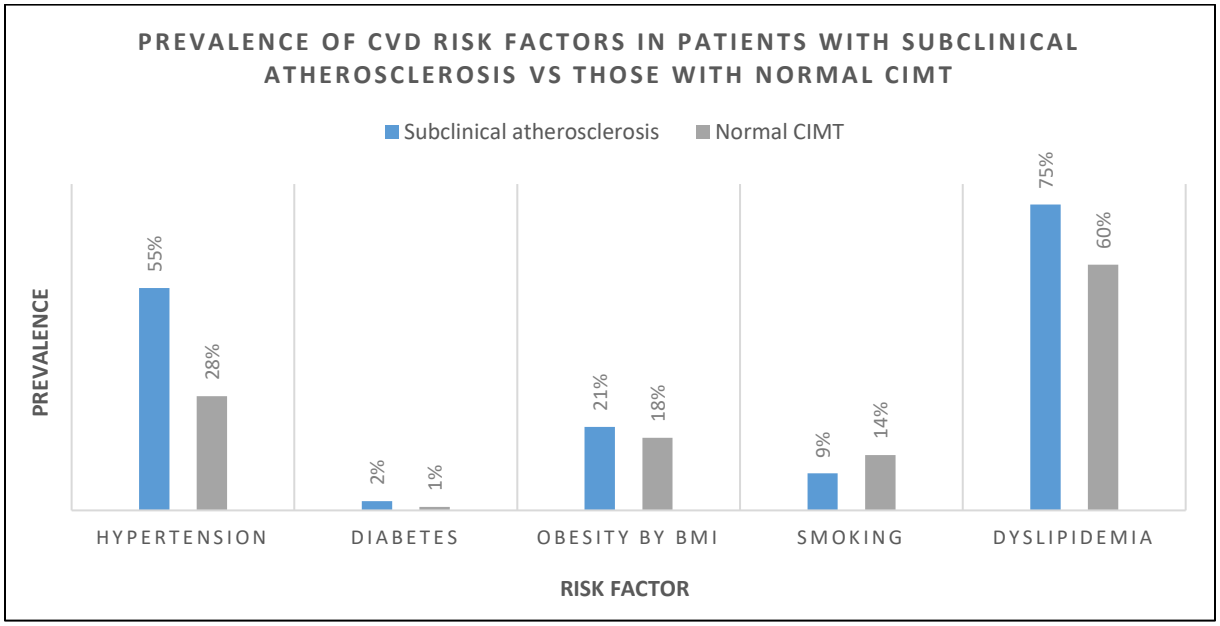
Summary of HIV related clinical characteristics

Study Variable	All study subjects (n = 162)	Subjects with CIMT >0.8mm (n = 44)	Subjects with normal CIMT (n = 118)	p value
Female (%)	101 (62.3)	27 (61.4)	74 (62.7)	0.875
Male (%)	61 (37.7)	17 (38.6)	44 (37.3)	0.880
Lowest CD4 count, mean (SD)	142.8 (128.0)	119.1 (106.1)	152.1 (134.9)	0.148
CD4 <200 (%)	122 (78.2)	37 (84.1)	85 (75.9)	0.266
Duration of HIV (months), mean (SD)	122.9 (55.9)	127.7 (45.2)	121 (59.4)	0.501
Duration of ART (months), mean (SD)	115.5 (46.6)	117.9 (45.4)	115 (47.2)	0.698
1st line ART (%)	78 (48.1)	22 (50)	56 (47.5)	0.773
2nd line ART (%)	78 (48.1)	22 (50)	56 (47.5)	0.773

8.2.3 Subclinical Atherosclerosis & Cardiovascular Disease Risk Factors

Only one of the patients with subclinical atherosclerosis did not demonstrate selected CVD risk factors. Majority of the patients had more than 2 CVD risk factors. In patients with subclinical carotid atherosclerosis, the commonest CVD risk factor was dyslipidemia which was observed in 75%, followed by hypertension 54.5%, age 47.7%, male gender 38.6%, obesity (by BMI) 20.5%, smoking 9.1% and diabetes 2.3% (figure 4).

Figure 4: Prevalence of CVD Risk Factors



Every 5-year increase in age was associated with 1.75-fold higher odds of having carotid atherosclerosis (OR 1.75; 95% CI 1.37-2.24; $p < 0.001$).

Only 2 (1.2%) of the patients were diabetic, of which one had visible carotid plaque. Both patients were aged more than 45 years. No patients in the study population had impaired fasting glucose.

Hypertension was present in 57 (35.2%) study participants. It was a significant factor associated with subclinical carotid atherosclerosis in our population (OR 3.09; 95% CI 1.51-6.33; $p = 0.002$). The mean systolic blood pressure in patients with an abnormal CIMT was significantly higher than those with a normal CIMT (140.25 ± 28.46 versus 132.75 ± 32.38; $p = 0.009$). Similarly, the mean diastolic blood pressure was also significantly higher in the group with an abnormal CIMT (84.45 ± 18.86 versus 79.72 ± 20.78; $p = 0.011$).

