SERUM BIOMARKERS FOR RISK OF CARDIOVASCULAR DISEASE IN PATIENTS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY IN HOMA-BAY COUNTY REFERRAL HOSPITAL, KENYA

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DECLARATION

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I KENNETH WEKE declare that this dissertation is my original work and has not been presented in any other institution of learning for the award of a degree or any other award.

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To my supervisors who believed in me and immensely shaped my thoughts as I worked on this project.

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ABBREVIATIONS

AMI	Acute myocardial infarction
ApoA-I	Apolipoprotein A-I
ApoB	Apolipoprotein B
ARV	Antiretroviral
BMI	Body mass index
CAD	Coronary artery disease
CAM	Cell adhesion molecule
CCC	Comprehensive care clinic
CD	Cluster differentiation
CRP	C-reactive protein
CVD	Cardiovascular disease
DAD	Data collection on adverse events of anti-HIV drugs
DM	Diabetes mellitus
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
HAART	Highly active antiretroviral therapy
HbA1c	Glycated hemoglobin
HDL	High density lipoprotein
HIV/AIDS	Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome
HLA	Human leukocyte antigen
hsCRP	high sensitivity C-reactive protein
ICAM-1	Intercellular cell adhesion molecule-1

IL-6	Interleukin 6
IQC	Internal quality control
LDL	Low density lipoprotein
Lp(a)	Lipoprotein (a)
Lp-PLA2	Lipoprotein-associated phospholipase A2
MI	Myocardial infarction
MPO	Myeloperoxidase
NRTIs	Nucleoside/nucleotide reverse transcriptase inhibitors
OxPL/ApoB	Oxidised phospholipids on ApoB100-containing proteins
PI	Protease inhibitor
RR	Relative risk
SMART	Strategies for management of anti-retroviral therapy study
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TTT	Transfusion transmissible infections
VCAM-1	Vascular cell adhesion molecule-1
VLDL	Very low density lipoprotein

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ABSTRACT

Background: Human Immunodeficiency Virus (HIV) continues as a major public health problem both in developing as well as developed nations. The prevalence of HIV in Kenya as determined by the National Aids Control Council in 2015 stands at six percent. However, regional variation exists with Homa-Bay County ranking highest at 25.7 percent. With the introduction of highly active antiretroviral therapy (HAART), a decline in morbidity and mortality from HIV has been observed. Recent studies have showed that a strong association exists between dysregulated concentrations of serum lipids and sugar, which are recognised markers of cardiovascular disease, and HAART use. However, it remains unclear if the findings of these studies would be observed at the Home-Bay County Referral Hospital. Thus, this study intended to correlate the serum concentrations of these markers with the duration of HAART use.

Research Question: Does prolonged use of HAART raise the levels of serum biomarkers for risk of CVD among HIV-positive individuals receiving care in Homa Bay County referral hospital?

Objective: To determine the correlation between levels of serum biomarkers for risk of CVD with the duration of HAART use among HIV-positive individuals receiving care in Homa Bay County referral hospital.

Specific Objectives:

- To determine serum concentration of HbA1C, TC, LDL-C, HDL-C and MPO in individuals using HAART.
- To calculate the proportion of the participants at low and high risk based on the the levels of their serum HbA1C, TC, LDL-C, HDL-C and MPO.

- To determine the correlation between HAART duration and increased levels of the biomarkers for risk of CVD.

Study Design: This was a descriptive cross-sectional study

Study Area: Participants recruitment and data collection was done in the Comprehensive Care Clinic at the Homa-Bay County Referral Hospital. The biochemical analysis was done at the Kenyatta National Hospital.

Study Population: Study population consisted of HIV-positive males and females of ages between 18 and 45 years, who had been on HAART for at least six months. The sample size was 120.

