PREVALENCE AND RISK FACTORS FOR PERIPHERAL ARTERIAL DISEASE AMONG HIV INFECTED PATIENTS AT THE MOI TEACHING AND REFERRAL HOSPITAL, ELDORET, KENYA

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DECLARATION

Student's declaration:

This research thesis is my original work and has not been presented for a degree in any other university. No part of this research thesis may be reproduced without prior written permission of the author or Moi University.

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DEDICATION

I dedicate this work to my family: Julia, Brilliant and Peace for their prayers, support and inspiration.

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ABSTRACT

Background: According to the United Nations programme on HIV/AIDS (UNAIDS 2017 Data book), Kenya has the fourth highest HIV infection burden in the world with majority of her HIV infected adult population on antiretroviral therapy (ART). Studies have shown that HIV infection and use of ART predispose to peripheral arterial disease (PAD), which is the narrowing and occlusion of arteries of the lower limbs mainly by atherosclerotic plaques. PAD is associated with major acute cardiovascular events and physical disability. Studies in High Income Countries have reported high prevalence of PAD in HIV infected population. However, prevalence in Kenya and sub-Saharan Africa has not been well described. Early detection of PAD leads to successful management and prevention of complications.

Objective: To compare the prevalence of peripheral arterial disease and associated risk factors in HIV infected versus non-HIV infected patients matched for age and sex.

Methods: This was a cross-sectional study conducted at the Moi Teaching and Referral Hospital (MTRH). The study population was all HIV infected and non-HIV infected patients aged ≥18 years in the Academic Model Providing Access to Healthcare (AMPATH) modules, adult medical wards and medical outpatient clinics at MTRH. A total of 235 study participants were enrolled by systematic random sampling between March and May 2017. An interviewer administered structured questionnaire was used to collect socio-demographic and clinical data. The Edinburgh Claudication Questionnaire (ECQ) and Ankle Brachial Index (ABI) were used as study tools (whereby, resting and post ABI squatting exercise measurements were taken). Blood samples were also collected for measurement of glycated haemoglobin and cholesterol levels. Data was analysed for prevalence with statistical significance set at $p<0.05$.

Results: A total of 235 participants were included in the final analysis, 118 HIV infected and117 non-HIV infected. Claudication suggestive of PAD was reported using ECQ in 20.3% of HIV infected compared to 6.8% of non-HIV infected participants ($P = 0.003$). Low ABI suggestive of PAD (ABI≤ 0.9 or >15% absolute drop on exercise) was reported in 18.0% of HIV infected compared to 2.6% of non-HIV infected participants ($P < 0.001$). The participants who had PAD tended to have the following predominant risk factors: being old (median age 46 IQR: 11), female (72.0%), having dysglycaemia (75.0%), high viral load (58.3%), being on second line ART (58.3%), overweight (56.2%), having dyslipidaemia (37.5%), and hypertension (25.0%).

Conclusion: There is a high prevalence of PAD as measured by ABI and ECQ in HIV infected compared to non-HIV infected adults in a tertiary medical centre in western Kenya.

Recommendation: Routine individualized screening of PAD, in those with more than one risk factor, based on guidelines by AHA/ACC, and monitoring of potential risk factors in HIV infected population.

LIST OF ABBREVIATIONS

ABI	Ankle Brachial Index
AACE	American Association of Clinical Endocrinologists and American College of Endocrinology
ACC	American College of Cardiology
ADA	American Diabetes Association
AHA	American Heart Association
AIDS	Acquired Immune Deficiency Syndrome
AMPATH	Academic Model Providing Access to Healthcare
ART	Anti-Retroviral therapy
BP	Blood Pressure
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DCCT	Diabetes Control and Complications Trial
DM	Diabetes Mellitus
ESC	European Society of Cardiology
HBA1c	Glycated hemoglobin
HIV	Human Immunodeficiency Virus
HDL-C	High Density Lipoprotein- Cholesterol
IREC	Institutional Research and Ethics Committee
LDL-C	Low Density Lipoprotein- Cholesterol

MTRH	Moi Teaching and Referral Hospital
NGSP	National Glycohemoglobin Standardization Program
PAD	Peripheral Arterial Disease
PI	Protease Inhibitor
SBP	Systolic Blood Pressure
UNAIDS	United Nations programme on HIV/AIDS
WHO	World Health Organization

OPERATIONAL DEFINITIONS

Peripheral Arterial Disease (PAD): this was defined as having a low Ankle Brachial Index (ABI≤ 0.9 or >15% absolute drop on exercise) and/or presence of claudication

Claudication: This was defined as definite claudication being pain in the calf and/or atypical claudication as having pain in thigh or buttock (in the absence of calf pain)

Dysglycemia: This was defined as glycated hemoglobin (HBA1c) ≥ 5.7%

Dyslipidemia: Dyslipidemia was defined as presence of Non HDL-c > 4.9mmol/L

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CHAPTER ONE: INTRODUCTION

1.0 INTRODUCTION

1.1 Background

Peripheral Arterial Disease (PAD) is the progressive constriction and blockage of arteries of the lower extremities, mainly by deposition of atherosclerotic plaques (Beckman JA et al. 2018, Topakian R, et al. 2010).

The causal pathway for PAD in HIV infection is multifactorial: HIV infection, high viral load, low CD+ 4 count, use of ART, increasing age, presence of high burden of cardiovascular risk factors and genetic predisposition have all been shown to synergistically predispose to atherosclerosis, leading to PAD (Beckman JA et al., Hadigan C et al. 2017, Periard D et al. 2008, Gupta N et al. 2013). The use of ART, especially protease inhibitors, leads to metabolic changes predisposing to development of atherosclerosis and PAD (Hadigan C et al. 2017). HIV infection can directly cause immune induced inflammation and endothelial dysfunction that promote atherosclerosis (Freiberg MS. et al. 2013). This is accelerated in the presence of unsuppressed viral load and low CD+ count. However, patients who are on good ART that have successfully suppressed the viral load and increased CD+ count may still develop atherosclerosis that progress to PAD. This can be prevented by controlling other PAD risk factors (Freiberg MS. et al. 2013): HIV infected persons tend to have a great burden of CVD risk factors which predispose them to atherosclerosis and PAD (Hadigan C et al. 2017).

When atherosclerosis develops before 50 years of age, it is defined as premature or early onset atherosclerosis. HIV infected patients have been shown to develop early onset atherosclerosis (Lorenz MW et al. 2006, Charakida et al. 2009), putting them at an

increased risk for progression of PAD (Harris LM et al. 1996). Therefore, HIV infected population needs early screening, diagnosis, prevention and treatment of PAD before complications develop. Studies have shown that early screening promotes early detection and intervention (Khan NA, 2006): Early detection of PAD leads to successful management and prevention of complications (ACC/AHA 2017, ESC 2017).

PAD has high morbidity and mortality: intermittent claudication, critical limb ischaemia, leg ulcer and gangrene, myocardial infarction and stroke. However, management and prevention of complications can be successfully achieved when PAD is detected early, by screening those with increasing risk of developing PAD, like HIV infected population (Hadigan C et al. 2017).

The prevalence of PAD has tended towards being more in infected persons in comparison to the non-HIV infected counterparts, mainly in studies done in USA and Europe, where the prevalence has been reported to range between 10.0% and 25.6% (Gupta N et al. 2013, Periard D et al. 2008, Palacios et al. 2008, Ashraf Y. Qaqa et al. 2010). The prevalence in Kenya and Sub-Saharan region has not been well defined, despite the region having the greatest burden of HIV in the world. According to the UNAIDS 2017 Data book, Kenya's HIV burden is the fourth highest in the world. Most of her HIV infected adult population is on antiretroviral therapy. Therefore, there is need to establish the prevalence of PAD in our region, in order to bring to attention the need to be aware of its presence when dealing with HIV infected patients for their better management.

When the arteries of the lower limbs become narrowed by atherosclerotic plaques, ischaemic changes develop which present with leg pain when walking which gets

alleviated with rest. This is referred to as intermittent claudication. Severely reduced blood supply, as a result of narrowed arterial walls, leads to bluish skin coloration and cold extremities. When there is accelerated atherosclerosis, occlusion of the arterial walls can develop leading to necrosis, skin ulcer and/or gangrene which may necessitate amputation (ESC 2017, McGee Sr. 1998). Generalized atherosclerosis may affect the heart leading to myocardial infarction, or brain arteries leading to stroke. Thus, presenting with PAD is a surrogate marker of generalized atherosclerosis in the body, predisposing to stroke and myocardial infarction (ACC/AHA 2017, ESC 2017, McGee Sr. 1998).

Only about 10 percent of people with PAD present with symptoms while the remaining 90% are either asymptomatic or have atypical presentation. This leads to late diagnosis with complications. Therefore, there is need for early screening for early detection and intervention (Khan NA, 2006).

Both ABI and ECQ have high sensitivity and specificity for screening and diagnosing PAD. These two tools have been validated locally, are available, cheap and easy to use (The DAD study Group, 2007, Hirsch AT et al. 2005). Edinburg claudication questionnaire (ECQ) is a tool which has been validated worldwide with high specificity (91.3%) and sensitivity (99%) for diagnosing those with classic and typical symptoms (The DAD study Group, 2007). An ABI that is equal to or less than 0.9 is diagnostic of PAD (ACC/AHA 2017, Wang JC, 2005).

In those with PAD, improved outcomes can be achieved through controlling the CVD risk factors such as stopping smoking, reduced fatty food intake and reducing body

hyperlipidemia, controlling hypertension and supervised exercise (ACC/AHA 2017, Fokkenrood HJ et al. 2014). Medical and surgical interventions are usually employed in management of PAD (ACC/AHA 2017, Bedenis, et al. 2014, Hauk L et al. 2013).

According to the UNAIDS 2017 Data, Kenya's HIV infection burden is the fourth highest in the world, with adult HIV infection prevalence of 4.8% (UNAIDS Data book 2017). Over 64% of HIV infected adults, in Kenya, are on ART (WHO 2015, UNAIDS Data 2017). AMPATH HIV module clinics at the MTRH serve over 15,000 HIV infected population in the whole of western Kenya, with almost all of them on ART. This high HIV burden and use of ART could predispose this HIV infected population to PAD (Hadigan C et al. 2017).

The burden of PAD among the population infected with HIV has not been defined in Kenya. There is no published study on the same in Kenya or in the region, despite having high burden of HIV infection.

Recently, unpublished master's dissertation by Kiplagat among the HIV infected patients attending care at AMPATH, in MTRH showed a high burden of risk factors for PAD: dyslipidaemia (70%), and dysglycaemia (15.3%). A study by Bloomfield et al., in Bungoma East District among 4037 adults with median age of 35 (IQR: 26-50) years in Western Kenya showed that 7% of the population used tobacco while 16% of the population reported alcohol use. In the same study, the proportion of women and men with hypertension was reported as 7.4% and 11.2%, respectively and they reported prevalence of overweight/obesity as 10.6 and 22.6%, respectively.

1.2 Problem Statement

There is a high HIV infection burden in this region. Kenya has an adult HIV prevalence of 4.8% (UNAIDS Data book 2017). The Academic Model Providing Access to Healthcare (AMPATH), at Moi Teaching and Referral Hospital (MTRH) covers the whole of western Kenya with over 1500 HIV infected population under care.

There is increased usage of ART (UNAIDS Data book 2017), with over 90% of the patients infected with HIV under care in AMPATH on ART. The use of ART, especially protease inhibitors, tends to lead to metabolic changes predisposing to development of atherosclerosis and PAD (Hadigan C et al. 2017).

Bloomfield et al. and Kiplagat KN, have recently demonstrated a tendency of infected adults in western Kenya, under AMPATH care, to have a high burden of PAD risk factors.

Because of these three factors, high HIV burden, increased usage of ART and the increasing burden of risk factors for PAD, this population infected with HIV is predisposed to atherosclerosis and, therefore, to PAD.

PAD has high morbidity and mortality: intermittent claudication, critical limb ischaemia, leg ulcer and/or gangrene, myocardial infarction and stroke.

1.3 Study Justification

There is insufficient data since the burden of PAD in Kenya and sub-Saharan African has not been well defined.

PAD is mostly asymptomatic (90%) and late presentation comes with complications, hence the need for early screening and diagnosis (Khan NA, 2006).

Early screening and detection of PAD by ECQ and ABI (Khan NA, 2006) have been shown to lead to successful management and prevention of complications (ESC/AHA 2017).

Both ABI and ECQ have high sensitivity and specificity for screening and diagnosing PAD. These two tools have been validated locally, are available, cheap and easy to use (The DAD study Group, 2007, Hirsch AT et al. 2005)

Therefore, there is need to establish the prevalence of PAD in our region, in order to bring to attention the need to be aware of its presence when dealing with HIV infected patients for their better management.

1.4 Research Question

1. What is the prevalence and risk factors for peripheral arterial disease among HIV-infected patients compared with non-HIV infected patients?

1.5 Research Objectives

1.5.1 Broad Objective

To assess the prevalence of peripheral arterial disease, using Ankle Brachial Index (ABI) measurement and Edinburg Claudication Questionnaire (ECQ) in HIV infected and non HIV infected patients at the Moi Teaching and Referral Hospital.

1.5.2 Specific Objectives

1. To compare the prevalence of peripheral arterial disease among HIV-infected versus non-HIV- infected patients at the MTRH.
2. To describe the traditional risk factors (hypertension, dysglycaemia, dyslipidaemia, smoking, obesity) of peripheral arterial disease in adult HIV infected patients and non HIV-infected group, matched for age and sex at the MTRH.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

The prevalence of PAD has tended towards being more in those infected compared to the non- HIV infected counterparts, mainly in studies done in USA and Europe, where the prevalence has been reported to range between 10.0% and 25.6% (Gupta N et al. 2013, Periard D et al. 2008, Palacios et al. 2008, Ashraf Y. Qaqa et al. 2010).

The causal pathway for PAD in HIV infection is multifactorial: HIV infection, high viral load, low CD+ 4 count, use of ART, increasing age, presence of high burden of cardiovascular risk factors and genetic predisposition have all been shown to synergistically predispose to atherosclerosis, leading to PAD (Hadigan C et al. 2017, Periard D et al. 2008, Gupta N et al. 2013).

Prevention, reduction and/or reversal of PAD progression can successfully be achieved with early detection and control of risk factors in those at high risk.

This early detection can successfully be achieved using ECQ and/or ABI.

2.2 The burden of PAD in HIV infected Adults

The true burden of PAD among the population infected with HIV has not been well defined in the world: the findings of different studies report marked variations. Daniel Periard et al in their study in USA, 2008, recorded a PAD (ABI<0.9) prevalence of 20.7% in a multiracial HIV infected population aged >40 years (average age 49.5 years). Palacios et al in their study recorded a PAD (ABI≤0.9) prevalence of 10.2% in a mainly male (82.8%) consecutive HIV infected patients aged ≥50 years (average age 58.6 years).