20 (12.4%) patients in the study population were classified as current or former smokers. 4 of these had an abnormal CIMT. In this data set, smoking status was not associated with an increased risk of subclinical carotid atherosclerosis (OR 0.64; 95% CI 0.20-2.02; $p = 0.441$).

Dyslipidemia was present in 64.2% of the study participants. 75% of the patients with subclinical carotid atherosclerosis had dyslipidemia, which was observed in only 60.2% of those who had a

normal CIMT. The difference approached but did not reach statistical significance (OR 1.99; 95% CI 0.9-4.3; p = 0.083). The commonest abnormality in lipid profile amongst patients with carotid atherosclerosis was elevated LDL occurring in 65.9%, followed by hypercholesterolemia in 63.6%, hypertriglyceridemia in 31.8% and decreased HDL in 11.4%. Abnormal lipid profiles significantly associated with subclinical atherosclerosis included a high total cholesterol (OR 5.1; 95% CI 2.4-10.8; p < 0.0001) and a high LDL cholesterol (OR 3.25; 95% CI 1.57-6.72; p = 0.002).

18.5% of the study population had an BMI >30. The mean BMI of all study participants was 25.36 $\pm$ 9.64. In our models, patients with subclinical atherosclerosis and those without, were equally likely to have a BMI >30 kg/m^2 (OR 1.02; 95% CI 0.42-2.44; p = 0.971). Similarly, waist circumference and waist-to-hip ratio were not associated with subclinical atherosclerosis (p = 0.486 & 0.924 respectively).

On univariate analysis, the odds ratios for dyslipidemia, hypertension, age and diabetes were more than 2-fold. However, only hypertension and age attained statistical significance. Risk factors that were significant at univariate analysis (age, hypertension, total cholesterol & LDL) were entered into a multivariate analysis model to determine the factors independently associated with subclinical atherosclerosis. Only age was independently associated with subclinical atherosclerosis on multivariate analysis using logistic regression.

A summary of the CVD characteristics is illustrated in tables 8 & 9 below.

Table 8: CVD Risk Factors Odds Ratios in Patients with Subclinical Atherosclerosis

CVD Risk Factors Odds Ratios

Risk Factor	CIMT > 0.8mm Frequency (%)	Normal CIMT Frequency (%)	Odds Ratio	p value
Dyslipidemia	33 (75)	71 (60.2)	1.99 (0.9-4.3)	0.083
Hypertension	24 (54.5)	33 (28)	3.09 (1.51-6.33)	**0.002**
Age (M>45, F>55)	21 (47.7)	33 (28)	2.35 (1.15-4.81)	**0.02**
Male gender	17 (38.6)	44 (37.3)	1.06 (0.52-2.16)	0.875
Obesity (BMI >30)	9 (20.5)	21 (17.8)	1.02 (0.42-2.44)	0.971
Smoking	4 (9.1)	16 (13.6)	0.64 (0.20-2.02)	0.441
Diabetes	1 (2.3)	1 (0.85)	2.72 (0.17-44.47)	0.483

Table 9: CVD Characteristics in All Patients

Summary of CVD characteristics in all subjects

Study Variable	All Subjects (n = 162)	CIMT >0.8mm (n = 44)	Normal CIMT (n = 118)	p value
Age, mean (SD)	45.9 (9.0)	51.8 (7.5)	43.7 (8.5)	**<0.001**
Hypertension (%)	57 (35.2)	24 (54.5)	33 (28)	**0.002**
Systolic BP, mean (SD)	134.8 (16.3)	140.3 (14.5)	132.8 (16.5)	**0.009**
Diastolic BP, mean (SD)	80.9 (10.5)	84.5 (9.6)	79.7 (10.6)	**0.011**
Diabetes (%)	2 (1.2)	1 (2.27)	1 (0.85)	0.483
FBS, mean (SD)	4.65 (0.5)	4.68 (0.37)	4.64 (0.54)	0.605
Obesity, BMI>30 (%)	30 (18.5)	9 (20.5)	21 (17.8)	0.976
BMI, mean (SD)	25.4 (4.92)	24.4 (3.8)	23.1 (4.24)	0.274
Waist Circumference, mean (SD)	84.7 (10.5)	85.7 (11.0)	84.4 (10.3)	0.486
Waist-Hip Ratio, mean (SD)	0.83 (0.06)	0.83 (0.05)	0.83 (0.06)	0.924
Dyslipidemia (%)	104 (64.2)	33 (75)	71 (60.2)	0.083
Total cholesterol, mean (SD)	4.84 (1.14)	5.6 (1.22)	4.55 (0.98)	**<0.001**
LDL, mean (SD)	2.68 (0.97)	3.27 (1.02)	2.46 (0.85)	**<0.001**
HDL, mean (SD)	1.43 (0.5)	1.47 (0.39)	1.42 (0.54)	0.542
Triglycerides, mean (SD)	1.73 (1.49)	1.89 (0.94)	1.67 (1.65)	0.393
Smoking (%)	20 (12.3)	4 (9.1)	16 (13.6)	0.594