Methodology: Systematic random sampling technique was used to collect blood samples from participants after they gave consent to participate in the study. Four (4) ml non-fasting blood samples was collected aseptically from the antecubital vein and was then processed for biochemical analysis of Total-Cholesterol (TC), Low Density Lipoprotein- Cholesterol (LDL-C), High Density Lipoprotein-Cholesterol (HDL-C), Glycated Haemoglobin A1c (HbA1c), Lipoprotein-associated Phospholipase 2 (Lp-PLA2) and Myeloperoxidase (MPO). At the same time, demographic data and medical history of the participants were collected by use of a study questionnaire. Data entry was done in an excel spreadsheet. Descriptive statistics (mean, median and standard deviation) were used to analyse continuous variables. Chi-square test was used to test for the significance association between age and gender with HAART duration. Logistic regression was then used to test for the independent association between raised levels of the biomarkers with gender, age and HAART duration.

Results: Majority of the participants (77%) were married and 64.2% had attained primary education. Most of the subjects (65%) were in self-employment. Majority of the participants

(67.5%) had been on HAART for more than sixty months. Most of the study participants had TC, LDL-C, HDL-C, HbA1c, Lp-PLA2 and MPO levels within the reference interval. The proportions with elevated levels above the reference interval were as follows; 14.2% (TC), 5.8% (LDL-C), 2.5% (HDL-C), 4.2% (HbA1c), 24.2% (Lp-PLA2) and 44.9% (MPO). Using logistic regression analysis, no significance correlation between gender and raised laboratory parameters was found. The same was reported for the different age categories except for 39 - <45 years, which had significantly increased TC level; OR = 1.57 (CI: 0.14 – 17.29, p = 0.021). Raised levels of biomarkers were not significantly correlated to HAART duration except for TC and Lp-PLA2 for HAART duration >60 months (OR = 1.62, CI: 0.28 – 9.43, p = 0.045 and OR = 1.65, CI: 0.43 – 6.39, p = 0.047 respectively).

Conclusion: Majority of the participants who had at least one derangement in the laboratory parameters being abnormal had been on HAART for more than sixty months. Dysregulated concentrations of the serum biomarkers were not significantly associated to gender. Age was significantly associated to HAART duration. Serum concentrations of TC and Lp-PLA2 showed significant association between raised serum levels with the duration of HAART.

1.INTRODUCTION

1.Background Information

Highly active antiretroviral therapy (HAART) has become more available to individuals who have Human immunodeficiency virus infection/Acquired Immunodeficiency Syndrome (HIV/AIDS) worldwide. There is a growing concern that the metabolic dysregulation, inflammation and endothelial dysfunction that are associated with HIV and HAART may increase cardiovascular risk and lead to cardiovascular diseases. In Kenya, Homa-Bay County has the highest HIV prevalence, which is at 25.7 percent[1,85]. The use of HAART in this county is also on the upper side. It would be expected that there would be an increased risk for cardiovascular diseases. We, therefore, set out to describe the cardiovascular risk profile of HIV-positive individuals receiving HAART in the Homa Bay County Referral Hospital in Kenya.

The process leading to the deposition of leucocytes, lipids, calcium and other substances in the intima of the artery and subsequently forming a plaque defines atherosclerosis. It usually occurs in the medium to large arteries. With increased deposition, the plaques continue to grow to large sizes that reduce blood flow through the artery significantly. Eventually, some plaques may even rupture forming a thrombus that can move to smaller arteries and completely block them.

Research evidence points out that HIV infection and HAART increase the risk for atherosclerosis[2]. The virus itself and HAART in the presence of other traditional risk factors can accelerate the progression of cardiovascular disease (CVD)[2]. Identification of biomarkers is useful in the prediction of CVD risk among those on HAART. Biomarkers of metabolic dysregula-

tion, inflammation, and endothelial dysfunction according to the studies conducted, potentially point the mechanisms by which HAART and HIV infection affect the cardiovascular system.