Ashraf Y. Qaqa et al in USA, 2010 consecutively enrolled 113 HIV infected subjects (mean age of 47±10 years) with normal resting ABI who were then subjected to post exercise ABI (using either a treadmill or walking plantar flexion). A peripheral arterial disease prevalence of 26.5% was reported in the study.

These findings of different studies with marked variations call for the need to know the burden contributed by PAD in Africa, in general, and Kenya in particular, considering that Kenya's HIV burden is the fourth highest in the world (UNAIDS Data book 2017).

A USA study by Gupta N et al. (2013) recorded a PAD prevalence of 28.5% against 12.5% (p value 0.0069) by ECQ and 10% against 1.3% (p value 0.006) by ABI among the infected compared to the non- infected adult participants.

Between 40 to 60 years, the burden of PAD in general population stands at 1% to 3% (Murabito JM et al. 1996, Agarzwal S, 2009).

2.3. Peripheral arterial disease Risk factors

There is a tendency of adults infected with HIV to have a great burden of CVD risk factors which predispose them to atherosclerosis and PAD (Hadigan C et al. 2017).

There is also a tendency of the following to independently predict PAD in persons infected with HIV: HIV infection itself, use of PIs, old age, smoking, hypertension, dysglycaemia, dyslipidaemia, and alcohol intake (Hadigan C et al. 2017, Periard D et al. 2008, Gupta N et al. 2013).

Bloomfield et al. and Kiplagat KN, have recently demonstrated a tendency of infected adults in western Kenya, under AMPATH care, to have a high burden of PAD risk factors.

2.4. HIV infection independently predicts PAD

HIV by itself has been found to independently predict PAD (Periard D et al. 2008, Gupta N et al. 2013). This is probably because HIV infection can directly cause immune induced inflammation and endothelial dysfunction that promote atherosclerosis (Freiberg MS. et al. 2013). This is accelerated in the presence of unsuppressed viral load and low CD+ count. However, patients who are on good ART that have successfully suppressed the viral load and increased CD+ count can still develop atherosclerosis that progress to PAD. This can be prevented by controlling other PAD risk factors (Freiberg MS. et al. 2013).

2.5. Increasing age

Although PAD is a disease of old age, HIV has been shown to lead to early development of PAD that worsens with age (Lorenz MW et al. 2006, Charakida et al. 2009). Early-onset atherosclerosis, or premature atherosclerosis, is defined as PAD presenting prior to 50 years of age. HIV-infected population with early-onset atherosclerosis have increased risk of progressing to PAD (Harris LM et al). Therefore, HIV infected population needs early screening, diagnosis, prevention and treatment of PAD before complications develop.

2.6. Antiretroviral therapy (ART)

The use of ART, especially protease inhibitors, leads to metabolic changes predisposing to development of atherosclerosis and PAD (Hadigan C et al. 2017). Protease Inhibitors have been shown to cause dysglycaemia, which predispose to atherosclerosis: Kaletra

(lopinavir/ritonavir) and darunavir are associated with dyslipidaemia while saquinavir causes dysglaycaemia. Dolutegravir, which is a widely used integrase strand transfer inhibitor is associated with dysglycaemia. Protease inhibitors are mostly used as second line ART to suppress viral load and raise the CD+ count. Therefore, as long as the viral load is suppressed and CD+ count is raised by using the PIs, the CVD risk factors (dyslipidaemia and dysglycaemia) need to be well controlled to prevent development and progression of PAD (Fontas Em Van Leth F et al. 2004, Lucher TF et al. 2003).

Moreover, ART has enabled HIV infected population to live long and this has also predisposed them to develop PAD which increases with age (Hadigan C et al. 2017). However, although PAD is a disease of old age in the general population, HIV seems to lead to early development of PAD that increases with age, which carries a poor prognosis (Lorenz MW et al. 2006, Charakida et al. 2009).

2.7. Dyslipidaemia in HIV

There is a tendency of HDL-c and LDL-c levels to be decreased and plasma triglycerides levels to be increased in HIV infection (Grunfed C et al. 1992) and this tends to correlate with accelerated PAD (Unit ES, 2005). In HIV infection, dyslipidaemia is mostly caused by the use of most protease inhibitors (PIs), although the effect will differ with the individual PI (Fontas Em et al. 2004). Patients diagnosed with PAD have a tendency of having increased levels of total cholesterol (Vogelberg KH et al. 1975) and reduced levels of HDL-c (Bradby GV et al. 1978). Thus, non-HDLc which is the difference between total cholesterol and HDLc is a better marker and predictor of PAD.

2.8. Dysglycaemia in HIV

Patients infected with HIV on ART, especially protease inhibitors, have higher risk of developing dysglycaemia and therefore, PAD: presence of HIV and PIs promote insulin resistance and dysglycaemia (Lucher TF et al. 2003, Brown TT et al. 2005, Rudich Am Ben -Romano R et al. 2005, Butt AA et al. 2009). Dysglycaemia is a poor prognostic factor in PAD (Bundó M et al. 2010). Dysglycaemia leads to development of symptomatic PAD with complications (Murabito JM et al. 1997).

2.9. Hypertension in HIV

Hypertension causes shear stress leading to damage to endothelium, causing atherosclerosis and eventually to PAD (Ridker PM et al. 2001). HIV-infected adult population tends to have high burden of hypertension (Bloomfield et al. 2007, Seaberg EC et al. 2005, Rooke T et al. 2013). Hypertension promotes progression of PAD (Palumbo PJ et al. 1991).

2.10. Smoking in HIV

Tobacco use is the leading modifiable cause of PAD. Smoking causes inflammation an endothelial damage, leading to atherosclerosis and PAD (Rahman MM et al. 2007). There is a high tendency of developing PAD in active cigarette smokers, but no association has been shown for other forms of tobacco exposure (Agarwal S, 2009). Smoking tends to cause early onset PAD (Powell JT et al. 1994). Smoking confers poor prognosis in PAD (Lu L et al. 2014). Stoppage of smoking decreases PAD morbidity (Conen D et al. 2011).

The worsening of PAD tends to correlate with pack years (Triant VA et al. 2007).

2.11. Pathogenesis of vascular disease in HIV infection

The pathogenesis pathway for PAD in HIV infection is multifactorial: HIV infection, high viral load, low CD+ 4 count, use of ART, increasing age, presence of high burden of cardiovascular risk factors and genetic predisposition have all been shown to synergistically predispose to atherosclerosis, leading to PAD. The initial steps in the PAD pathogenesis are mainly inflammation and endothelial dysfunction which lead to atherosclerosis. HIV infection, by itself, and all the CVD causations have been shown, in several studies, to cause PAD through inflammation and endothelial dysfunction (Reingold JM et al. 2008, Francisci D et al. 2009, Seingneur M et al.1997).

2.11.1 Inflammation

HIV infection presents with several marker of inflammation, comprising CRP and IL-6. The HIV immune induced inflammation causes damage to endothelial cells leading to endothelial dysfunction that promotes atherosclerosis and PAD. The ART, especially protease inhibitors, and the cardiovascular risk factors (old age, hypertension, dyslipidaemia, dysglycaemia, smoking and alcohol) have also been shown to promote inflammatory reactions that lead to endothelial dysfunction (Reingold JM et al. 2008).

2.11.2. Endothelial dysfunction

HIV can directly cause endothelial dysfunction as an initial step in atherosclerosis leading to PAD. All the other causative factors for PAD (smoking, hypertension, dyslipidaemia, dysglycaemia) have been shown to cause endothelial damage that promote atherosclerosis, progressing to PAD (Francisci D et al. 2009, Seingneur M et al. 1997).

2.11.2. Atherosclerosis

Immune induced inflammation and endothelial dysfunction promotes deposition of atherosclerotic plaques. Progressive buildup of the atherosclerotic plaques leads to narrowing and blockage of arterial lumen. This diminishes supply of blood to the lower limbs (Francisci D et al. 2009, Seingneur M et al. 1997).

2.12. Clinical Presentation

Narrowing of arterial lumen, as a result of atherosclerotic deposition, causes reduced blood supply. Ischaemic changes present as pain when walking which is alleviated by resting. This is termed as intermittent claudication. The reduced blood supply also causes the lower limb arterial pulses to be reduced or absent. The limb becomes cold to touch and assumes bluish colour due to compromised blood supply. When blood supply is severe reduced, pain will be present both when the patient is walking or at rest. This is referred to as critical limb ischaemia. When there is complete occlusion of the arterial lumen, the part of the limb distal to the blocked lumen undergoes necrosis, predisposing to gangrene formation.

2.13. Lower Limb PAD is suggestive of generalized atherosclerosis

PAD is an indicator of a generalized atherosclerosis. Therefore, presence of atherosclerosis in the lower limb predisposes to myocardial infarction and cerebrovascular accidents (ACC/AHA 2017).

2.14. Screening and diagnosis of PAD

Lower limb PAD, can easily and cheaply be screened and diagnosed using either ECQ or ABI. For those who are symptomatic, they can easily be diagnosed using either ECQ or ABI. For those who are asymptomatic, ABI is usually used. Both of these two tools are highly sensitive and specific and have been validated worldwide. (Hirsch AT et al. 2005).

2.14.1. Edinburgh Claudication Questionnaire (ECQ)

The ECQ is employed in screening and/or diagnosing symptomatic PAD, based on presence or absence of claudication (pain). Definite claudication is having calf pain. Atypical claudication is having buttock or thigh pain, without calf pain. However, ECQ cannot be used in those who are asymptomatic. During early presentation, only 10% of those who have lower limb PAD are symptomatic. Therefore, ECQ, can only screen for this 10% of the symptomatic PAD patients. The ECQ is a tool which has been validated worldwide with high specificity (91.3%) and sensitivity (99 %) for diagnosing those with classic and typical symptoms (The DAD study Group, 2007).

2.14.2. Ankle Brachial Index (ABI)

The ABI is employed in screening and/or diagnosing PAD in those who are either asymptomatic or symptomatic. The ABI is also highly sensitive and specific and easily available. There is diminishing supply of blood to the lower extremities, distal to the narrowed or blocked part. Therefore, the distal arterial pulses of the lower limb become reduced or absent. ABI uses a normal blood pressure machine. However, instead of a stethoscope, a hand held doppler machine is used to detect the reduced or absent arterial

pulses. Systolic ankle pressure and brachial pressures are taken all the arms and ankles. The ABI is derived by dividing the highest ankle pressure with the highest systolic brachial pressure. When ABI measure is equal to or less than 0.9 at rest, it is considered as suggestive of PAD. However, when the ABI at rets is normal or borderline, the patient can be subjected to an exercise (such as supervised squatting or treadmill). A post exercise ABI is then measured and if there is more than 15 percent drop in ABI, it is also considered as diagnostic for PAD. Subjecting the patient to exercise increases the ABI sensitivity for picking mild PAD (Hirsch AT et al. 2005).

2.15. Prevention and management of PAD

Controlling of the modifiable cardiovascular causative factors, in those at high risk of PAD, has been shown to lead to prevention, reduction or reversal of progression. Stopping smoking, reduced intake of fatty food, weight reduction, good glycaemic control, control of hypertension and dyslipidaemia and supervised exercise have all been shown to lead to prevention, reduction or reversal of progression of PAD in those at risk. Medical interventions using antiplatelets such as aspirin, cilostazol and pentofixylline and statins are used. However, those with advanced disease may require surgical interventions. Gangrenous foot may have to be amputated. Angioplasty and atherectomy may be used in those with severe atherosclerosis. Studies have shown that early screening promotes early detection and intervention (Khan NA, 2006): Early detection of PAD leads to successful management and prevention of complications (ACC/AHA 2017).

CHAPTER THREE: METHODOLOGY

3.1 Study Design

This was a cross sectional study design comparing the prevalence of peripheral arterial disease and associated factors in HIV-infected to non-HIV infected adult counterparts. The design was chosen because it is best suited for determination of prevalence and description of associated risk factors, without necessarily showing causal effects.

3.2 Study Setting

The study was conducted at the AMPATH HIV modules, adult medical wards and medical out-patient clinics at the MTRH.

The AMPATH program is an association between Moi University, College of Health Sciences, MTRH and a group of North American academic medical centres led by Indiana University that provides a comprehensive HIV care in western Kenya. The AMPATH centre at MTRH serves over 15,000 HIV infected patients in over 144 satellite clinical sites in both urban and rural western Kenya over the last 15 years. There are four AMPATH modules; three adult modules and one paediatric/youth module clinic where HIV infected patients receive care. Participants for this study were recruited from modules 1, 2 and 3.

This is a National Referral Hospital in Kenya, located in Eldoret, Kenya. It is an 800- bed capacity, Moi University affiliated hospital, serving a broad mix of rural, urban poor and urban middle class population. It serves a population over 16 million people in the whole of the larger western Kenya and is the primary care centre for the 300,000 urban population of Eldoret town.

The adult medical wards have two wards i.e. Umoja and Amani wards. The Umoja ward admits male patients while Amani ward admits female patients. Each ward has a bed capacity of 42 to cater for the large number of in-patients.

3.3 Study Population

3.3.1 Target population

The target population was adult HIV infected patients enrolled into care in the AMPATH modules and non-HIV infected counterparts admitted in the adult medical wards and those attending medical outpatient clinics in the MTRH. The participants were put into two groups based on their HIV status: HIV infected and non HIV infected study groups. Approximately over 15,000 HIV infected patients on ART are enrolled in AMPATH's urban MTRH with about 3,000 seen every month.

3.4 Sample Size

Based on the sample size calculation, as demonstrated below, we anticipated enrolling a minimum of 200 and a maximum of 236 HIV-infected and non-HIV infected adult patients. This sample size, with a minimum of 100 and a maximum of 118 patients in each study group, was powered to 80% to detect the difference in the prevalence of peripheral arterial disease between the HIV Infected and the sex and age matched non HIV infected.

According to the National Health and Nutrition Examination Survey (NHANES: Agarwal S, 2009, Selvin E et al. 2004), the prevalence of PAD is 1% percent between the ages of 40 and 49 and 2.5 percent between the ages of 50 and 59 (Agarzwal S, 1999 - 2000).

In a USA study by Gupta N et al. (2013), a PAD prevalence of 10% against 1.3% (p 0.006) by ABI and 28.5% against 12.5% by ECQ (p 0.0069) in infected against non-HIV infected adult participants was recorded.

In this present study we assumed the prevalence of PAD in the non HIV infected population to be 1.0%, and in the patients infected with HIV to be 10%, based on the NHANES (Agarwal S, 2009, Selvin E, et al. 2004) and Gupta N et al studies respectively. We determined the sample size such that with 80% certainty and 95% confidence we could be able to detect at least a 9% difference in the prevalence of PAD between the HIV infected and the non-HIV infected groups.