8.2.4 Framingham Risk Scores

Based on the CVD risk factors demonstrated, the study patients were distributed according to the Framingham risk score categories of <10% (low risk), 10-20% (moderate risk), and >20% (high risk). In accordance with CVD risk stratification, subclinical atherosclerosis was found in 21% (26 out of 124) of the low risk category, 47.6% (10 out of 21) of the moderate risk and 47.1% (8 out of 17) of the high-risk category.

Patients with subclinical atherosclerosis had a significantly higher median Framingham risk score compared to their counterparts with no atherosclerosis demonstrated (7.6% vs 3.9%; $p < 0.001$).

Table 10: Distribution Across Framingham Risk Score Categories

Distribution Across Framingham Risk Score Categories

Framingham Risk Score	All study subjects (n = 162)	Subjects with CIMT >0.8mm (n = 44)	Subjects with normal CIMT (n = 118)	p value
<10%	124 (76.5%)	26 (59.1%)	98 (83.1%)	
10-20%	21 (13%)	10 (22.7%)	11 (9.3%)	
>20%	17 (10.5%)	8 (18.2%)	9 (7.6%)	
Median (IQR)	4.6 (2.4-9.4)	7.6 (5.3-13.7)	3.9 (2.0-7.2)	**<0.0001**

8.3 OBSERVER VARIABILITY

8.3.1 Inter-observer Variability

Carotid scans for twenty-three patients were done by 2 separate radiologists to assess variability in measurement of CIMT & detection of plaque, using the intra-class correlation coefficient (ICC) & Cohen's kappa coefficient respectively. The analysis showed a strong agreement in the observations by the 2 radiologists; ICC value 0.95; $p < 0.0001$ & kappa value of 0.82; $p < 0.0001$, for CIMT measurement and plaque detection respectively.

8.3.2 Intra-observer variability

8 patients had their carotid scans repeated on a later date by the same radiologist who performed it on the first occasion, to assess for reproducibility of results. The analysis involved two different observers and found an acceptable agreement in the results of the 2 scans (ICC value 0.92; $p < 0.0001$ and kappa value of 1.0; $p = 0.005$) for CIMT measurement and plaque detection respectively.

9.0 DISCUSSION

In this study, we set out to determine the prevalence of subclinical carotid atherosclerosis in HIV infected patients and explore associations with HIV related and traditional CVD risk factors at a resource limited setting in Kenya. We recorded a 27.2% prevalence of subclinical carotid atherosclerosis. We explored CVD risk factors in the patients with subclinical atherosclerosis and found an increased prevalence of traditional cardiovascular risk factors. Patients with subclinical atherosclerosis, as compared to those without, were older (mean age 51.8 years vs 43.7 years, $p < 0.001$), were more likely to have hypertension ($p = 0.002$), and had higher total, and LDL cholesterol ($p < 0.001$).

In a cross-sectional study in Botswana, Mosepele *et al* found a discordance in CVD risk as shown by CIMT as compared to the ASCVD risk score (2013 pooled cohort equation) in a virally suppressed HIV infected population, with a greater number of patients being classified as high risk using the CIMT.[95] This suggests that using traditional risk scoring systems could possibly underestimate the actual CVD risk in HIV infected patients. A possible explanation to these observations is the fact that the aforementioned risk prediction tools only take into consideration traditional CVD risk factors only whereas the HIV population could have combination of other risk factors related to inflammation and metabolic disturbances that accentuate the CVD risk.

Our study population was fairly young (mean age 46 years) and comprised predominantly of females (62.3%). This finding is consistent with other studies done on HIV patients in Kenya [47,96,97] and estimates by NASCOP that approximately two-thirds of all HIV infected patients in Kenya are female.[1] Our study sample was therefore a fair representation of the HIV population in Kenya.

Our prevalence was higher than that recorded in a recent study by Ssinabulya *et al* in Uganda, who found a prevalence of 18%, despite using a lower cut-off of 0.78mm for CIMT.[23] Their study population was much younger at median age of 37 years, and included both ART naïve and ART experienced patients. We did not include an ART naïve group in this study, due to the introduction of new national ART guidelines in 2016, which recommend treatment for all HIV infected patients regardless of WHO stage or CD4 count.[15] Thirdly, our patients had a longer

median duration of ART exposure (10.9 vs 7 years). The duration of HIV exposure could influence the thickening of the intima-media complex and development of plaques.[9] Finally, we recorded a higher mean BMI, SBP and DBP which could be possible explanations for the higher prevalence of subclinical carotid atherosclerosis in our study.