2.LITERATURE REVIEW

1.Epidemiology of CVD in HAART

According to the World Health Organisation (WHO), an approximate number of 17.3 million people die annually from CVD, which represents about 30% of all the global deaths. Islam et al.[3] conducted a meta-analysis, which reported an increased risk of CVD in HAART-exposed and HAART-naïve when compared to HIV-uninfected people to be 1.61 and 2.00 relative risk (RR) at 95% (1.43-1.83 and 1.7-2.37) confidence interval respectively. Clark et al.[5] conducted a study in South Africa, which involved 3641 study subjects and they reported 39% incidence risk of CVD. In Western Kenya, Bloomfield et al.[6] reported 7.4% prevalence of hypertension and obesity of 7.4% in HIV-positive individuals. Studies have also been done on novel markers of CVD where their levels have been observed to be raised in HAART use. Ross et al.[7], Cleveland, reported increased levels of Myeloperoxidase (MPO) in 861 subjects on HAART. Another study done by Mangili et al.[8], Boston, reported 75% abnormal level of Lipoprotein-associated phospholipase A2 (Lp-PLA2) in 341 subjects.

1.1.CVD RR for HAART Naïve Compared to HIV-uninfected Persons

Lang et al.[9]conducted a study in France that reported a relative risk (RR) of myocardial infarction (MI) to be 1.5. Another study done in Denmark by Obel et al.[10] reported a RR of ischemic heart disease for HIV-infected compared to uninfected individuals to be 1.39 and 2.12 for the pre-HAART and HAART duration respectively.

1.2.CVD RR for HAART Exposed Compared to HIV-uninfected Persons

Klein et al.[11] carried out a study that compared 6702 HAART-exposed individuals with uninfected ones and approximated the MI RR to be 1.78 at 95% CI (1.43, 2.22). A study by Islam et al.[3] after adjusting traditional risk factors (blood pressure, diabetes, smoking, age, sex, cholesterol and left ventricular hypertrophy) compared 80 HAART (PI-based) exposed with 256 uninfected individuals and estimated CVD RR to be 2.4 at 95% CI (1.69-3.46).

1.3.CVD RR for HAART-exposed Compared to Treatment-naïve

According to a meta-analysis by Islam et al.[3] HAART-exposed has a 52% higher risk of CVD than HAART-naïve. Aboud et al.[12] reported a RR of CVD and CHD for HAART-exposed and HAART-naïve after adjusting gender and age to be 1.13 and 1.02 respectively at 95% CI. Again, Islam et al.[3] reported a RR of CVD to be 1.41 at 95% CI (1.2, 1.65; $p<0.001$) for PI-based HAART compared to non-PI-based HAART.

2.Aetiopathogenesis of CVD in HAART-Naïve and Exposed

Understanding the cause of CVD in HAART-naïve and those on HAART is a phenomenon that is difficult to understand. However, recent scientific explorations have demonstrated underpinning evidence that links aetiopathogenesis of CVD in HAART-naïve and exposed to a number of combined determinants[13]. The factors can be due to the inherent effects of the viral infection, the impact of HAART, a high prevalence of several traditional risk factors among these population as well as the presence of other infections that often occur in HIV-infected individuals especially hepatitis C virus[13].

2.1.HIV Infection

A study by Strategies for Management of Anti-Retroviral Therapy Study (SMART)[13] demonstrated HIV as a potential CVD risk in HIV-positive individuals. One-half of the study population had a random assigning of HAART-interruption plan while the other had a continuous HAART plan. Patients assigned to the discontinuous plan showed an unexpected cardiovascular outcomes compared to those who were on a continuous plan. Thus, the results pointed out that HAART has a lower influence on CVD than uncontrolled HIV infection[13]. Different proposed mechanisms attempt to explain the influence of HIV infection on CVD. Some of the mechanisms explaining this occurrence include endothelial damage, persistent immune activation and inflammation, higher oxidative stress, increased thrombotic activity and indirect metabolic disorders[13].

HIV infection causes activation of the immune response thereby activating various inflammatory pathways[13]. Subsequently, this leads to the release of cytokines as well as the endothelial adhesion molecules expression, which then enhance adhesion and transmigration of leukocytes[15,2]. There is a link between activation of the immune system, inflammation, and endothelial dysfunction. Endothelial action by various cytokines often changes its functionality[11]. The HIV itself also causes damage to endothelial cells directly by increasing its permeability, which then promotes apoptosis and enhances the expression of adhesion molecules including ICAM-1, VCAM-1, and E-selectin[15'16].