The sample size to compare two proportions was computed using the following formula (Hulley S et al. 2007).

$$n = \frac{\left(Z_{1-\alpha/2} \times \sqrt{(p_1 + p_2) \times (2 - p_1 - p_2)/2} + Z_{1-b} \times \sqrt{p_1(1-p_1) + p_2(1-p_2)} \right)^2}{(p_1 - p_2)^2}$$

$$= \frac{\left(1.96 \times \sqrt{(0.01 + 0.1) \times (2 - 0.01 - 0.1)/2} + 0.84 \times \sqrt{0.01(1-0.01) + 0.1(1-0.1)} \right)^2}{(0.01 - 0.1)^2}$$

$$= 100$$

Where p_1 is the proportion of PAD among the non-HIV-infected patients and p_2 is the proportion of PAD among HIV infected patients.

$z_{1-\alpha/2}$ is the $100(1-\alpha/2)$ percentile of the standard normal distribution under type I error while $z_{1-\beta}$ is the $100(1-\beta)$ percentile of the standard normal distribution under type II error.

$p_1 - p_2$ gives the effect size.

For each group we needed to enroll at least 100 participants.

Correcting for a potential nonresponse rate of 15% (r =0.15) will give us:

$\frac{n}{(1-r)} = \frac{100}{(1-0.15)}$ = 118 per arm.

For each group we needed to enroll 118 participants.

3.5 Sampling Technique

Systematic random sampling technique was used to select the participants for the study. For each group a sampling interval of five was used as described below.

HIV Infected Patients

The HV infected participants were enrolled at the AMPATH module clinics. There were three AMPATH module clinics each with 60 patients visiting per day. This implies that in three months the population size of HIV non-infected patients was

N = 60 patients per day x 5 days x 4 weeks x 3 months

= 3600 patients.

Given that the population size is sufficiently large, the sampling interval (k) was decided on a priori. Every fifth HIV infected patient meeting the inclusion criteria was sampled and enrolled in the study.

Non HIV Infected Subjects

The non HIV infected patients were enrolled from the medical adult (Nyayo) wards. All patients admitted at Nyayo ward are offered free HIV testing and these non-infected ones actually had confirmed negative testing.

Each day, there were 10 new patients being admitted in the wards. This implies that in three months the population size of HIV non-infected patients was

N = 10 patients per day x 5 days x 4 weeks x 3 months

= 600 patients.

Based on the population size and the sample size the sampling interval (k) = 600 / 118 = 5. In this group we chose to sample every fifth participant in order to get similar sampling intervals.

The medical outpatient clinics run on every Tuesday and Thursday. Every Tuesday and Thursday, there were 50 new patients being seen in the medical outpatient clinics. This implies that in three months the population size of HIV infected and non-infected patients was

N = 50 patients per day x 2 days x 4 weeks x 3 months

= 1200 patients.

Based on the population size and the sample size the sampling interval (k) = 1200 / 236 = 5. In this group we chose to sample every fifth participant in order to get similar sampling intervals.

Each of the two study groups had equal number of study participants. Recruitment was done until the desired sample size was attained in each of the two groups. The unique medical file outpatient number of every participants was recorded to prevent repeat recruitments.

3.6 Eligibility Criteria

3.6.1 Inclusion criteria

1. Persons aged 18 years and above with confirmed HIV status
2. Informed written consent
3. Persons receiving care at MTRH between March and May 2017

3.6.2 Exclusion criteria

1. Subjects with significant physical disability that precluded test procedures: deep venous thrombosis (DVT), recent surgery, ulcers, casts or bandages that could not be compressed by pressure cuffs.

3.7 Study Procedure

3.7.1 Recruitment of subjects

Subjects were recruited at the AMPATH outpatient HIV module clinics, medical wards, and outpatient medical clinics in MTRH, concurrently, between March and May 2017, by systematic random sampling, whereby every fifth subject was approached. Participants who met the criteria were approached and requested to participate in the study. Figure 1 below shows a summary of the study flow algorithm. Recruitment of HIV infected subjects into the study was done in the triage room and at the waiting lobby, as they waited to be seen by the clinicians. The recruitment of non HIV infected and HIV infected subjects into the study was done in the adult medical wards and outpatient medical clinics at the MTRH, after undergoing standard routine serological testing by the AMPATH staff and confirming their HIV status.

The purpose of the study and the potential benefits were explained to the participants individually in a language they understood and all their questions were answered. Those who met the inclusion criteria and consented to participate in the study were enrolled after signing informed consent forms (Appendix I and II).

The participants were then recruited either into the HIV infected (exposed) group or non HIV infected (comparison) study group. The two study groups had equal number of

participants (118 subjects in each group). The HIV infected subjects and non HIV infected subjects were matched for sex and age (±5years). The age was self-reported and confirmed from the documents (clinical data and/or national identity card). A predesigned matching matrix was used to match for age (±5). Recruitment was done until the desired sample size was attained in each of the two study groups.

3.7.2 Study Procedures

After recruitment the participants' socio-demographic characteristics and clinical data were collected by the study questionnaire (Appendix I) and Edinburg Claudication Questionnaire (Appendix II). Subsequently, they had their anthropometric measurements (height – Appendix I and body weight (Appendix I) and blood pressure taken (Appendix VII). Then, Ankle Brachial Index measurement was done at rest and post squatting exercise (Appendix VIII).

Those with PAD, dysglycaemia and/or dyslipidemia were referred to their respective clinics for education and appropriate care. The same information was communicated to the attending clinicians. The algorithm for the study procedure is summarized in Figure 1 below.

3.7.3 Laboratory procedures

Blood sample (about 10ml) was drawn from the venipuncture for HBA1c (Appendix IV) and Lipid profile (Appendix VI) from each participant. The blood samples were then taken to the laboratory after labelling. The results would come the following day. The principal researcher and/or assistant researcher would make a phone call to the subjects who had participated in the study and inform them about the clinical results. Those with

PAD, dysglycaemia and/or dyslipidaemia were referred to their respective clinics for education and appropriate care. The same information was communicated to the attending clinicians, through official clinical referral notes. The algorithm for the study procedure is summarized in Figure 1 below.

3.8 Data Variables

3.8.1 Primary outcome variables

1. Peripheral Arterial Disease (PAD) was defined according to AHA and ESC (2017) as Ankle Brachial Index (ABI) ≤ 0.9 (Appendix VIII) and/or presence of claudication (Appendix I).
2. Claudication was defined by the Edinburg Claudication Questionnaire (Appendix I) as:
    i. Definite claudication being pain in the calf
    ii. Atypical claudication as having pain in thigh or buttock (in the absence of calf pain).

3.8.2 Secondary data variables

1. Dysglycemia was defined according to ADA (2017) as a HBA1c ≥5.7

   This was standardized by drawing venous blood and putting in sample bottles that had anticoagulants. The samples were sent to the MTRH level II point of care laboratory that is NGSP satisfied and does calibrations every 6 monthly to the DCCT Reference values. The HBA1 was measured within 12 hours of drawing the venous blood (appendix V).
2. Dyslipidaemia was defined according to the American Association of Clinical Endocrinologists and American College of Endocrinology Guidelines for

management of dyslipidemia and prevention of cardiovascular disease (AACE, 2017 guidelines) as presence of Non HDL-c > 4.9mmol/L.

3. Blood pressure: Hypertension was as defined according to JNC 7 as BP ≥140/90 mmHg, as measured after 5 minutes of rest or being on any antihypertensive drugs or having a previous history of hypertension (Appendix VII).
4. Body Mass Index (BMI) was defined according to ADA 2017 guidelines as the ratio of height and weight, expressed as kg/m2 (Appendix I).
5. Alcohol intake(Appendix I)
6. Cigarette smoking (Appendix I)

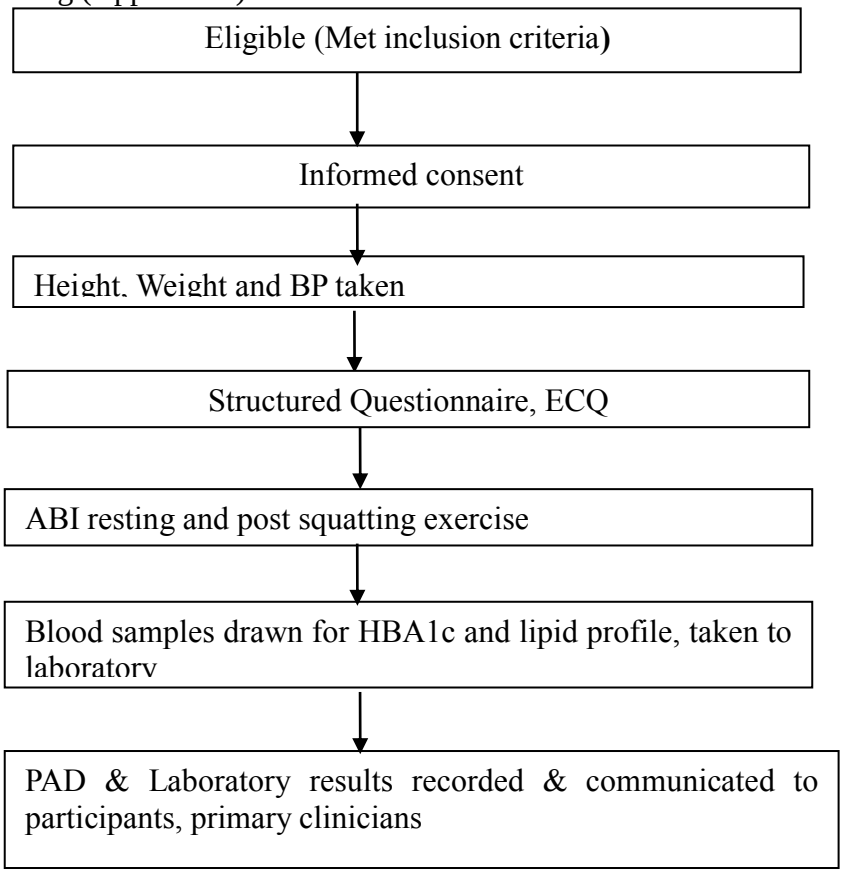

Figure 1: Algorithm of study procedure

3.9 Data management

Materials and data collection: A standardised interviewer administered questionnaire was used to collect data from the subjects' demographic and clinical information. The only unique identifier questionnaire contained was the subject's birth dates and the study number. A master list was maintained which linked the study number to subject's identifiers in order to provide primary care providers with the results of laboratory studies. The master list was maintained in the study computer.

Data cleaning: Data was cleaned during collection, data entry and analysis. Data collection sheets and questionnaires were examined closely for completeness and errors at the end of each week during data collection. Missing data was not included in the analysis. There was no need to collect more data since the sample size had already been inflated to factor in missing data and non-responders.

Data entry: double entry was done to minimize error. Data entry and cleaning were done using computer excel database. Data was entered into computer Microsoft excel before exporting to R STATA software version 13.

Data protection and security: paper records were kept by lock and key not accessible to anyone. Computer was password protected, with up to date Kaspersky antivirus, internet firewall protected and with a backup.

Sharing: data was encrypted when sharing through internet. Identifiable descriptions like date of birth were removed.

Duration: questionnaire will be kept for 7 years for legal safety of the raw data.

Statistical Data Analysis

Descriptive statistics such as mean and the corresponding standard deviation (SD) were used to summarize continuous variables (e.g. age, BMI, non HDLC among others) that assumed the Gaussian distribution. Continuous variables that violated the Gaussian assumptions such as SBP, DBP, and HBA1c among others were summarized using the median and the corresponding inter-quartile range (IQR). Gaussian assumptions were assessed using Shapiro and Wilk test. Categorical variables such as gender, categorized BMI, categorized SBP, and categorized DBP among others were summarized using frequencies and the corresponding percentages.

Association between the HIV status and independent continuous variables that assumed the Gaussian distribution such as age, BMI, non HDLC among others were compared using independent samples t-test. Two sample Wilcoxon rank-sum test was used to compare independent continuous variables that violated the Gaussian assumptions. Pearson's Chi Square test was used to assess the association between HIV status and independent categorical variables.

Two sample Wilcoxon rank-sum test was used to compare independent continuous variables that violated the Gaussian assumptions. Fisher's exact test was used whenever the Chi Square assumptions were violated.

The prevalence of PAD were reported alongside the corresponding 95% confidence intervals (95% CI).

Interpretation of data was done using STATA version 15.

3.10 Ethical Considerations

Prior to initiating research study, research proposal was acknowledged by the department of internal medicine, AMPATH and MTRH and approved by the Institutional Research and Ethics Committee (IREC).

Patients who were recruited for the study were informed that participation is voluntary and they could withdraw from the study at any point. The purpose of the study was explained at an appropriate educational level to ensure understanding and all participants were required to sign a consent form delineating the objectives of the study and their rights.

Participants were informed that the project did not carry monetary benefits and all tests performed for the study incurred no fees to participants.

Patient data were de-identified and entered into a research database. Data was stored into one master database. The principal investigator and research assistant upheld the highest level of confidentiality and privacy for all participants.

CHAPTER FOUR: RESULTS

4.1 Screening and Recruitment of Participants

Altogether, 266 participants (133 infected and 133 non-HIV infected patients) were screened for the study between March and June 2017. Of these, 236 (118 infected and 118 non-HIV infected patients) fulfilled the inclusion criteria, and were recruited. Figure 2 shows the recruitment schema for the study.