Our prevalence was however notably lower than that reported in European and American studies. In an Italian study done on ART naïve individuals with a mean age of 42 years, a 41.7% prevalence of subclinical atherosclerosis was reported, despite a higher cut-off value of 0.9mm for CIMT being used.[98] Majority (80%) of the patients in that study were male, and half of them were smokers. Parra *et al* reported a 65% prevalence of subclinical atherosclerosis in a cross-sectional observational study of a Spanish cohort (mean age 37 years).[99] Over 80% of patients in his study were smokers, and had worse dyslipidemias than our patients, both of which remain important CVD risk factors. A higher prevalence of traditional CVD risk factors could thus explain the higher prevalence of subclinical atherosclerosis in western HIV cohorts.

Vasculopathy in HIV infection is related to depletion of CD4 cells and persistent immune activation. This modulates chronic inflammation of the arterial wall during the initial phases of atherosclerosis.[72,73] In addition, viral proteins gp120 and Tat are thought to be toxic to vascular endothelial cells rendering them dysfunctional, a process which is pivotal in the early stages of atherosclerosis.[36,37] Whereas the durations of HIV infection and ART exposure were longer in the group of patients with subclinical carotid atherosclerosis, our study failed to find a significant association between these factors and subclinical atherosclerosis ($p = 0.501$ and 0.698 respectively). Evidence for this lack of association is supported by other studies.[100,101] In a cohort of 327 HIV infected patients, Mangili *et al* used both the CIMT and CAC score to demonstrate CVD risk. Despite there being a trend towards an increased CVD risk with some HIV specific risk factors, CIMT and CAC score were predicted mostly by traditional CVD risk factors.[101]

In our study, we used the lowest recorded CD4 count as a marker of HIV disease severity, since inflammation is thought to be maximal at the lowest CD4 level. Despite the fact that the nadir CD4 was lower in the group with subclinical atherosclerosis, we did not demonstrate significant association between the nadir CD4 count and subclinical atherosclerosis ($p = 0.226$). Kaplan *et al* reported low CD4 count as a major predictor of increased risk of carotid lesions in both HIV infected men and women.[81] In that study, direct comparison to HIV uninfected patients was

done, and it was noted that HIV patients with lower CD4 counts had significantly more carotid lesions. We lacked a HIV negative control group to make such comparisons. Secondly, the lowest recorded CD4 count does not necessarily mean a low CD4 count at present. We noted that the lowest CD4 count in the majority of our patients was recorded at the time of initiation of ART. As immune reconstitution occurs over time with the use of ART, CD4 count rises too, thus mitigating the effects of inflammation and specific pathogen exposures which would contribute to accelerated atherosclerosis. Finally, in Kaplan's study, imaging included sections of the internal carotid artery, whereas we restricted our scans to the CCA and carotid bulb. Our finding, however, is comparable to that of previous published work. In a larger HIV cohort with a similar comparative age (48 years) but slightly longer duration of HIV infection (156 months) sampled from the FRAM study, Delaney *et al* did not report an association between markers of HIV disease severity or ART drug classes, and CIMT.[100] It is important to note that using the lowest CD4 count may be a subjective marker of severity of HIV disease. Laboratory measurement of markers of systemic inflammation, such as the CRP or IL6, which correlate better with CVD in HIV are more objective.[31–33] In our study, we did not measure these, and as such we could not look for such correlations.

In the group with subclinical atherosclerosis, we did not find a difference in the proportions of patients on 1st line or 2nd line therapy ($p = 1.000$). There have been conflicting reports from studies that investigated the relationship between CIMT and use of PI containing ART regimens. In a multicenter prospective cohort study, Mercie *et al* investigated the impact of CVD factors and ART use on progression of CIMT, and found a relationship between PI use and CIMT on univariate analysis.[102] However, at multivariate analysis, the effects of PI use disappeared, and only traditional CVD risk factors appeared to be significantly associated with an increased CIMT. In a smaller case-control study, it was reported that disturbances in lipids, but not use of ART, was significantly associated with an increased CIMT.[103] PI's are known to unfavorably impact CVD risk factors through their associated metabolic changes, which translates into a higher CVD risk. Possibly, it takes longer for these metabolic changes to reflect on the changes in CIMT values. The cross-sectional nature of our study did not allow us to investigate the progression of changes in the CIMT over time, thus we could not explore this possibility.