Several studies show strong evidence that links HIV infection to immune activation as indicated by markedly increased plasma levels of various activation markers on monocyte and macrophages, which encompass sCD14, sCD163, and CD14+/CD16+ monocyte expansion[17,

[14]. Additionally, these studies point that HIV infection has an association with an elevated fraction of activated CD8 T-lymphocytes human leukocytes antigen (HLA)-DR+CD38+. The role played by monocyte/macrophages is pivotal in the start and progression of atherosclerosis. Therefore, with altered endothelial functionality, the atherosclerotic plaque develops due to increased apoptosis and expression of the adhesion molecules[13]. Consequently, macrophages phagocyte modify the lipoproteins in the atherosclerotic plaques, which then promote chemotactic and proinflammatory cytokine and mediate cholesterol efflux from the arterial wall[18]. One study reported elevated proportion of two principal types of monocytes, which are CD14+ and CD16+ and have been marked as proinflammatory because of their higher potency in the antigen presentation and increased expression of proinflammatory cytokines[18]. Furthermore, the virus impairs the pathway of adenosine triphosphate-binding cassette transporter A1 by acting via the viral protein Nef, which then inhibits cholesterol efflux from the macrophages to HDL particles. Thus, promoting accumulation of foam macrophages in the atherosclerotic plaques[19]. Increased thrombotic activity has also been linked with immune activation and inflammation in which there are elevated levels of biomarkers including fibrinogen, D-dimer, and von Willebrand[20].

HIV-induced oxidative stress blocks the DNA repair mechanisms; hence favouring the accumulation of oxidative lesions[21]. Other studies have exhibited the role that HIV infection plays in the imbalance of lipid metabolism. Deranged lipid metabolism in HIV-positive individuals results from the decreasing levels of plasma HDL-C and apoA1 caused by the virus. Additionally, HIV reduces the clearance of the LDL particles while increasing the levels of triglycerides and VLDL-C thereby promoting atherogenesis[21]. Moreover, such a pattern depicts a high prevalence of proatherogenic small and dense LDL particles. Norata et al.[22]

pointed that in a proinflammatory state, the circulating HDL particles are usually functionally less active and thus less atheroprotective, which limit their ability to carry out cholesterol efflux.

2.2.HAART

Initially, the observed increased CVD risk in HIV-positive patients was ascribed to metabolic derangement linked to HAART; this was particularly due to the impacts of viral protease inhibitors (PI)[3]. The initiation of PI in the clinical practice showed a coincidence with the first cases of ischemic heart disease reported in patients with HIV[23]. Currier et al.[24] conducted an epidemiological study that confirmed the association between HAART and CVD risk. Additionally, D.A.D study (Data Collection on Adverse Events of Anti-HIV Drugs) was more representative and demonstrated a substantial rise in acute myocardial infarction (AMI) incidence after exposing HAART-naïve to HAART[25]. Mechanisms of HAART-mediated factors are due to metabolic and plasma lipid changes, inflammation, and effects of HAART initiation on endothelial dysfunction and damage.

2.3.Lipid and Metabolic Alterations

Leclercq and Blanc[4] pointed that antiretroviral drugs cause metabolic imbalance, which promotes the development of hypertriglyceridemia, hypercholesterolemia, and insulin resistance as well as type 2 diabetes mellitus (T2DM). Such metabolic imbalance can occur singly or as a part of other disorders including lipodystrophy and metabolic syndrome[4]. HIV patients treated with nucleoside/nucleotide analog reverse transcription inhibitors (NRTIs) and PI drugs have reported such metabolic alteration, which is typically an atherogenic pattern[78]. While differences may exist between individual drugs within the PI class regarding lipid al-

tering effects, most studies have strongly linked PI treatment to CVD[4]. Even though the new generation of these drugs has significantly reduced their lipid altering effects, their initiation is often linked to increasing levels of plasma lipids.