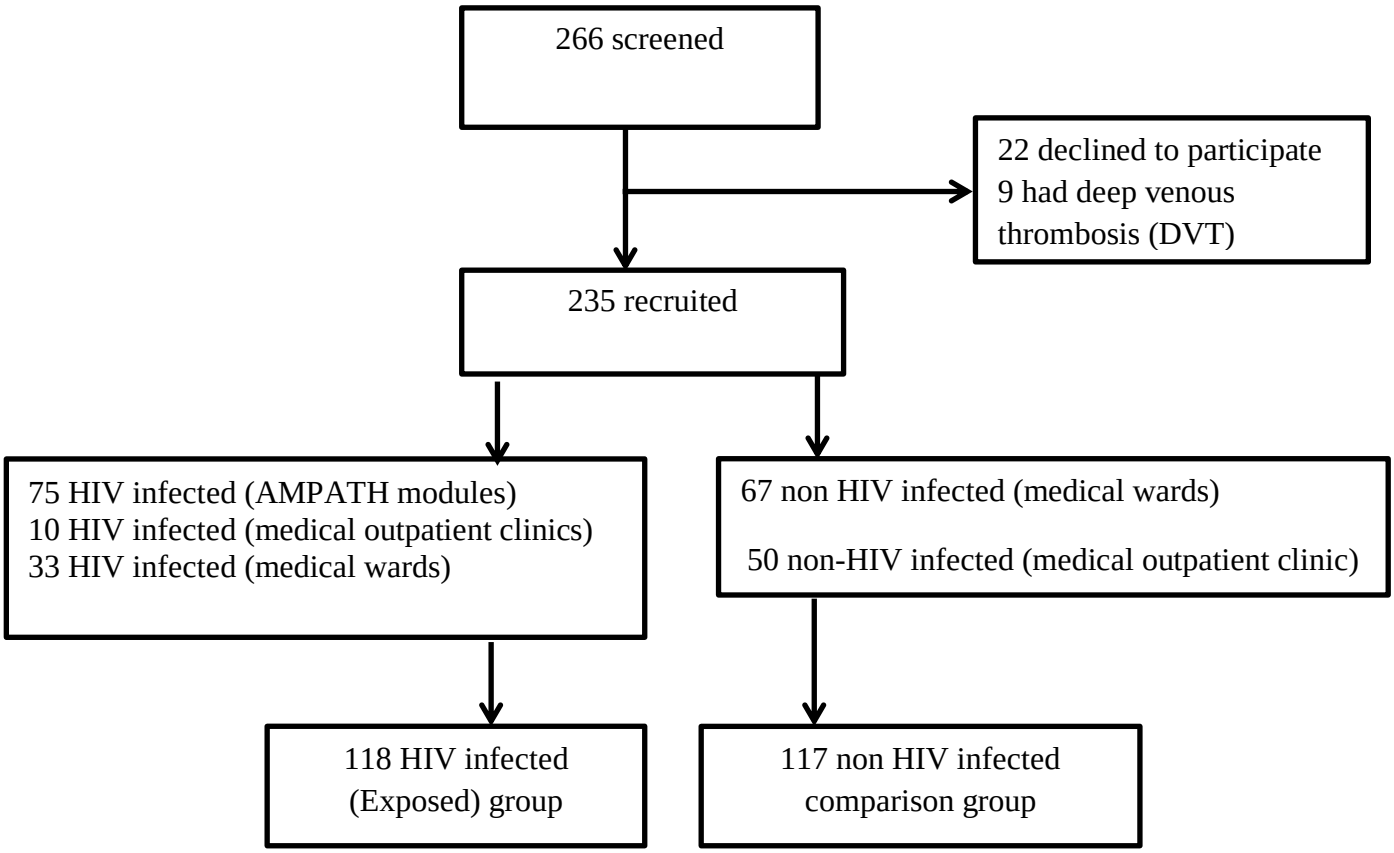

Figure 2: Recruitment Schema

4.2 Sociodemographic Characteristics

Table 1: Demographic characteristics

Characteristics	**HIV Status**		Statistical Comparison
	Infected (N = 118)	Non Infected (N = 117)	
	Mean (SD) or Median (IQR) or n (%)		**P – Value**
Age (Years)	41.8 (9.5)	39.6 (9.2)	0.064[t]
Male	60 (50.9%)	58 (49.6%)	0.845[c]

[c] Pearson's Chi Square test; [t] Independent samples t-test.

Altogether, 235 participants were enrolled in the study, 118 HIV infected, and 117 non-HIV infected. Table 1 presents the comparison of demographic characteristics by the HIV status.

The mean age between the HIV infected and the non-HIV infected was statistically the same, 41.8 (SD: 9.5) years vs. 39.6 (SD: 9.2) years, p = 0.064. The proportion of the male participants among the infected and among the non-HIV infected were similar, 50.9% vs. 49.6%, p = 0.845. This is because our participants were matched for sex and age.

4.3 Clinical Characteristics

Table 2: Clinical characteristics

	HIV Status		
	Infected (N = 118)	Non Infected (N = 117)	Statistical Comparison
Characteristics	**Mean (SD) or Median (IQR) or n (%)**		**P – Value**
BMI (Kg/m^2)	23.6 (2.9)	24.6 (3.8)	0.019[t]
< 18.5	15 (12.7%)	7 (6.0%)	
18.5 – 24.9	58 (49.2%)	56 (47.9%)	0.094[c]
25.0 – 30.0	30 (25.4%)	35 (29.9%)	
> 30.0	15 (12.7%)	19 (16.2%)	
Non HDLC	3.4 (1.0)	3.0 (1.1)	0.010[t]
< 4.0	90 (76.3%)	97 (82.9%)	
4.0 – 4.8	18 (15.3%)	13 (11.1%)	0.601[c]
4.8 – 5.7	6 (5.1%)	5 (4.3%)	
> 5.7	4 (3.4%)	2 (1.7%)	
Dyslipidemia (non HDLC > 4.91)	9 (7.6%)	6 (5.1%)	0.304[c]
HBA1c	4.8 (4.1, 6.5)	5.0 (4.2, 6.0)	0.728[w]
Dysglycaemia ≥5.7%	30 (25.4%)	14 (12.0%)	0.006[c]
SBP (mm Hg)	120.0(110.0,130.0)	130.0(128.0, 138.0)	<0.001[w]
95 – 119	22 (18.6%)	25 (44.4%)	
120 – 139	85 (72.0%)	48 (52.1%)	0.003[c]
140 – 160	11 (9.3%)	44 (37.6%)	
DBP (mm Hg)	70.0 (60.0 – 75.0)	80.0 (70.0 – 85.0)	<0.001[w]
< 70	20 (16.9%)	22 (37.6%)	
70 – 79	78 (66.1%)	48 (41.0%)	
80 – 89	12 (10.2%)	23 (18.0%)	0.006[c]
90 – 99	8 (6.8%)	24 (20.5%)	
Hypertension status			
Normal BP	57 (48.3%)	22 (18.8%)	
Prehypertension	50 (42.4%)	67 (57.3%)	0.006[c]
Hypertension	11 (9.3%)	28 (23.9%)	
Alcohol use	14 (11.9%)	2 (1.7%)	0.002[c]
Smoking	3 (2.5%)	3 (2.6%)	0.992[c]
ART (Anti-Retroviral Therapy)			
First Line	80 (67.8%)	NA	NA
Second line	32 (27.1%)	NA	NA
Third line	6 (5.1%)	NA	NA
Viral Load (copies/ml)			
<1000 copies/ml	96 (81.4%)	NA	NA
>1000 copies/ml	22 (18.6%)	NA	NA

SBP – Systolic Blood Pressure; DBP – Diastolic Blood Pressure; BMI – Body Mass Index;

Normal BP - SBP < 120 & DBP < 90; Prehypertension -SBP: 120 – 139 | DBP: 80 – 89; Hypertension - SBP ≥ 140 | DBP ≥ 90; [c] Pearson's Chi Square test; [t] Independent samples t-test; [w] Two sample Wilcoxon rank-sum test

Table 2 presents the comparison of clinical characteristics by the HIV status. Non-HIV infected participants had a significantly higher mean BMI, 24.6 (SD: 3.8) kg/m^2 compared to the HIV infected, 23.6 (SD: 2.9) kg/m^2, p = 0.019. HIV infected participants were more likely to have normal weight compared to the non-HIV infected, 49.2% vs. 47.9%, p = 0.094.

HIV infected participants had significantly higher average non-HDLC levels compared to the non-HIV infected, 3.4 (SD: 1.0) vs. 3.0 (SD: 1.1), p = 0.010.

There was evidence of pronounced presence of dyslipidaemia among the HIV infected participants compared to the non-HIV infected, 7.6% vs. 5.1%, p = 0.304.

The proportion of participants with dysglycaemia (HBA1c ≥ 5.7%) was significantly higher among the infected participants compared to the non-HIV infected, 25.4% vs. 12.0%, p = 0.006.

The median systolic blood pressure was significantly high among the non-HIV infected participants compared to the HIV infected, median (IQR): 130.0 (IQR: 128.0, 138.0) vs. 120.0 (110.0, 130.0) mm Hg, p <0.001. Similarly, the median diastolic blood pressure was significantly high among the non-HIV infected compared to the HIV infected, median (IQR): 80.0 (IQR: 70.0, 80.0) vs. 70.0 (IQR: 60.0, 75.0) mm Hg, p <0.0001.

The proportion of pre-hypertensive (SBP: 120-139 & DBP < 80 or DBP: 80-90 and SBP < 120) participants was significantly high among the non-HIV infected compared to the HIV infected participants, 57.3% vs. 42.4%, p = 0.006. The proportion of hypertensive (SBP: ≥140 | DBP ≥ 90) participants was high among the non-HIV infected (23.9%) compared to the HIV infected (9.3%), p = 0.006.

The proportion of infected participants who reported alcohol use was significantly higher than those reporting alcohol use among the non-HIV infected, 11.9% vs. 1.7%, p = 0.002. The proportion who reported smoking among the two groups were similar, 2.5% vs. 2.6%, p = 0.992.

All HIV infected participants were on ARV with the proportion of those on first line being 67.8%, second line 27.1% and third line 5.1%.

Majority of the proportion of HIV infected participants had suppressed viral load (81.4%), with only 18.6% having high viral load.

4.4 Prevalence of Peripheral Arterial Disease in Hiv Infected and Non Hiv Infected Patients

Table 3: Peripheral arterial disease by ABI and ECQ

	HIV Status		Statistical
	Infected (N = 118)	Non-Infected (N = 117)	Comparison
Variable	**Median (IQR) or n (%)**		**P – Value**
PAD			
PAD = Low ABI (ABI ≤ 0.9)	21 (18.0%)	3 (2.6%)	
Borderline (ABI: 0.91– 1.0)	63 (53.0%)	33 (28.2%)	<0.001 [w]
Normal (ABI: 1.0 – 1.3)	34 (29.0%)	81 (69.2%)	
PAD = Claudication present	24 (20.3%)	8 (6.8%)	0.003[c]

ABI – Ankle Brachial Index; PAD – Peripheral Arterial Disease; [c] Pearson's Chi Square test; [w] Wilcoxon rank-sum test, [c] Pearson's Chi Square test

Table 3 describes the prevalence of peripheral arterial disease by Ankle Brachial Index (ABI) and by Edinburgh Claudication Questionnaire (ECQ).

Based on ABI, PAD was found in 18.0% (95% CI: 11.0 – 26.0) among the HIV infected, and in 2.6% (95% CI: 0.5, 7.0) among the non-HIV infected participants. This finding shows that infected participants had a higher prevalence of PAD compared to the non-HIV infected, p <0.001.

By ABI, the proportion of infected participant who had borderline ABI compared with the non-HIV infected was 53.0% vs 28.2%, p <0.001.

The proportion of infected participants who had normal ABI compared with non-HIV infected was 29.0% vs 69.2%, $p < 0.001$.

Based on ECQ, the results demonstrated evidence of significantly higher proportion of participants with claudication among the infected compared to the non-HIV infected, 20.3% vs. 6.8%, $p = 0.003$

Table 4: Secondary Descriptive Data on all participants with PAD

	Participants with PAD		
	Total (N = 32)	HIV infected (N = 24)	Non-HIV infected (N = 8)
Characteristics	**Mean (SD) or Median (IQR) or n (%)**		
Age (Years)	46.3 (11.4)	45 (12)	54 (31)
Male Female	9 (28.1) 23 (71.9)	3 (12.5) 21 (87.5)	6 (75.0) 2 (25.0)
Mean BMI (Kg/m^2) Overweight 25.0 – 30.0 Obesity > 30.0	25.1 (3.9) 18 (56.2) 6 (18.8)	25.1 (3.9) 13 (54.2) 4 (16.7)	24.0 (3.4) 5 (62.5) 2 (25.0)
Mean Non HDLC Dyslipidaemia (non HDLC > 4.91)	4.2 (1.4) 12 (37.5)	4.5 (1.4) 9 (37.5%)	3.6 (1.4) 3 (37.5)
Mean HBA1c Dysglycaemia (HBA1c≥5.7)	8.1 (6.3, 9.9) 24 (75.0)	8.1 (6.3, 9.9) 18 (75.0%)	4.9 (4.0, 6.0) 6 (75.0)
Low ABI	24 (75.0)	21 (87.5%)	3 (37.5)
Claudication present	32 (100.0)	24 (100.0)	8 (100.0)
Mean SBP (mm Hg)	135.0 (130.0, 138.0)	128.0 (112.0, 130.0	135.0 (130.0, 138
Mean DBP (mm Hg)	85.0 (80.0, 88.0)	70.0 (68.0, 80.0)	85.0 (80.0, 88.0)
Hypertension (SBP ≥ 140 | DBP ≥ 90)	8 (25.0)	3 (12.5)	5 (62.5)
Alcohol use	2 (6.3)	2 (8.3)	0 (0.0)
Smoking	0 (0.0)	0 (0.0)	0 (0.0)
ART (Anti-Retroviral Therapy) First Line Second Line Third Line	 9 (28.1) 14 (43.8) 1 (3.1)	 9 (37.5) 14 (58.3) 1 (4.2)	 NA NA NA
Viral Load (copies/ml) <1000 copies/ml >1000 copies/ml	 10 (31.3) 14 (43.7)	 10 (41.7) 14 (58.3)	 NA NA

A total of 32 participants who were enrolled in the study were diagnosed with PAD, 24 HIV infected, and 8 non-HIV infected.

Table 4 presents the secondary data on the demographic and clinical characteristics of all the 32 participants who had PAD.

All the 32 participants who had PAD had a median age of 46.3 (IQR: 11.4) years. However, Non – HIV infected participants who had PAD tended to be older than their HIV infected participants with PAD, 54 (IQR: 31) years vs 45 (IQR: 12) years.

The dominant characteristics among the 32 participants who had PAD were that: a high proportion of them tended to be female (72.0%) and to have overweight (56.0%) and dysglycaemia (75.0%). All of the 32 participants with PAD had symptomatic claudication (100.0%) but only 75.0% of them had PAD based on low ABI (ABI equal to or less than 0.9 or more than 15% absolute drop post squatting exercise).

A high proportion of 75.0% of the non-HIV infected participants who had PAD tended to be male, while a high proportion of 87.5% of the HIV infected participants who had PAD tended to be female.

A high proportion of 62.5% of non-HIV infected participants who had PAD tended to have hypertension, while only 12.5% of the infected participants who had PAD tended to have hypertension.

A high proportion of 58.3% of the infected participants who had PAD tended to be on second line ART. A high proportion of 58.3% of the infected participants who had PAD also tended to have a high, unsuppressed viral load of > 1000 copies/ml.