In this study, traditional CVD risk factors played a key role in their association with subclinical carotid atherosclerosis. Patients with subclinical atherosclerosis were older (51.8 years vs 43.7 years, $p < 0.001$). The impact of increasing age on subclinical atherosclerosis as measured by CIMT has previously been demonstrated in several HIV studies.[85,98] In the HERMES study, De Socio *et al* demonstrated that both the chronological age and vascular age as being significantly associated with carotid atherosclerosis in ART naïve HIV infected patients.[98] In ART experienced patients, Currier *et al* reported age as the strongest predictor of CIMT.[85]

Hypertension was a key factor associated with subclinical atherosclerosis in our study. The overall prevalence of hypertension in our patients was 35%. A local study in the general population (mean age 33.4 years), conducted in an urban slum in Nairobi, found hypertension prevalence rate of 23%.[104] The higher prevalence in HIV patients could be explained by the HIV related arterial stiffness that occurs earlier in infected patients, as a result of changes in vascular endothelium.[105] It has also been reported from the Multicenter AIDS Cohort Study that prevalence of hypertension is higher with longer duration (>2 years) of ART therapy.[106] Similarly, Baekken *et al* found that cumulative use of ART for more than 5 years was associated with an increased prevalence of hypertension.[107] Recommendations from a recent study conducted in Netherlands suggest intensified monitoring and treatment of hypertension as part of HIV care, to avert CVD events.[108]

Majority (75%) of our patients with subclinical atherosclerosis had some form of dyslipidemia. Previous local studies have found a high prevalence of dyslipidemia among HIV infected patients.[47] Dyslipidemias in HIV is largely driven by dietary practices, physical inactivity and the impact of ART. We found that only one of the patients with dyslipidemia in our study was on a statin, signifying that dyslipidemia in this group was largely untreated. Patients with subclinical atherosclerosis demonstrated a significantly atherogenic lipid profile: mean total cholesterol 5.60 (SD 1.22), mean LDL 3.27 (SD 1.02) and mean triglycerides 1.89 (SD 0.94), all of which are above the acceptable low-risk limits. Elevated LDL level is described in the literature as the most atherogenic dyslipidemia.[93] This was the major dyslipidemia recorded in our patients with subclinical atherosclerosis, occurring in 66% of patients. We found a positive correlation between elevated total cholesterol & LDL levels and subclinical atherosclerosis ($p < 0.001$). This finding is consistent with various previous studies. In the Ugandan study, a high level of LDL-

cholesterol was associated with subclinical atherosclerosis.[23] Mercie *et al* found an association between elevated total cholesterol and subclinical atherosclerosis.[102]

Overall, the prevalence of smoking in our study was 12.3%. Another local study done earlier in HIV infected patients found a similar low prevalence of 9%.[47] We did not find significant association between cigarette smoking and subclinical atherosclerosis. Vasculopathy associated with smoking has been described in the literature as a strong risk factor for the development and progression of carotid lesions.[109] A possible explanation for the lack of association in our study could be a due to chance owing to a small sample size. Notwithstanding this low rate of cigarette use in our models, smoking remains a critical cardiovascular hazard that should be tended to in every patient as part of lifestyle modification therapy.

We did not demonstrate an association between obesity and subclinical atherosclerosis. A number of previous studies in HIV population have previously demonstrated an increased CIMT with obesity.[23,102] The lack of association in our study could be explained by the fact that we did not capture the duration of obesity, which plays an important role in increasing the CVD risk. In HIV infection, loss of weight may initially occur prior to initiation of ART. Once initiated on ART, the improved health and subsequent weight gain results in a progressive increase in the BMI, which can then accentuate the CVD risk. We could not satisfactorily address the duration of obesity in our study due to the cross-sectional design.

Diabetes was an infrequent finding in our study population with a prevalence of 1.2%. As such association between diabetes and subclinical atherosclerosis could not be addressed by our study.

10.0 CONCLUSIONS

1. A high prevalence of subclinical carotid atherosclerosis in HIV infected patients on ART was recorded.
2. Advancing age, hypertension and elevated levels of total cholesterol & LDL were significant cardiovascular risk factors in HIV infected patients taking ART.
3. No significant associations were found between HIV specific risk factors and subclinical carotid atherosclerosis.

11.0 LIMITATIONS OF STUDY

1. Inability to recruit ART naïve group.
2. The study design was cross-sectional in nature therefore may not bring out associations with progression of disease.

12.0 IMPLICATION OF STUDY & RECOMMENDATIONS

We found a relatively high burden of atherosclerotic cardiovascular disease in our HIV cohort, occurring prematurely in a fairly young population. This was largely driven by traditional CVD risk factors such as advancing age, hypertension and elevated levels of total and LDL cholesterol. Optimizing blood pressure control and treating dyslipidemias are potential targets for intervention. If left untreated, these factors can lead to clinical cardiovascular events such a stroke and myocardial infarctions in these young patients. In our resource limited setting, it may not be cost-effective to screen HIV infected patients for subclinical atherosclerosis, thus, emphasis needs to be laid on screening and timely management of the aforementioned risk factors, to recognize individuals at an increased risk of future CVD events.

Further prospective studies in a larger HIV population should be carried out to provide incremental information about the risk factors of atherosclerosis, and document any morbidity or mortality from cardiovascular disease in this population.

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APPENDICES

Appendix 1: SCREENING PROFORMA

Study No ☐☐☐☐

Age …….....

Date of Birth ☐☐ ☐☐ ☐☐☐☐

Contact: P.O. Box……

Telephone…….