2.4.Inflammation in HAART-exposed

There is a constant up-regulation of inflammatory markers in patients receiving HAART, which could be due to a number of mechanisms including persistent low-level viral replication, drug toxicity as well as other factors. Madden et al.[26] and Reingold et al.[27] showed elevated levels of fibrinogen in women as well as increased levels of hsCRP in men, all of whom were on HAART[26,27]. Additionally, the two studies observed higher levels in those patients on PI compared to the NRTI-based treatment regimen. Additionally, another study is consistent with increased hsCRP levels in HIV-positive patients receiving HAART when compared to the HAART-naïve counterpart[28]. Thus, these findings reinforce the significance of metabolic and lipid alterations as key mediators of HAART-linked inflammation. According to DAD study, exposure to didanosine and abacavir showed an increased risk of AMI[29].

2.5.Effects of HAART on Endothelial Dysfunction and Damage

There is a short-term improvement in endothelial dysfunction and damage caused by HIV infection upon initiation of HAART. Wong et al.[30] performed a longitudinal study, which demonstrated decreased levels of markers of endothelial activation. Mechanisms leading to PI-associated endothelial dysfunction are intricate; however, they may include insulin resistance, increased oxidative stress, and lipoprotein alterations. Additionally, the PI-linked endothelial damage shows variation with the individual drugs in the PI class. However, a study by SMART showed net protective effects of HAART on CVD in patients receiving

HAART[14]. HAART suppresses viral replication thereby reducing immune activation, inflammation, and endothelial dysfunction. Thus, endothelial dysfunction and inflammatory biomarkers are at lower levels in HAART-exposed than HAART-naïve patients. Other studies have also demonstrated that immune activation diminishes with the initiation of HAART[31]. Further, the studies showed that HAART-treated patients had a slightly lower fraction of pro-inflammatory CD14+/CD16 monocytes compared to HAART-naïve[31].

According to SMART, HAART did not fully reverse endothelial dysfunction, immune activation, inflammation in HIV-positive patients[14]. The study involved two large populations and demonstrated elevated plasma levels of hsCRP, D-dimer and IL-6 in HAART-exposed patients compared to healthy subjects even after the suppression of viral replication[32]. The study was consistent with one of the case-control in which the levels of hsCRP and sVCAM-1 remained elevated in HIV patients even after HAART suppression as compared to healthy individuals[9]. Additionally, Syed et al.[33] demonstrated that HIV infection and HAART increases the plasma levels of myeloperoxidase (MPO). The results from most of these investigations indicate HAART as a key factor in reducing CVD in HIV patients receiving HAART. However, HAART-patients maintain a state of immune activation, endothelial dysfunction, and inflammation, which explains the high prevalence of CVD in HIV-positive patients receiving HAART even with a stable suppression of HIV replication.

2.6.Host/Traditional CVD Risk Factors

Currier et al.[24] demonstrated a high prevalence rate of some traditional CVD risk factors including T2DM, smoking, and dyslipidaemia in HIV-infected individuals. Various cohort studies have documented the significance of these cardiovascular risk factors and demon-

strated a robust linkage between CVD and HIV infection, which is stronger compared to linking HAART to CVD[21]. However, as earlier stated, both HIV infection and HAART use have the potential to induce diabetes and dyslipidemia[9].

Lifestyle: Smoking and dietary habits. Joy et al.[34] demonstrated that HIV patients had substantially greater consumption of trans-, saturated and total fat as well as cholesterol. They attributed this to a compensatory mechanism for the lipoatrophy. Another study observed a reduced incidence of dyslipidaemia in HAART-exposed patients upon initiation of low saturated fat and hypo-caloric diet compared to subjects who never went a dietary intervention[35].