CHAPTER FIVE: DISCUSSION

5.1 Prevalence of Peripheral Arterial Disease

5.1.1 Prevalence of Peripheral Arterial Disease by Edinburg Claudication Questionnaire and Ankle Brachial Index

This cross-sectional study has revealed a trend towards a PAD prevalence that is high among infected adults compared to non-HIV infected counterparts. This high prevalence can be attributed, probably, to the following: HIV infection, high viral load, low CD+ 4 count, use of ART, increasing age, presence of high burden of cardiovascular risk factors and genetic predisposition, all of which have been shown to synergistically predispose to atherosclerosis, leading to PAD (Beckman JA et al., Hadigan C et al. 2017, Periard D et al. 2008, Gupta N et al. 2013). HIV infection can directly cause immune induced inflammation and endothelial dysfunction that promote atherosclerosis (Freiberg MS. et al. 2013). This is accelerated in the presence of unsuppressed viral load and low CD+ count. However, patients who are on good ART that have successfully suppressed the viral load and increased CD+ count may still develop atherosclerosis that progress to PAD. This can be prevented by controlling other PAD risk factors (Freiberg MS. et al. 2013): HIV infected persons tend to have a great burden of CVD risk factors which predispose them to atherosclerosis and PAD (Hadigan C et al. 2017). Most of the HIV infected adult population are on ART (UNAIDS Data 2017).The use of ART, especially protease inhibitors, leads to metabolic changes predisposing to development of atherosclerosis and PAD (Beckman et al. 2018, Hadigan C et al. 2017). Moreover, the use of ART has enabled patients infected with HIV to live long and this has also predisposed them to develop PAD which increases with age (Beckman et al. 2018,

Hadigan C et al. 2017). Although PAD is a disease of old age, HIV has been shown to lead to early development of PAD that worsens with age (Lorenz MW et al. 2006, Charakida et al. 2009). Early-onset atherosclerosis, or premature atherosclerosis, is defined as PAD presenting prior to 50 years of age. HIV-infected population with early-onset atherosclerosis have increased risk of progressing to PAD (Harris LM et al).

HIV infected persons tend to have a great burden of CVD risk factors which predispose them to atherosclerosis and PAD (Beckman et al. 2018, Hadigan C et al. 2017).

Most studies in USA and Europe have reported high prevalence of PAD in those infected compared to those not infected with HIV. This study was comparable to similar studies, done in Switzerland and USA as follows:

A similar study was done in Switzerland in 2008 by Periard et al. Ninety two HIV infected adult participants aged ≥40 years (mean of 49.5 years) were consecutively enrolled. Only 23.9% of them were women. PAD was defined as having claudication, based on ECQ, and/or having abnormal ABI. ABI diagnostic of PAD was an ABI < 0.9 or more than 25% absolute drop in ABI post exercise. A PAD prevalence of 15.2% by ECQ and 20.7% by ABI was recorded.

This study was comparable to that done in Switzerland by Periard et al. in terms of similar methodology, whereby pre and post exercise ABI measurements were used. The slightly higher prevalence of PAD can be attributed to the much older participants (mean age 49±7.4 years) compared to the participants in our study (mean age 41±10 years), considering that PAD increases with age.

This study was comparable to that done in USA by Gupta N et al. 2013, who recorded a PAD prevalence of 28.5% against 12.5% (p value 0.0069) by ECQ and 10% against 1.3% (p value 0.006) by ABI among those infected compared to those not, matched for age but not sex. ABI diagnostic of PAD was defined as less than 0.9 or more than 15% absolute drop in ABI post squatting exercise.

This study was comparable to that done in USA by Gupta N et al. in terms of similar methodology, whereby pre and post exercise ABI measurements were used. However, this study differed from that by Gupta N et al. in terms of the definition of PAD, whereby PAD was defined as ABI less than or equal to 0.9 or more than 15% absolute drop in ABI on exercise in this study, but defined as ABI less than 0.9 or more than 15% absolute drop on exercise by Gupta N et al. Thus, whereas in this study ABI =0.9 was considered as PAD, the same was excluded by Gupta N et al. which could explain the smaller prevalence of 10% compared to the 18%.

This study was comparable to that done in USA by Ashraf Qaqa et al. in terms of methodology, whereby post exercise ABI measurements were used. However, this study was different from that of Ashraf Qaqa et al. in terms of the definition of PAD, whereby PAD was defined as ABI less than or equal to 0.9 or more than 15% absolute drop on exercise in this study, but defined as an absolute decrease in post exercise ABI (eABI) >15% or a fall in post exercise ankle systolic pressure (eASP) by at least 20mmHg, by Ashraf Qaqa et al, which probably increased the sensitivity of low ABI measurement, thus higher prevalence of 26.5% than in the present study of 18%. The higher prevalence could also be attributed to the older participants (mean age 46±10.2 years) compared to the participants in our study (mean age 41±10 years).

Although PAD is a disease of old age, HIV has been shown to lead to early development of PAD that worsens with age (Lorenz MW et al. 2006, Charakida et al. 2009). A mean age of 45.0 (SD: 12) years was found among the infected patients. Early-onset atherosclerosis, or premature atherosclerosis, is defined as PAD presenting prior to 50 years of age. Patients with early-onset PAD tend to have a severe disease and worse prognosis (Harris LM et al). Thus, there is need for establishment of early intervention programs to prevent development of PAD in this HIV infected population.

In this present study, there was a higher PAD prevalence despite a small burden of hypertension, overweight and obesity among those infected in comparison to the non-infected participants. This was similar to Gupta N et al and Beckman et al who also in their studies reported higher PAD prevalence despite similar or reduced atherosclerotic risk factor burden among the infected in comparison to the non-infected patients. They supported their findings by demonstrating that HIV infection independently predicted PAD (Gupta N et al. 2013, Beckman et al. 2018). Periard D et al and Ashraf Q et al, in their studies, did not have non-HIV infected comparison groups. However, in this present study, there was a higher burden of dysglycaemia, dyslipidemia and alcohol intake among those infected compared to the non-infected participants. This was comparable to Kwiatkowska W et al. 2014, who reported higher prevalence of lipid disorders and diabetes mellitus among those infected in comparison to the non-infected group. This was also consistent with Hadigan C et al, 2017, who have reported that infected persons tend to have increased burden of PAD risk factors. The findings were also comparable to the two previous studies by Bloomfield et al, and Kiplagat KN, done in the same HIV

infected population catchment area, that have demonstrated that infected patients in western Kenya tend to have high burden of PAD risk factors.

Kiplagat KN (2015, unpublished master's dissertation in Moi University) in his study found the prevalence of newly diagnosed dyslipidemia to be 70% (CI: 64.5, 75.2) and dysglycaemia to be 15.3% (CI: 11.4, 19.9); diabetes mellitus and impaired fasting glucose being 2.3% and 12% respectively in HIV infected patients attending care at MTRH. In the same study, a prevalence of 2% of newly diagnosed hypertension, 9% of smoking, 30% of alcohol use, 25% of overweight and 14% of obesity were reported among the HIV infected participants. A peri-urban population based study in Bungoma East District, by Bloomfield et al., among 4037 adults with median age of 35 (IQR: 26-50) years in Western Kenya showed that 7% of the population used tobacco while 16% of the population reported alcohol use. In the same study, the proportion of hypertension in men was 11.2% and in women was 7.4%. The proportion of overweight/obesity in men was 10.6% and 22.6% in women.

The prevalence of overweight in our infected participants of 25.4% is comparable to that by Kiplagat KN, who reported prevalence of overweight of 25.0% and Bloomfield et al. reported overweight/obesity prevalence of 10.6 and 22.6%, among men and women, respectively. The similarity, in these three studies, can be attributed to similar patient characteristics and environmental factors. However, Bloomfield et al. had a merged prevalence of overweight/obesity, unlike in our study.

The proportion of HIV infected participants with obesity of 15 (12.7%) is comparable to the study by Kiplagat KN, who reported prevalence of obesity of 14% and also to

Bloomfield et al. reported overweight/obesity prevalence of 10.6 and 22.6%, among men and women, respectively. The similarity can also be attributed to similar patient characteristics and environmental factors.

About 9 (7.6%) of the HIV infected participants were noted to have dyslipidemia. Both Gupta N et al. and Periard D et al. recorded a slightly higher prevalence of 13.0%. The similarity can be attributed to similar methodology of determining dyslipidemia, using non-HDLc measurements. The slightly higher prevalence can be attributed to patient characteristics, in that the USA and Switzerland studies were mainly multiracial study populations, who were also much older than participants in the current study, which consisted mainly of the black Kenyan Africans. However, the study by Kiplagat KN did not use non-HDLc for estimation of dyslipidemia. Patients with PAD often present with increased levels of total cholesterol (Vogelberg KH et al. 1975) and low levels of HDL-c (Bradby GV et al. 1978). Non-HDLc, which is the difference between total cholesterol and HDL-c, is highly effective predictor of PAD.

The proportion of HIV infected participants with dysglycaemia was 30 (25.4%) which is comparable to that reported by Kiplagat KN of 15.3%. This similarity can also be explained by the similar patient characteristics and environmental factors. The slightly higher prevalence can be attributed to the difference in methodology in that whereas we used HBA1c, Kiplagat's study used fasting blood sugar measurements. HBA1c is highly effective at predicting cardiovascular events such as PAD, and dysglycaemia is a poor prognostic factor in PAD (Bundó M et al. 2010). Dysglycaemia leads to development of symptomatic PAD with complications (Murabito JM et al. 1997).

The proportion of the infected participants who had hypertension was 11 (9.3%). Bloomfield et al. had a hypertension prevalence of 11.2% in men and 7.4% in women. This similarity can also be explained by the similar patient characteristics and environmental factors. However, Kiplagat's study only reported a prevalence of 2% among the newly diagnosed hypertensives, unlike in this study that reported presence of both past and current history of hypertension. Hypertension independently predicts PAD (Periard D et al. 2008, Gupta N et al. 2013). There is a tendency of hypertension to confer poor prognosis to PAD patients (Palumbo PJ et al.1991).

The proportion of HIV infected participants using alcohol was 14 (11.9%). Bloomfield et al, had a prevalence of 16.0% and the study by Kiplagat KN reported a prevalence of 32.0%. The participants in all the three studies came from the same catchment area. However, in this present study we looked at the history of current alcohol usage only, as opposed to having a history of past and/or present alcohol usage, as in the two previous studies.

The proportion of HIV infected participants smoking cigarette was 3 (2.5%). Bloomfield et al, had 7.0% and Kiplagat KN reported a prevalence of 9.0%. The participants were from the same catchment area in all these studies. However, in this present study we looked at the history of current smoking only, as opposed to having a history of past and/or present smoking, as in the two previous studies. Smoking independently predicts PAD (Lu Let al) and smoking cessation decreases morbidity related to PAD (Conen D et al. 2011).

This study reported the PAD prevalence of 2.6% by low ABI and 6.8% by Edinburg Claudication Questionnaire, among the non-HIV infected participants. These findings are comparable to Oyelade BO et al. 2012 and Taylor P et al. 2011, who have reported that the burden of PAD in Africa ranges from 2% and above, in the general population

All the participants who had PAD tended to have the following predominant risk factors: being old (median age, 46.3 IQR: 11.4), female (71.9%), overweight (56.2%), having dysglycaemia (75.0%), dyslipidaemia (37.5%), high viral load (58.3%), being on second line ART (58.3%), and hypertensive (25.0%). Periard D et al. and Gupta N et al. have previously found these risk factors to independently predict PAD.

A high proportion of participants diagnosed with PAD were females (71.9%) compared to the males (28.1%). This was consistent with previous studies that showed that PAD prevalence is higher among women than men in black African race (Norgren L et al. 2007). However, other studies have shown that African men have more PAD burden than women (Allison MA et al. 2009, Oyelade BO et al. 2012).

5.2 Study Strengths and Limitations

Strengths:

This was a comparative study: a comparative arm (non-HIV infected) highly enriched understanding of PAD in our region.

The matching by sex and age of participants in this study improved our understanding of difference in prevalence of PAD between those infected with HIV and those not infected.

To detect contrast in prevalence between infected participants and non-HIV infected counterparts, the study was powered at 80%.

The use of post exercise ABI was used to improve sensitivity.

The use of both ABI measurement and ECQ ensured that there was increased sensitivity and specificity, to ensure that less severe forms of peripheral arterial disease could not be missed.

Limitation:

Predictors and associations of PAD were not ascertained. We have overcome this by describing the unique dominant characteristics of participants who had PAD.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study shows that there is a high prevalence of PAD, as measured by ABI and ECQ, in HIV infected compared to non-HIV infected adults in a tertiary medical centre in western Kenya. The participants who had PAD tended to have the following predominant risk factors: older age, female, having dyslipidemia, dysglycaemia, high viral load, being on second line protease based ART, overweight and hypertensive.

6.2 Recommendation

1. Routine individualized screening of PAD, in those with more than one risk factor, based on guidelines by AHA/ACC, and
2. Monitoring of potential risk factors in HIV infected population.

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APPENDICES

APPENDIX I: QUESTIONNAIRE AND DATA COLLECTION FORM

CARDIOVASCULAR HEALTH EXAMINATION QUESTIONNAIRE PERIPHERAL ARTERIAL DISEASE STUDY (adopted from kirui's study)

SOCIODEMOGRAPHIC INFORMATION

1. **Sex**

☐ Male ☐ Female

2. **Date of birth |__|__| / |__|___| / |__|__|__| (DD|MM|YYY)**

PERSONAL MEDICAL HISTORY

Now I'm going to begin with some general questions about your health.

1. **What is your HIV status:** ☐ **positive** ☐ **Negative**

   Date of HIV test: |__|__| / |___|___| / |___|___|___|___| (DD|MM|YYY)

   If HIV negative skip to question #2

- When were you diagnosed with HIV infection?
  |__|__| / |___|___| / |___|___| **(Give at least month and year)**
  (DD|MM|YYY)
- Do you take medications (ART) for HIV? ☐ Yes ☐ No
- If yes, which of the following HIV medication for you **currently** take? (select all that apply)
  - I. Atazanavir (ATV) ☐
  - II. Darunavir ☐
  - III. Efavirenz (EFV) ☐
  - IV. Lamivudine (3TC) ☐
  - V. Lopinavir (LPV) ☐
  - VI. Nevirapine (NVP) ☐
  - VII. Ritonavir (r) ☐
  - VIII. Stavudine (d4T) ☐

IX. Tenofovir (TDF) ☐

X. Zidovudine (AZT) ☐

XI. others ☐ → Please list medication

i. _____

ii. _____

iii. _____

- When did you start taking your HIV Medications? |__|__| /|___|___|___|___|**(MM|YYY)**
- Have you ever had your HIV medications changed or stopped? Yes ☐

  No ☐
- When was your medication changed or stopped? |__|__| /|___|___|___|___| **(MM|YYY)**
- Which HIV medication were you taking before? (select all that apply)

I. Atazanavir (ATV) ☐

II. Darunavir (DRV) ☐

III. Efavirenz (EFV) ☐

IV. Lamivudine (3TC) ☐

V. Lopinavir (LPV) ☐

VI. Nevirapine (NVP) ☐

VII. Ritonavir (r) ☐

VIII. Stavudine (d4T) ☐

IX. Tenofovir (TDF) ☐

X. Zidovudine (AZT) ☐

XI. Others ☐ → Please list medications

i. _____

ii. _____

iii. _____

2. Have you ever been told by a doctor that you have:

	Yes	**No**	**Don't know**
a. High blood pressure or take medications for high blood pressure? **IF YES → STOP HERE!!!**	☐	☐	☐

3. **Have you ever been told by a doctor that you have:**

	Yes	No	Don't know
a. Diabetes or take any medications for diabetes?	☐	☐	☐

IF <u>HIV NEGATIVE</u> AND HISTORY OF DIABETES

Stop here!!!