Year diagnosed with HIV ☐☐☐☐

Year of initiation of ART ☐☐☐☐

Year of switch to 2nd line ART ☐☐☐☐

Duration on ART (months) ……

PATIENT DEMOGRAPHICS

Gender

- ☐ Male
- ☐ Female

Marital Status

- ☐ Single ☐ Married ☐ Divorced ☐ Widowed
- ☐ Separated

Usual Residence ……

Occupation

☐ Self-employed ☐ Employed ☐ Unemployed ☐ Retired

☐ Student

Level of Education

☐ None ☐ Primary School ☐ Secondary School ☐ Tertiary

☐ Other (specify)

……

ELIGIBILITY

1. Are you willing to participate in the study:

SUBCLINICAL ATHEROSCLEROSIS IN HIV INFECTED PERSONS AT MBAGATHI DISTRICT HOSPITAL COMPREHENSIVE CARE CLINIC?

☐ Yes

☐ No

2. Are you willing to return on another date for carotid imaging?

☐ Yes

☐ No

3. Which of the following tests have you had done recently (in the last 6 months)? Tick where appropriate
    1. CD4 count
    2. Viral load
    3. HBA1C
    4. RBS
    5. Lipid Profile
    6. Serum creatinine
4. Have you ever had any of the following? (Tick where appropriate)

☐ Been told by a doctor that you have coronary heart disease?

☐ Heart attack

- ☐ Angina pectoris (chest pain due to insufficient blood flow to the heart)
- ☐ Coronary bypass surgery
- ☐ Coronary angioplasty (ballooning)
- ☐ Been told by a doctor that you have abdominal aortic aneurysm
- ☐ Transient ischemic attacks (transitory strokes)
- ☐ Blockage of an artery of the legs
- ☐ Stroke

Appendix 2: CONSENT EXPLANATION FORM

Research Title: Subclinical Carotid Atherosclerosis In HIV Infected Patients At Mbagathi District Hospital Comprehensive Care Clinic

I am Dr. Zaheer Harunany, a medical doctor currently doing a masters' degree in Internal Medicine at the University of Nairobi. I am conducting a research project to evaluate subclinical atherosclerosis in HIV patients, for which we request your participation.

What does the study involve?

HIV infection can cause problems with the blood vessels. When people infected with HIV infection are initiated on HIV medicines for the first time, these medicines are called first line. If the first line do not control the HIV infection, we call this treatment failure. When treatment failure occurs the patient is put on second line medicines. In addition to HIV infection itself, both first line and second line medicines can cause problems with blood vessels in patients taking these medicines. These problems can lead to stroke and heart attacks.

We do not have any studies that investigated blood vessel problems in patients infected with HIV in Kenya. This study aims to look into this issue in order to educate and treat our patients better.

If you agree to participate you was asked some questions about your HIV infection and HIV medicines. A doctor will perform a physical examination on you. An ultrasound scan of your blood vessels in the neck was performed to look for any problems. You will not be required to pay for the scan. The project will also provide you with transport to and from the clinic on the day of the study.

How do I benefit from the study?

As a patient infected with HIV, or taking HIV medicines, you will get to know if these are affecting your blood vessels. If there is a problem, your doctor will talk to you about what to do next. Your doctor will also educate you on how to stay healthy.

Are there any risks of participation?

Risks involved in the clinical assessment and doppler ultrasound scanning are going to be negligible. Doppler ultrasound is non-invasive and not painful. No laboratory tests are going to be conducted in this study therefore blood samples shall not be withdrawn from your body.

Do I have to take part?

Taking part in this study is voluntary. You shall not be forced to participate in the study. Should you agree to take part you was given this information sheet to keep and was expected to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. You will still receive all treatment that you should get even without participating in the study.

Confidentiality

Any data collected for this study will only be accessible to authorized persons. This will minimize accidental disclosure to any unauthorized personnel. Results will only be made available to the patient and his/her primary care provider. It was the responsibility of the principal investigator that patient confidentiality is maintained.

Thank you for taking the time to read this information sheet.

KIAMBATISHO 2: IFOMU YA MAELEZO KUHUSU UTAFITI HUU

Mada: Ugonjwa wa kukusanyika mafuta katika mishipa ya damu ya shingo (carotid) kwenye wagonjwa walioathiriwa na HIV katika hospitali ya Mbagathi.

Mimi ninaitwa Daktari Zaheer Harunany. Ninasomea shahada ya juu katika matibabu ya watu wazima katika chuo kikuu cha Nairobi. Ninafanya utafiti kutathmini ugonjwa wa kukusanyika mafuta katika mishipa ya damu ya shingo (carotid) kwenya watu ambao walioambukizwa na HIV. Ninaomba ujiunge na utafiti huu.

Je, nimealikwa kwa nini?