Metabolic factors. As with the general population, HIV-positive individuals have similar predisposing factors for developing diabetes, dyslipidaemia or metabolic syndrome in spite of individual variation[35]. However, other factors coexist in HIV patients that include HAART, HIV infection itself as well as other coinfections, which explain the high rate of markedly abnormal lipid and carbohydrate metabolism in this population.

2.7.Other factors

Additional factors other than HIV infection and HAART also play a role in the development of CAD. Some of these factors include the presence of chronic coinfections. As with the HIV infection, the presence of chronic coinfections favours immune activation, endothelial dysfunction, inflammation and increased generation of reactive oxygen species[36]. There is a high prevalence of subclinical atherosclerosis in the general population with chronic coinfection. Atherosclerotic plaques, AMI, and stroke have been observed in HIV patients with chronic coinfection[37]. Underlying infection with a herpes family of the virus has been demonstrated

to have atherogenic effects because of their immense contribution to inflammation and immune activation.

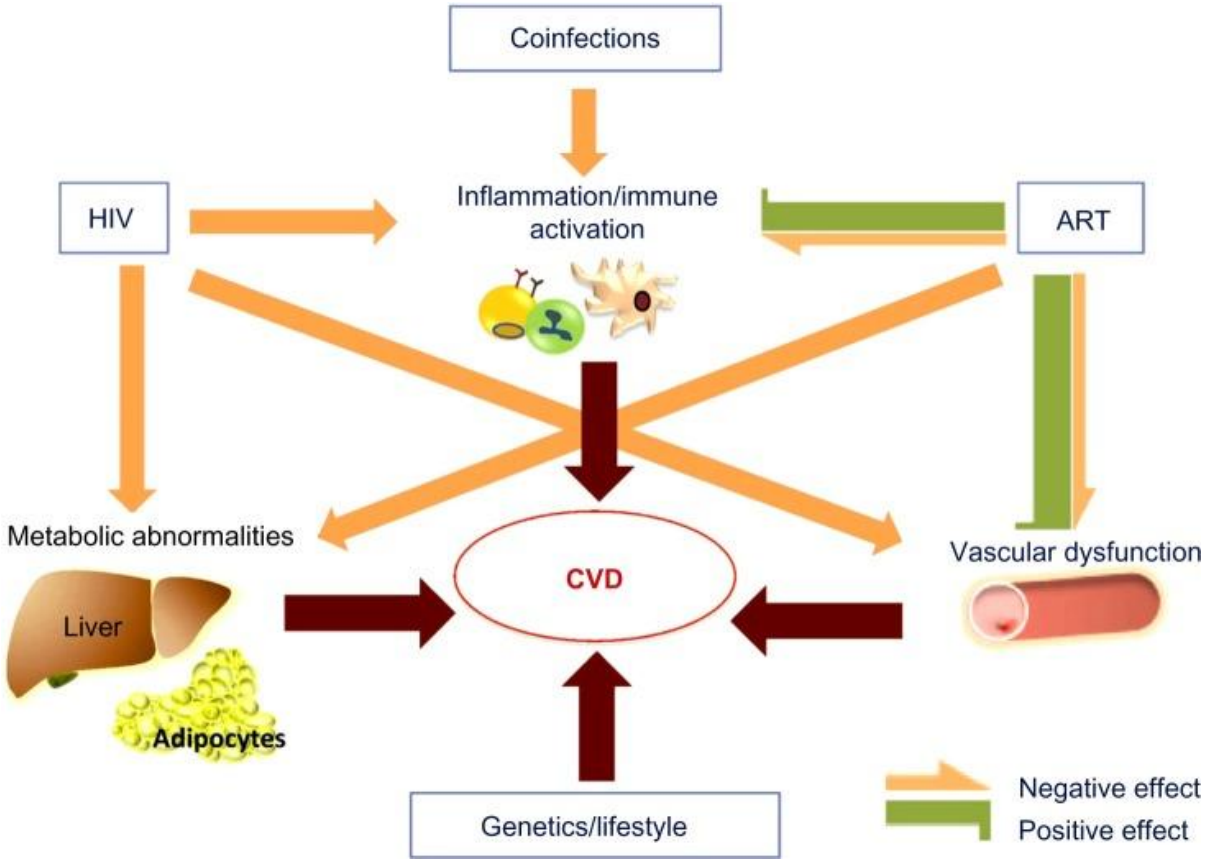

Figure 2.2.1: Diagrammatic summary of the determining factors of CVD risk in HIV-positive individuals, showing the interplay of the mechanisms involved in the development of CVD in this population[13].