4. **Have you ever been told by a doctor that you have?**

	Yes	No	Don't know
a. High cholesterol	☐	☐	☐
b. Stroke	☐	☐	☐
c. Heart attack or myocardial infarction	☐	☐	☐
d. Peripheral arterial disease	☐	☐	☐

TOBACCO USE AND SMOKE EXPOSURE

Next I will ask you some questions about tobacco use and exposure.

1. Do you currently smoke or have you smoked in the past?

☐ Never → skip to question #9

☐ Currently smoking

☐ Quit smoking

☐ Declines to answer

2. About how old were you when you first smoked part or all of a cigarette?

   |___|___| Years

3. If you **<u>quit smoking</u>**, how many years did you smoked before quitting?

   |___|___| Years

4. If you **<u>quit smoking,</u>** how many years has it been since you quit smoking?

   |____|___| Years

5. If you **<u>quit smoking,</u>** how many packs per day did you use to smoke on average?

   |____|___| packs per day (1 pack= 20 cigarettes)

6. If you are **currently smoking,** how many days a week do you smoke?

   |___|/7 days

7. If you are **currently smoking,** on average how many packs per day do you smoke?

   |___|___| . |___|___| packs per day (1 pack= 20 cigarettes, 5 cigarettes/day= 0.25 pack)

8. If you are **currently smoking,** how many years have you been smoking?

   |___|___| Years

9. Does anyone else living in your household smoke cigarettes?

☐ Yes

☐ No → skip to section E

10. In your house is smoking allowed…

☐ Always ☐ Allowed only at some times or in some places ☐ Never allowed

ALCOHOL USE

1. How often do you have a drink containing alcohol?

☐ Never → skip to section F

☐ Monthly or less

☐ 2 to 4 times a month

☐ 2 to 3 times a week

☐ 4 or more times a week

2. How many standard drinks containing alcohol do you have on a typical day?

☐ 1 or 2

☐ 3 or 4

☐ 5 or 6

☐ 7 or 9

☐ 10 or more

3. How often do you have six or more drinks on one occasion?

☐ Never

☐ Less than monthly

☐ Monthly

- ☐ Weekly
- ☐ Daily or almost daily

4. What kind of alcoholic drink do you drink?

- ☐ Chang'aa
- ☐ Liquor
- ☐ Beer
- ☐ Wine
- ☐ Busaa

I will take your pulse and blood pressure twice now before continuing with the interview, and twice again later.

PREPARE SUBJECT FOR BLOOD PRESSURE MEASUREMENT ACCORDING TO INSTRUCTIONS

1. Which arm is being used for blood pressure (BP) measurement:

   ☐ Right Arm ☐ Left Arm

2. **Pulse** rate per Minute |__|__|__|
3. **SYSTOLIC BP** |__|__|__|
4. **DIASTOLIC BP** |__|__|__|

USE DIFFERENT ARM FOR REPEAT BLOOD PRESSURE MEASUREMENT.

5. Which arm is being used for blood pressure (BP) measurement:

   ☐ Right Arm ☐ Left Arm

6. **Pulse** Rate per Minute |__|__|__|
7. **SYSTOLIC BP** |__|__|__|
8. **DIASTOLIC BP** |__|__|__|

ANTHROPOMETRY

Now I would like to take some additional measurements like your weight, height

A. Measure and record <u>WEIGHT</u>

1. |___|___|___| . |___| Weight in kilograms (Kg) to one decimal place

---

B. Measure and record <u>HEIGHT</u>

1. |____|___|___| . |___|___| Height in centimeters (cm) to two decimal places

---

_Measure and record <u>WAIST</u> circumference

1. |___|___|___| . |___|___| in centimeters (cm) to two decimal places

---

_Measure and record <u>HIP</u> circumference

1. |___|___|___| . |___|___| in centimeters (cm) to two decimal places

PREPARE SUBJECT FOR ANKLE BRACHIAL INDEX (ABI) MEASUREMENT ACCORDING TO INSTRUCTIONS

USE ARM BRACHIAL FOR ANKLE BRACHIAL INDEX (ABI) MEASUREMENT.

1. Which arm is being used for blood pressure (BP) measurement:

   ☐ Right Arm ☐ Left Arm

2. **SYSTOLIC BP** |__|__|__|

USE ANKLE FOR ANKLE BRACHIAL INDEX MEASUREMENT.

3. Which ankle is being used for Ankle Brachial Index (ABI) measurement:

   ☐ Right Ankle ☐ Left Ankle

4. **SYSTOLIC BP** |__|__|__|

LABORATORY TESTING

Blood Draw Date: |___|___|/|___|___|/|___|___|___|___|

Day/Month/Year

Time: |___|___|:|___|___| AM/PM

Collected by (Initials): |___|___|

Delivered to lab by (Initials): |___|___|

LAB:

Hemoglobin A1C

☐ Collected ☐ Not collected

Specimen ID number |___|___|___|___|___|___|___|___|

Lipid Panel

☐ Collected ☐ Not Collected

Specimen ID number |___|___|___|___|___|___|___|___|

CHART REVIEW DATA for HIV POSITIVE PATIENTS

LAB	**DATE (DD/MM/YY)**	**VALUE**
Most recent HDL	|___|___|___|	
Most recent LDL	|___|___|___|	
Most recent Triglycerides	|___|___|___|	
Most recent Total Cholesterol	|___|___|___|	

EDINBURGH CLAUDICATING QUESTIONNAIRE (ECQ)

1. Do you get a pain or discomfort in either leg on walking? YES NO
2. Does this pain ever begin when you are standing still or sitting? YES NO
3. Does this pain occur if you walk uphill or hurry? YES NO
4. Does this pain occur if you walk at an ordinary pace on the level? YES NO
5. What happens if you stand still?
   - Usually continues more than 10 minutes
   - Usually disappears in 10 minutes or less

6. In what part of your leg do you feel the pain

INTERPRETING ECQ

A definite claudicantis one who indicates pain in the calf, regardless of whether pain is also marked in other sites;

Atypical claudication is made if pain is indicated in the thigh or buttock, in the absence of any calf pain.

No claudication if pain is indicated in the hamstrings, feet, shins, joints or appears to radiate, in the absence of any pain in the calf.

Appendix II: Consent Form- English

PREVALENCE AND RISK FACTORS OF PERIPHERAL ARTERIAL DISEASE AMONG HIV PATIENTS

MOI UNIVERSITY COLLEGE OF HEALTH SCIENCES/MOI TEACHING AND REFERRAL HOSPITAL

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

INFORMED CONSENT FORM

Lead Researcher:

Francis Ochieng, MBChB, Moi University, School of Medicine

Contact Person: Dr. Francis Ochieng at 0724306216

Researchers' statement

We are asking you to be in a research study. The purpose of this consent form is to give you the information you would need to help you decide whether to be in the study or not. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything less about the research or this form that is not clear. When we have answered all your questions, you can decide if you want to be in the study or not. We will give you a copy of this form for your records.

PURPOSE OF THE STUDY

The purpose of the study is to learn about the prevalence and risk factors of peripheral arterial Disease in the HIV population and compare it to HIV uninfected individuals.

STUDY PROCEDURES

After you have read, agreed and signed this consent form, you will have more information collected today during the clinic visit. If you meet the requirements to join the study, the evaluation will take about 1 hour to complete. If you cannot spend the extra time today or complete all testing, we will ask you to return to clinic within the next 1 month to complete the evaluation.

If you decide not to take part in this study or if you do not meet the eligibility requirements, we may still use some of your information.

Evaluations:

As part of the study, you will have the following evaluations:

1. You will have a physical examination to include measurement such as your blood pressure.
2. About 10ml of blood will be taken from your arm for standard lab tests.
3. You will have an Ankle Brachial Index (or ABI) which evaluates narrowing by atherosclerosis (fat) of arterial blood vessels of your hands and feet. Blood pressure Scuff attached to a hand held sound wave machine will be attached to your arms and then to your ankles to measure this activity. The sound waves and blood pressure show the blood pressures at your arms and ankles.

RISKS, STRESS, OR DISCOMFORT

Taking bloods may cause some discomfort, bleeding, or bruising where the needle enters the body, light-headedness, and in rare cases, fainting or infection. There is no risk associated with having an ankle Brachial Index measurement.

Interviews will contain some questions of a sensitive nature. This information will be confidential but should you close not to answer, it will in no way affect your treatment or relations with the clinic. There are no costs to you for participating in the study. In the event of study-related injury, illness, or distress contact: Dr. Francis Ochieng at 0724306216.

BENEFITS OF THE STUDY

If you participate in this study there may be direct benefit to you. You will learn about your health status as regards your cardiovascular risk, diabetes status and cholesterol levels. You will also learn about any Blood Pressure changes in your legs and arms that we would be able to evaluate with Ankle Brachial Index. If any of these is found to be abnormal, it shall be communicated to you and your primary physician to facilitate further management.

SOURCE OF FUNDING

The study will be personally funded by the principal researcher.

CONFIDENTIALITY OF RESEARCH INFORMATION

The information you provide, ankle Brachial Index and blood test results will be shared with the research team in this study. However it will not be possible to identify you individually from this information. The study team will provide you with an identification number. The identification number (not your name or other information that could be used to identify you) will be used during analysis of the study. Data linking your unique ID number and name will be encrypted and stored electronically. Only the study staff investigators will have access to this information. All other data will be collected and stored encrypted electronically. Sharing this information will also not identify you. Government or university staff sometimes review studies such as this one to make sure they are being done safely and legally. If a review of this study takes place, your records may be examined. The reviews will protect your privacy. The study records will not be used to put you at legal risk of harm.

There are some limitations to this protection. We will voluntarily provide the information to the institutional Review and Ethics Committee (IREC) of Moi University.

National privacy regulations may not apply to these groups; however, they have their own policies and guidelines to assure that all reasonable efforts will be made to keep your personal information private and confidential.

OTHER INFORMATION

Taking part in this research study is voluntary. You may choose not to take part in the study. You could still receive other treatments. Saying no will not affect your rights to health care services. You are also free to withdraw from this study at any time. If after data collection you choose to quit, you can request that the information provided by you be destroyed under supervision and thus not used in the research study. You will be notified if new information becomes available about the risk or benefits of this research. Then you can decide if you want to stay in the study.

COMPENSATION

You will receive no financial payments as a result of participating in this study.

RESEARCH-RELATED INJURY

If you think you have a medical problem or illness related to this research, contact Dr. Francis Ochieng at 0724306216 right away. He will refer you for treatment.

Printed name of study staff obtaining consent Signature Date

Subject's statement

This study has been explained to me. I volunteer to take part in this research. I have had a chance to ask questions. If I have questions later about the research, or if I have been harmed by participating in this study, I can contact one of the researchers listed on the first page of this consent form. If I have questions about my rights as a research subject, I can contact Moi University Institutional Review Ethics Committee (IREC) 053 33471

Printed name of subject Signature Date

(Must be dated by the participant if literate)

Signature of Witness Signature Date

(If participant is illiterate) (Must be dated by witness)

Appendix III: Consent Form- Kiswahili

PREVALENCE AND RISK FACTORS OF PERIPHERAL ARTERIAL DISEASE AMONG HIV PATIENTS

MOI UNIVERSITY COLLEGE OF HEALTH SCIENCES/MOI TEACHING AND REFERRAL HOSPITAL

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

FOMU YA KIBALI CHA KUSHIRIKI KWENYE UTAFITI

Jina la mtafiti mkuu:

Francis Ochieng, MBChB, MMed, Moi University School of Medicine

Jina la mtu wakuwasilisha kuhusu utafiti: Dr. Francis Ochieng 0724306216

Taarifa kutoka kwa utafiti

Unaombwa kushiriki katika utafiti huu. Dhumuni ya hii fomu ya idhini ni kukupa habari Zaidi kuhusu utafiti huu ili iweze kukusaidia kuamua kama ungependa kushiriki katika utafiti huu au hapana. Tunaomba uisome hii fomu kwa makini. Watafiti wataongea na wewe kuhusu utafiti huu na uko huru kuuliza maswali wakati wowote kuhusiana na chochote tutakachokifanya katika utafiti huu, hatari na manufaa ya kushiriki katika utafiti huu na pia haki zako kama mshiriki wa utafiti huu. Tukishakujibu maswali yako yote, utachagua kushiriki kwenye huu utafiti au kukataa. Kama utakubali kushiriki katika utafiti huu, utaombwa kuweka sahihi katika hii fomu ya idhini. Utapewa nyaraka kuweka kwa ukumbusho wako.

LENGO LA UTAFITI

Madhumuni ya utafiti huu ni kuelewa wingi wa magonjwa ya mafuta kwenye mishipa ya damu kati ya watu wanaougua ugonjwa wa ukimwi, na kulinganisha wingi huu na watu ambao hawana ugonjwa wa ukimwi.

UTARATIBU WA UTAFITI

Baada ya kusoma, kukubali na kuweka sahihi kwa fomu ya idhini utaulizwa maswali kuhakikisha kuwa unayo mahitaji yote ya kushiriki katika utafiti huu. Ukikubali kushiriki, itakuchukua muda wa nusu saa kukamilisha utafiti huu. Ikiwa hautaweza kukamilisha utafiti huu leo, tutaomba urudi katika muda wa mwezi moja ukiwa tayari kushiriki.

Ukiamua kutoshiriki kwenye utafiti huu, ama kama hautatimiza matakwa yote ya utafiti huu, huenda tukatumia majibu yako kama vile miaka na jinsia yako.

Tathmini

Mambo haya yatafanyika kwenye utafiti huu:

1. Vipimo vya Mwili vile uzito, urefu, na shinikisho la damu vitachukuliwa.
2. Damu kiwango cha mililita kumi kitatolewa kutoka kwa mkono ili kufanyiwa vipimo kwenye maabara.
3. Utafanyiwa utafiti wa mishipa ya damu (ABI), kutumia kifaa chenye aina ya presha iliyobandikwa kwenye miguu na mikono. Hiki kifaa huchunguza kiwango cha presha ya miguu na mikono yako.
4. Utafanyiwa utafiti wa kiwango cha presha kwenye mishipa ya damu ya miguu na mikono (ABI). Hiki kifaa kinatumia mawimbi ya sauti kuangalia kiwango cha presha kwenye miguu na mikono yako.

ARI ZINAZOAMBATANA NA KUSHIRIKI KATIKA UTAFITI

Kutoa damu inaweza kukukosesha starehe, kuvunja damu, ama kuviimba mahali shindano imedunga, kichwa nyepesi na kwa visa vichache kusilahi ama kuambukizwa magonjwa. Hakuna hatari yoyote inayotokana na ankle brachial index, lakini huenda ukasumbua kidogo unapopimwa presha.