HIV inaweza kusababisha matatizo ya kukusanyika mafuta katika mishipa ya damu. Wagonjwa walioambukizwa na HIV huanza madawa ya kuzuia virusi katika miili yao. Kuna aina tofauti tofauti za dawa za kutibu HIV. Mbali na virusi za HIV zenyewe, dawa za HIV zinaweza kusababisha matatizo ya mishipa ya damu, na huenda ikaleta shida zingine mwilini kama kiharusi na mshtuko wa moyo.

Nchini Kenya, tafiti za kuchunguza matatizo ya mishipa ya damu kwa wagonjwa walioambukizwa na HIV hazijafanywa. Kwa hivyo, utafiti huu unalenga kuangalia suala hili, na itatusaidia kuweka mikakati ya mapema kutambua matatizo ya mishipa ya damu ili kuwaelimisha na kutibu wagonjwa wetu bora zaidi. Ukikubali kushiriki, utaulizwa baadhi ya maswali kuhusu HIV na dawa ambazo unatumia. Ukiingia utafiti huu utapimwa mwili wako kikamilifu, kisha utapigwa picha ya mishipa ya damu ya shingo. Picha hiyo hailipishwi, na pia utapewa nauli ya kuja hospitali kuu ya Kenyatta siku ya utafiti.

Je, nitanufaika kivipi kwa kujiunga na utafiti huu?

Ukijiunga na utafiti huu, utapata kujua kama ugonjwa wa HIV, au kutumia dawa za HIV zinaathiri mishipa yako ya damu. Matokeo ya picha yatawasilishwa kwa mgonjwa pamoja na daktari wake ili kuhakikisha wagonjwa wanapata matibabu bora. Kukipatikana tatizo lolote katika mishipa yako ya damu, daktari wako atakueleza njia bora za matibabu.

Je, kuna athari za kujiunga na utafiti huu?

Athari ya kushiriki katika utafiti huu ni kidogo sana. Hakuna uchungu wowote utasikia ukipigwa picha ya mishipa ya damu ya shingo, maanake picha ya mishipa sio kama sindano. Katika utafiti huu, vipimo vya maabara havitafanywa kwa hivyo sampuli za damu hazitatolewa kwa mwili wako.

Je, ni lazima kujiunga na utafiti huu?

La! Uamuzi kuingia utafiti huu ni wako, unaingia kwa hiari yako. Ukiamua kujiunga na utafiti huu, utapewa fomu hii ya maelezo na kusaini kartasi ya makubaliano. Ukiingia utafiti huu, una uhuru wa kutoka wakati wowote bila ya kutupatia sababu zako za kutoka. Bado utaendelea kupata matibabu yako yote ya kawaida hata bila kuendelea na utafiti huu.

Je, rekodi zangu binafsi na matokeo ya picha yangu yatawekwa siri?

Rekodi zako za matibabu na matokeo yote yatakayojulikana kutoka utafiti huu yataangaliwa na watafiti walioidhinishwa pekee yao. Tunatumaini kwamba kufanya hivi itapunguza uwezekano ya watu nje ya utafiti huu kutambua mambo yako binafsi. Matokeo yatapeanwa kwa mgonjwa binafsi ama kwa mtu yule wa karibu aliyeidhinishwa kupokea matokeo ya matibabu yake.

Asante kwa kuchukua muda wako kusoma maelezo haya.

Appendix 3: PATIENT CONSENT FORM FOR PARTCIPATION IN THE STUDY

I, the undersigned have read and fully understood the explanation given to me regarding this project. All my questions have been answered satisfactorily by the investigators. I hereby consent to my participation in this study.

SIGNED: …… (Patient)

WITNESS: ……. (Principal Investigator or Research Assistant)

Date: …….

CONTACTS

For further information, you may contact any of the following:

1. Dr. Zaheer Harunany (Principal Investigator)
P.O Box 71200-00610, Nairobi.
Tel: 0720 703251

2. Dr. Loice Achieng (Lead Supervisor)
Department of Internal Medicine & Therapeutics, University of Nairobi.
Tel: 0722 576984

3. Professor A. N. Guantai
Chairman of Kenyatta National Hospital/University of Nairobi Ethics and Research Committee.
P.O Box 20723, Nairobi.
Tel: 020-2726300, extension 44102.

KIAMBATISHO 3: KARATASI YA MAKUBALIANO

Nimesoma na kukubaliana na maelezo nimepewa kuhusu utafiti huu. Maswali yangu yote yamejibiwa kwa ukamilifu na Daktari Zaheer na watafiti wenzake.

Nimekubali kuingia utafiti huu.

Sahihi ……. (mgonjwa)

Shahidi ……. (mtafiti mkuu ama msaidizi wake)

Tarehe ……

WANAOHUSIKA:

Kwa maelezo zaidi, unaombwa uwasiliane na watu wafuatao:

1. Daktari Zaheer Harunany - Mtafiti Mkuu
Sanduku la Posta 71200-00610, Nairobi.
Simu: 0720 703251

2. Daktari Loice Achieng (Msimamizi Mkuu)
Idara ya matibabu ya watu wazima, Chuo kikuu cha Nairobi.
Simu: 0722 576984

3. Profesa A.N. Guantai
Mkurugenzi wa Idhaa ya Uadilifu kwenye utafiti, hospitali kuu ya Kenyatta.
Sanduku la Posta 20723, Nairobi.
Simu ya Ofisi: 020-2726300-ugani 44102

Appendix 4: STUDY PROFORMA

Date: …… Study No: | | | | |

Date of Birth: | | | | | | | | | Age: ……

Year of Diagnosis of HIV: | | | | |

Duration of HIV since diagnosis ……..

Year of Initiation of ART: | | | | |

Duration of ART (months) ……

Current ART regimen: □ 1st line □ 2nd line

Specify Regimen ……..

Duration on 1st line ART (months) ……

Duration on 2nd line ART (months) ……..

Latest CD4 Count …….

Lowest CD4 Count ……..

CD4 at ART initiation …….

WHO Stage of HIV …….

Viral Load ……..

A. DEMOGRAPHICS

5. Gender ☐ Male ☐ Female

6. Marital status

   ☐ Single ☐ Married ☐ Divorced ☐ Widowed ☐ Separated

7. Residence

   ……

8. Occupation

   ☐ Self-Employed ☐ Employed ☐ Unemployed ☐ Retired

   ☐ Student

9. Education Level

   ☐ None ☐ Primary School ☐ Secondary School ☐ Tertiary Level

☐ Other (specify)

……

B. CHRONIC ILLNESSES

10. Diabetes ☐ Yes ☐ No

Duration (Years) …….

11. Hypertension ☐ Yes ☐ No

Duration (Years) ……

C. PAST MEDICAL HISTORY

12. Have you ever had any of the following? (Tick where appropriate)

☐ Been told by a doctor that you have coronary heart disease?

☐ Heart attack

☐ Angina pectoris (chest pain due to insufficient blood flow to the heart)

☐ Coronary bypass surgery

☐ Coronary angioplasty (ballooning)

☐ Been told by a doctor that you have abdominal aortic aneurysm

☐ Transient ischemic attacks (transitory strokes)

☐ Blockage of an artery of the legs

☐ Stroke

D. FAMILY HISTORY

13. Did or does any of your relatives suffer from diabetes? ☐ Yes ☐ No

Specify ☐ Father ☐ Mother ☐ Brother/Sister ☐ Children

Other (specify)

……

14. Did or does any of your relatives suffer from hypertension? □ Yes □ No

    Specify □ Father □ Mother □ Brother/Sister □ Children

    Other (specify) ……

15. Did any of your first-degree relatives (father, mother, brothers, sisters or children) suffer from heart attack, stroke or sudden death? If male <55 years, if female <65 years)

    ☐ Yes □ No

E. SMOKING

16. Do you smoke cigarettes now?

    ☐ Yes □ No

17. On average how many cigarettes do you smoke per day? …….. cigarettes/day.

18. Did you ever smoke cigarettes regularly in the past?

    ☐ Yes □ No

19. When did you stop smoking cigarettes regularly? Year ……..

    If in the last 12 months:

    ☐ Less than 1 month ago □ 1-6 months ago □ 6-12 months ago

20. What is the highest average daily number of cigarettes you have ever smoked for as long as a year? ……. cigarettes/day.

21. For how many years have you been smoking cigarettes? ……years.

22. Smoking status: □ Current Smoker □ Former Smoker □ Non –Smoker

F. ALCOHOL INTAKE

23. Do you drink alcohol?

☐ Yes □ No

G. CURRENT MEDICATIONS

Are you currently on any of the following medications?

24. Drugs to lower blood sugar? (oral/injectable)

☐ Yes □ No

25. Blood pressure lowering drugs?

☐ Yes □ No

26. Lipid lowering drugs?

☐ Yes □ No

27. Antiplatelet drugs? (Aspirin/Clopidogrel)

☐ Yes □ No

H. PHYSICAL EXAMINATION

28. Height (m) ……..

29. Weight (kg) ……..

30. BMI (kg/m2) ……

31. Waist Circumference (cm) ……

32. Hip Circumference (cm) …….

33. Waist/Hip Ratio ……

34. Blood Pressure ……

I. CIMT MEASUREMENT

COMMON CAROTID ARTERY IMT MEASUREMENTS						
	Right (mm)		**Left (mm)**		**Summary**	
IMT	Max	Mean	Max	Mean		
Lateral far wall					Mean (mm)	
Lateral near wall					Maximum	
Posterior far wall					Minimum	
Posterior near wall					**Average**	
Anterior far wall					Max Region (mm)	
Anterior near wall					Maximum	
					Minimum	
Plaque	Right (cm)		Left (cm)			
Plaque 1					**Average**	
Plaque 2						