Pia maswali utakayo ulizwa leo yanaweza kuwa maswali nyeti kwako. Mazungumzo au taarifa yeyote tutakayopata toka kwako itatunzwa kwa siri kubwa na kutoka jibu ni hiari yako. Kama utakataa kushiriki kwa huu utafiti, bado utaendele kupokea matibabu unayohitaji, na haita adhiri uhusiano wako na cliniki hii. Hautalipishwa ada kushiriki katika utafiti huu. Kama unatatizo lolote linalohusiana na utafiti unaweka kuwasiliana na Dr. Francis Ochieng **0724306216** aweze kukusaidia.

MANUFAA YA KUSHIRIKI KATIKA UTAFITI

Kuna manufaa kwako unapo shiriki katika utafiti huu. Utajifunza Zaidi kuhusu mangwoja ya moyo na jinsi kujikinga. Pia utajkua hadhi yako kuhusu kisukari na chembe za matufata katika damu yako. Hii itasaidia waugo wako kujua jinsi gani wakusaidia ikijulikana una matatizo hayo.

WADHAMINI WA UTAFITI HUU

Jina: Dkt. Francis Ochieng

USIRI WA TAARIFA ZA UTAFITI

Matokeo ya utafiti huu itapewa daktari wako na mashirika yanayoshirikiana katika utafiti huu. Walakini, haitawezekana kukutambua wewe kibinafsi kutoka kwa hii habari. Kikundi cha utafiti kitakupatia namba ya utambulisho. Na yautamubulisho (code) sio jina lako ama habari yoyote ambayo inaweza tumika kukutambulisha itatumika. Rekodi za utafiti ndiyo pekee yao watakuwa na funguo. Kuchapishwa kokote matokeo ya huu utafiti hautatumia jina lako kukutambulisha wewe binafsi. Rekodi zako zinaweza pitiwa naThe Institutional Review na Ethics Committee of Moi University.

Tafadhali kumbuka ya kwamba ni chaguo lako kushiriki ama kutoshiriki katika huu utafiti wa kufuatilia. Una kuacha kushiriki katika huu utafiti wakati wowote. Bado utaendelea kupata matibabu yako ya kiafya kikamilifu kama hautashiriki katika huu utafiti.

MALIPO

Hautalipwa ridhaa kwa muda wako uliotumia katika kumaliza uchunguzi unaohusiana na huu utafiti.

MAJERUHI YATOKANAYO NA UTAFITI HUU

Kama umejeruhiwa kwa sababu ulishiriki katika utafiti, mpigie Dr. Francis Ochieng **0724306216** haraka iwezekanavyo ili aweze kukupatia matibabu au kukutafutia njia ya kupata matibabu.

Jina la mwenye kukuchukua kibali cha kushiriki utafiti:

Tarehe:

Kwa kusingatia yote hapa juu, ninakkubali kushiriki katika huu utafiti. Nimesoma hii fom,u ya kukubali (ama imesomwa na nikaelezewa wazi), maswali yangu yote yamejibika na ninakubali kushiriki katika huu utafiti. Na ninakubali kupokea nakala ya fomu ya kukubali. Kama nina maswali Zaidi kuhusu utafiti ama majelaha yanayohusiana na utafiti, nitawasiliana na mtafiti Daktari Francis Ochieng kwa namba ya simu: 0724306216. Na maswali kuhusu haki zangu kama mshiriki katika utafiti ama malalamiko kuhusu utafiti, wasiliana na msimamizi wa IREC kwa namba ya simu: 053-203347

SAHIHI AMA ALAMA YA MSHIRIKI **Tarehe**

(Lazima tarehe iwekwe na mshiriki kama amesoma)

SAHIHI YA MSHAHIDI **Tarehe**

(Kama hana elimu ya kumwezesha kusoma lazima tarehe iandikwe na mshahidi)

APPENDIX IV: PROCEDURE FOR DRAWING VENOUS BLOOD

Venous blood was drawn for lipid profile and Glycated hemoglobin. The procedure was explained to the participant and verbal consent obtained. Universal safety procedures were observed. Venous blood draw was from the median cubital vein (in the antecubital fossa) of the less dominant upper limb.

Below is an overview of the steps that were followed:

1. Arm was selected and a tourniquet placed on the arm above the draw site. The median cubital vein was selected.
2. Site was cleansed with a sterile alcohol/methylated spirit preparation pad.
3. A needle was inserted into the vein and the collection tube was engaged.
4. Two milliliters of blood was collected into a vacutainer (plain) blood collection bottle.
5. Tourniquet was removed once the quantity of blood desired had been obtained.
6. A small qauze pad and Band-aid were placed on the venous blood drawn site.
7. The blood collection tube was labeled with the patient's information.
8. Blood collection tubes were batched until five samples were obtained before being taken to the laboratory for analysis.

Appendix V: Procedure For Glycated Haemoglobin (HbA1C)

Blood samples for determination of HBA1c were collected at a central laboratory at the time of recruitment by the laboratory technicians. HBA1c level was determined by spectrophotometry using the HBA1c analyzer (Bayer DCA 200R + Analyzer). See appendix 3 for procedure

HbA1c assay

HbA1c Analyzer used: Bayer DCA 2000R + Analyzer

CHEMICAL PRINCIPLES OF PROCEDURE:

Both the concentration of HBA1c and concentration of total hemoglobin were measured and the ratio reported as percentage HbA1c. All of the reagents for performing both reactions were contained in DCA 200R HbA1c reagent cartridge. For the measurement of total hemoglobin potassium ferricyanide was used to oxidize hemoglobin in the sample to methehemoglobin. The methehemoglobin did then complexed with thiocyanate to form thiocyanomethemoglobin, which was measured. The extent of colour development at 53-nm was proportional to the concentration of total haemoglobin in the sample. For the measurement of specific HbA1c, an inhibition of latex agglutination assay was used. An agglutinator caused agglutination of latex coated with HbA1c specific mouse monoclonal antibody. This was measured as an increase in absorbance at 531nm. HbA1c competed for the limited number of antibody-latex binding sites causing an inhibition of agglutination and a decreased scattering of light. The decreased scattering was measured as a decrease in absorbance at 531nm. The HbA1c concentration was then quantified using a calibration curve of absorbance versus HbA1c concentration. All measurements and calculations were performed automatically by the DCA 200+ Analyzer, and the screen displayed percentage HbA1C at the end of assay.

Testing Procedure

1. Procedure was explained to the subject/caregiver and consent obtained
2. The subject’s finger was cleaned with methylated spirit swabs and allowed a few seconds to dry.
3. The finger was then pricked using a lancet.
4. A drop of blood was placed at the end of a glass capillary allowing the glass capillary to fill.

5. A new reagent cartridge was opened up.
6. The plastic capillary holder was loaded into the DCA 200R + Analyzer.
7. Test results were automatically displayed in 6 minutes

Calibration

The instrument: The DCA 200+ Analyzer was calibrated by the manufacturer. Thereafter, the instrument automatically self-adjusted during first-time power up and during each assay. In the event of the system being unable to make appropriate internal adjustments, an error message was displayed.

Reagent:

The manufacturer calibrated the cartridge using parameters based on a DCCT reference method. The DCA 200 HbA1c test method was National Glycoheomoglobin Standardization program (NGSP) certified. The DCA 200 HbA1c test method was traceable to International Federation of Clinical Chemistry (IFCC) reference materials and test methods.

There was a calibration card for scanning for each lot cartridges; the calibration bar code was read by the instrument. This assessed the appropriate calibration values for the particular lot. If no calibration curve was in use, the instrument prompted the user to scan the calibration card.

Quality Control:

To assure quality of both testing and patient results for the HbA1c, the DCA 200+ system performed 48 optical, electronic, mechanical and reagent checked during the course of each specimen assay.

In addition to the above measures, each new lot had one kit used for assessing quality control by checking the HbA1c percent of healthy non diabetic persons.

Glycated hemoglobin 9HbA1c):

Less than 7%.....

7% to 7.5%.....

7.5% to 8%.....

8.0% to 8.5%.....

8.5% to 12.5%.....

More than 12.5%.....

Appendix VI: Procedure for Measuring Lipid Profile Components

Total Cholesterol Measurement

Method

CHOD- PAP- enzymatic colorimetric test for cholesterol with lipid clearance factor

Note: Cobasintegra 400 plus and Humastar 180 were the machines used at the MTRH laboratory; they run lipid profiles based on enzymatic colorimetry.

Principle

Cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine was formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase.

Reagent preparation

The reagent and standard were ready for use.

Reagent stability

The opened reagent was stable for two weeks at 15-2oC but remains stable up to the expiry date when stored at 2-8oC. Reagent manufacturer was Human.

Specimen

Serum, Heparinized or EDTA plasma

Assay

Wavelength: 500MM, Hg 546nm.

Optical path: 1 cm

Temperature: 20-25oC or 37oC

Measurement: Against reagent blank, only reagent blank series were required.

Apparatus

1. Clean test tubes
2. 1ML automatic pipette
3. 10ul automatic pipette
4. Test tube rack
5. Humalyzer- 300 machine

Procedure

1. Test tubes were labeled as blank, standard, control and test
2. 1 ml of working reagent was pipetted into each tube
3. 10 ul of standard was pipetted into tube labeled standard, 10 ul of sample to tube labeled test.
4. These were mixed well and incubated for 10 min. at 20- 25oC or 5 minutes at 37oC.
5. On the Humalyzer-3000 press running of test 9F10, location 6was done
6. Blank, standard, control then test were read
7. Quit was pressed to end the test.

Linearity

The test was linear up to cholesterol concentration of 750mg/dl (19.3mmol/L).

Samples were diluted with a higher cholesterol concentration (1:2) with physiological saline (0.99%) and the determination was repeated and the results were multiplied by 3.

Clinical interpretation

Total cholesterol was abnormal when over 5.2 mmol/L.

2.2. Measurement of HDL-C and LDL-C

Value of the test

HDL cholesterol is protective against atherosclerosis while LDL-C is atherogenic.

Principle

HDL-Cholesterol were specifically precipitated by phosphotungstic acid and magnesium ions and can then be removed by centrifugation, high density lipoprotein (HDL) remain in the supernatant. Determination of HDL cholesterol was performed using clear supernatant.

Reagent composition

Reagent A - Phosphotungstic Acid- 14mmol

Precipitant reagent - magnesium chloride- 2mmol

Reagent B - Cholesterol reagent

Standard - HDL cholesterol aqueous- 50mg/dl

The reagent used was manufactured by cypress Diagnostics.

Specimen- serum or plasma

Preparation

Reagent A and standard were ready for use.

Storage and stability

The reagents were stable up to the expiry date when stored at 2-8oC.

They had to be clear solutions; if turbidity or precipitation occurred the reagents had to be discarded.

Procedure

1. 1ml of serum was put into clean test tube and all 100 ul of the precipitant.
2. This was mixed well and allowed to stand at room temp for 10 minutes
3. This was mixed well again and centrifuged for 20 minutes at 4000 r.p.m.
4. The supernatant was separated into a clean cryovial and labeled.
5. The test tubes were labeled as blank, standard, control and test.

6. 1 ml of total cholesterol reagent was put into all the tubes.
7. 10 ul of the standard was added to the standard tube 10 ul of control to control tube and 10 ul of the supernatant to the test tube.
8. This was mixed well and incubated for 10 minutes at room temperature or 5 minutes at 37 o C.
9. Run test (F1) was pressed. Location 88 was entered and enter key pressed.
10. Blank button (F1) was read to end the test.

Calculation

LDL cholesterol = Total cholesterol- Triglycerides/5- HDL mg/dl

Or

LDL Cholesterol= Total cholesterol- Triglycerides/5- HDL cholesterol mmol/L

Non HDL Cholesterol = Total cholesterol – HDL cholesterol

2.3: Measurement of Triglycerides

Triglycerides are the main lipid present in human plasma,

They are formed in the intestinal mucosa by the esterification og glycerol and free fatty acids

Method

GPO-PAP-Method

Principle

Triglycerides are determined after enzymatic hydrolysis with lipase. The indicator is quinomeimide formed from hydrogen peroxide, 4-aminoastipyrine and 4-chlorophenol under the catalytic influence of peroxide.

Reagent stability

The reagents are ready for use. They remain stable up to the stated expiry date when stored at 2-8oC. Contamination must be avoided. The reagent used was manufacture by Human.

Note

Prior to use allow reagent to rest for at least 15minutes at room temperature.

Specimen

Heparinized plasma or EDTA plasma or serum

Note: Heparinized specimens usually generate turbidity of the sample-reagent mixture which leads to falsely elevated results. Triglyceride GPO Liquicolour test avoids these falsely elevated results through its built in lipid clearing factor (LCF). The LCF clears up totally turbidity caused by lipemic specimen.

Assay

Wavelength 500nm

Optical path – 1cm

Temperature: 20 – 25oC or 37oC

Measurement: Against reagent blank, only one reagent blank per series is required.

Apparatus

1. Clean test tubes
2. 1ml automatic pipette
3. 10 ul automatic pipette

Procedure

1. Test tubes were labeled as blank, standard, control and test
2. 1 ml of working reagent was pipetted into tube labeled blank, standard, control and test
3. 10 ul of control was pipetted into tube labeled control, 10 ul of standard into tube labeled standard and 10 ul of sample into tube labeled test

4. This was mixed and incubated for 10 minutes at 20-25oC or 5 minutes at 37oC
5. On humalyzer 300 F1 (run test) was pressed. Location 21 was entered and enter key pressed.
6. Blank, standard, control then test were read.
7. Quit was pressed to end the test

Linearity

The test is linear up to a triglyceride concentration of 100mg/dl or 1.4mmol/L. Samples with a higher concentration have to be diluted 1:4 with physiological saline (0.9%) and retested. Multiply the results by 5.

Clinical interpretation:

Increased when over 2.30mmol/L

Appendix VII: Procedure for Measuring Blood Pressure

AHA (American Heart Association) guidelines on blood pressure measurements were followed. A desk type mercury sphygmomanometer, calibrated monthly by MTRH maintenance department was used for BP measurements.

Cuff size- Appropriate cuff sizes was used based on upper arm circumference; 3 cuff sizes were available:

ARM CIRCUMFERENCE	**CUFF SIZE**
22-26 cm	Small adult cuff = 12 by 22 cm
27-34 cm	Adult cuff = 16 by 30 cm
35-44 cm	Large adult cuff = 16 by 36 cm

Position of the patient:

BP was taken with patient sitting on a chair with back supported and arm supported at the level of the heart on the desk. Patient was left to sit quietly for 5 minutes prior to BP measurements. Two BP measurements were taken one on each arm, 5 minutes apart. An average of the two readings was calculated and recorded.

Blood pressure machine:

A desk type manual mercury sphygmomanometer, model BK 1001, manufactured by Nanjing Everich Medicare Company was used. Three adult cuff sizes were available: small adult cuff, medium adult cuff and large adult cuff. The choice of the cuff to be used was based on the patient's upper arm circumference.

Appendix VIII: Procedure for Measuring Ankle Brachial Index

ABI MACHINE

Equipment and supplies for the ABI.

1. Portable Doppler with 8-10 MHz probe (Ultra Tec PD1v, 8MHz hand held Doppler ultrasound (South Wales, UK

2. A 5 MHz probe was used if a large amount of edema was present at the ankle.

3. Aneroid sphygmomanometer.

4. Ultrasound transmission gel.

5. Alcohol pads to clean the Doppler.

6. Gauze, tissue or pads to remove transmission gel from patient's skin.

7. Towels, sheets, or blankets to cover trunk and extremities.

8. Paper and pen for recording test results; calculator.

2. Pressure cuffs for ankles and arms should be long enough to fully encircle the limb. The cuff bladder width should be 40% of the limb circumference and long enough to cover 80% of the arm circumference

Typically, 12 cm wide cuffs were used for arms and 10 cm wide cuffs at the ankles.

Extra-large adult cuffs might be needed (14 cm).

PROCEDURE

Preparation of Patient and Environment

1. The ABI was performed in a quiet, warm environment to prevent vasoconstriction of the arteries

2. The patient was made comfortable by being allowed to rest for a minimum of 15 minutes prior to the test to allow pressures to normalize

3. The best ABI results were obtained when the patient is relaxed, comfortable, and has an empty bladder.

4. The procedure was explained to the patient.

5. Socks, shoes, and tight clothing were removed to permit placement of pressure cuffs and access to pulse sites by Doppler.

6. The patient was placed in a flat, supine position. One small pillow was placed behind the patient's head for comfort.

7. Prior to placement of the cuff, a protective barrier (e.g., plastic wrap) was applied on the extremities if any wounds or alterations in skin integrity were present.

8. Pressure cuffs was placed with the bottom of the cuff approximately 2-3 cm above the cubital fossa on the arms and malleolus at the ankle. Cuffs was wrapped without wrinkles and placed securely to prevent slipping and movement during the test.

9. The trunk and extremities were covered to prevent cooling.

Measuring Brachial Pressures with Doppler

1. After the rest period, the arm and ankle pressures were measured.

2. The arm was relaxed, supported and at heart level.

3. The brachial pulse was palpated to determine location to obtain an audible pulse.

4. Transmission gel was applied over the pulse site.

5. The tip of the Doppler probe was placed at a 45° angle pointed towards the patient's head until an audible pulse signal was obtained.

6. The pressure cuff was inflated 20-30 mmHg above the point where the pulse was no longer audible

7. The pressure cuff was deflated at a rate of 2-3 mmHg per second, noting the manometer reading at which the first pulse signal was heard and that systolic value was recorded.

8. The gel was cleaned from pulse site.

9. The procedure was repeated to measure the pressure on the other arm.

10. If a pressure was need to be repeated, a wait of 1 minute before re-inflating the cuff was done.

11. The higher of the right or left arm's brachial systolic pressures was used to calculate the ABI for both legs.

Measure Ankle Pressures with Doppler

The cuff was placed on the patient's lower leg with the bottom of the cuff approximately 2-3 cm above the malleolus.

1. Prior to placing the cuff, a protective barrier (e.g., plastic wrap) was applied on the extremity if any wounds or alterations in skin integrity were present.

2. Both dorsalis pedis and posterior tibial pulses were measured in each leg.

3. The pulses by were located by palpation or with the Doppler probe.

4. Transmission gel was applied to the pulse site.

5. The tip of the Doppler probe was placed at a 45° angle pointed towards the patient's knee until an audible pulse signal was obtained.

6. The pressure cuff was inflated 20-30 mmHg above the point where the pulse was no longer audible

7. The cuff was deflated slowly at a rate of 2-3 mmHg per second, noting the manometer reading at which the first pulse signal was heard and recorded that systolic value.

8. The gel was cleansed from pulse site.

9. The procedure was repeated to measure pressures on the other ankle.

10. If a pressure needed to be repeated, a wait of one minute before re-inflating the cuff was made.

11. The higher of the ankle pressures of each leg to was used to calculate the ABI for each leg.

Post exercise ABI

The participant was then requested to make 10 squats under supervision and then the same ABI measurement procedure as above was done and new ABI measurements recorded

Calculating the ABI

1. The higher of the dorsalis pedis or posterior tibial systolic pressure for each ankle was divided by the higher of the right and left systolic brachial pressures to obtain the ABI for each leg.

$$\text{ABI pressure} = \frac{\text{higher of either dorsalis pedis or posterior tibial systolic pressures}}{\text{Higher of the systolic brachial pressures}}$$

2. **Interpreting the ABI values**

1.0-1.4 = No blockage (PAD)

0.9 – 1.0 = Normal

≤ 0.9 = Narrowing of leg arteries (PAD)

0.7- 0.89 = Mild PAD

0.69- 0.4 = Moderate PAD

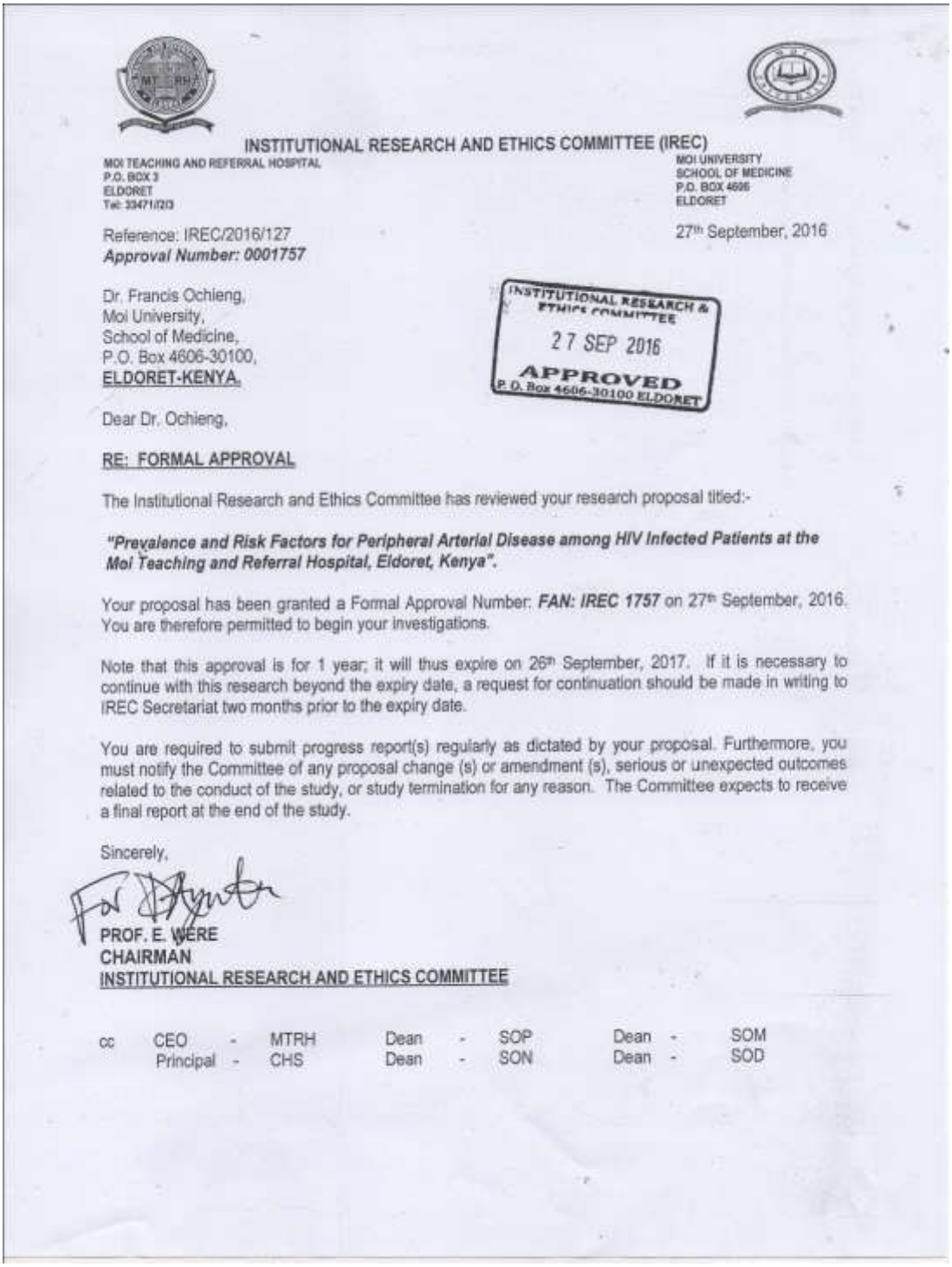
< 0.4 = Severe PAD

ABI >1.4 = Incompressible/stiff/rigid arteries (DM, CKD: Doppler U/S)

Data management

All clinical and laboratory data were entered in a questionnaire (Appendix I). The information was later stored electronically using a double entry system to minimize transcription errors.

Appendix IX: MTRH /IREC Approval Letters



INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2016/127
Approval Number: 0001757

27th September, 2016

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
27 SEP 2016
APPROVED
P. O. Box 4606-30100 ELDORET

Dr. Francis Ochieng,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Dr. Ochieng,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"Prevalence and Risk Factors for Peripheral Arterial Disease among HIV Infected Patients at the Moi Teaching and Referral Hospital, Eldoret, Kenya".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1757*** on 27th September, 2016. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 26th September, 2017. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

For

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
P. O. Box 3
ELDORET

Ref: ELD/MTRH/R.6/VOL.II/2008

30th September, 2016

Dr. Francis Ochieng,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

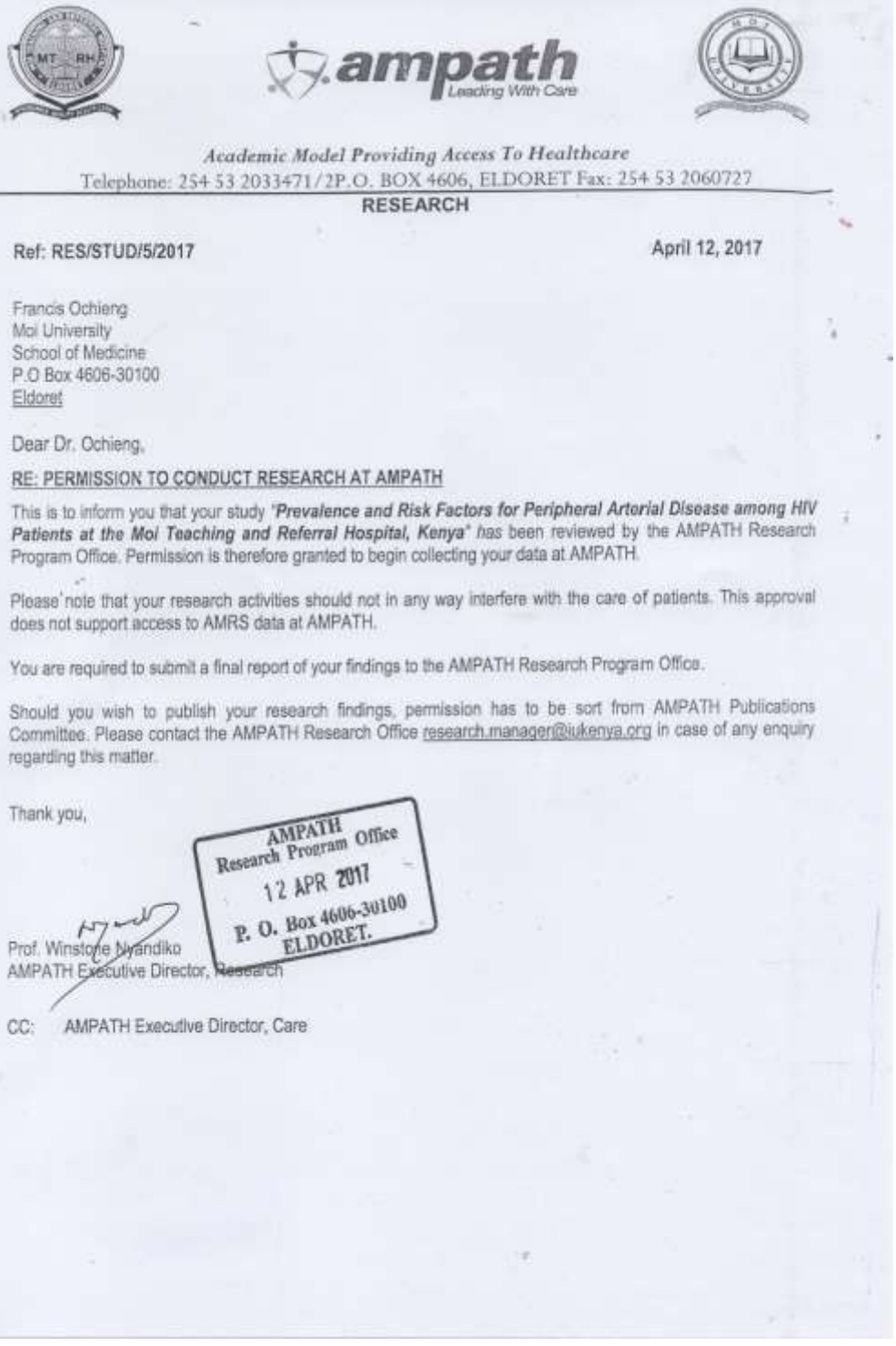
Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"Prevalence and Risk Factors for Peripheral Arterial Disease among HIV Infected Patients at the Moi Teaching and Referral Hospital, Eldoret, Kenya".

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

DR. WILSON ARUASA
CHIEF EXECUTIVE OFFICER
MOI TEACHING AND REFERRAL HOSPITAL

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM



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Telephone: 254 53 2033471/2P.O. BOX 4606, ELDORET Fax: 254 53 2060727

RESEARCH

Ref: RES/STUD/5/2017

April 12, 2017

Francis Ochieng
Moi University
School of Medicine
P.O Box 4606-30100
Eldoret

Dear Dr. Ochieng,

RE: PERMISSION TO CONDUCT RESEARCH AT AMPATH

This is to inform you that your study "***Prevalence and Risk Factors for Peripheral Arterial Disease among HIV Patients at the Moi Teaching and Referral Hospital, Kenya***" has been reviewed by the AMPATH Research Program Office. Permission is therefore granted to begin collecting your data at AMPATH.

Please note that your research activities should not in any way interfere with the care of patients. This approval does not support access to AMRS data at AMPATH.

You are required to submit a final report of your findings to the AMPATH Research Program Office.

Should you wish to publish your research findings, permission has to be sort from AMPATH Publications Committee. Please contact the AMPATH Research Office research.manager@iukenya.org in case of any enquiry regarding this matter.

Thank you,

AMPATH
Research Program Office
12 APR 2017
P. O. Box 4606-30100
ELDORET.

Prof. Winstone Nyandiko
AMPATH Executive Director, Research

CC: AMPATH Executive Director, Care