3.Persistent Inflammation and Immune Activation

Various mechanisms explain the occurrence of persistent inflammation and immune activation even after the initiation of HAART. One factor is the homeostatic drive and time to initiate HAART. Constant HIV replication often favours immune action and inflammation to the point that it remains irreversible even after initiating HAART. Thus, starting HAART before

advancement to this point is essential in suppressing viral replication and hence reduced immune/inflammatory responses. According to Burdo et al.[31], an early initiation of HAART can help reduce activation of monocytes and CD8 T-cells and reverse the condition to normal levels. Additionally, residual viral replication that is below the detection level of frequently used techniques has been demonstrated to promote persistent immune/inflammatory responses.

HIV infection damages the intestinal mucosa thereby promoting bacterial products translocation, which then induces immune activation[38]. There are severe damages to the lymphoid tissues linked to intestinal mucosa in acute HIV infection phases, which lead to a massive loss of T-cell lymphocytes. Thus, a full recovery of this lymphoid tissue may be impossible, which facilitates bacterial translocation.

4.Biochemical Markers of CVD

4.1.Lipoprotein and Lipid-related Markers

Lipoproteins function by transporting the hydrophobic cholesterol throughout the body. Based on their density, which is linked to the abundance of either apolipoprotein A-I (ApoA-I) or apolipoprotein B (ApoB), lipoproteins are different[39]. Therefore, according to their varying densities, they are named as very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) [79, 83, 84]. There is a link between high levels of cholesterol in ApoB-containing lipoprotein namely, VLDL and LDL with increased risk of CVD. Several studies have documented that ApoA-I-containing lipoprotein, which is HDL, shows an inverse relationship with CVD risk[40,41]. There is a disproportion of ApoB

present in VLDL and LDL, with almost 90% present in LDL but any measurement of non-HDL or ApoB captures these atherogenic particles[41].

4.2.Lipoprotein (a) [Lp(a)]

Lp(a) is an $ApoB_{100}$ molecule synthesised in the liver and is covalently linked to Apo(a)[42]. It has a similarity to both plasminogen and LDL since its Apo(a) component has a sequence of Kringle IV repeats similar to plasminogen, a fibrinolytic proenzyme, and its $ApoB_{100}$ component resembles LDL[43]. Similar to LDL, it is a proatherogenic and contributes to the progression of atherosclerosis[44,45,46] as well as the formation of foam-cell[47]. Some studies have demonstrated it as an atherosclerosis-specific marker, but it has no relationship with the risk of thrombosis[48,49]. Lp(a) correlates weakly with traditional cardiovascular risk factors including triglycerides, non-HDL cholesterol, fibrinogen, and $ApoB_{100}$, which points that it is an independent risk factor for CVD[82]. According to a recommendation made in 2013 by a consensus statement from the European Atherosclerosis Society, Lp(a) levels should be measured in patients with familial hypercholesterolemia[49].

4.3.Oxidized Phospholipids on $ApoB_{100}$-containing Proteins (OxPL/ApoB)

Oxidized lipids amplify the inflammatory response and have a central role in the progression of atherosclerosis and arterial plaques[50]. Lp(a) transports OxPL/ApoB, which is an oxidation-specific biomarker that circulates the plasma and deposit in the vascular wall thereby inducing a local inflammation[47].

4.4.Lipoprotein-associated Phospholipase A2 (Lp–PLA2)

Lp–PLA2 is a secretory phospholipase and a constituent of one of the fifteen groups, which include phospholipase A2 enzyme superfamily[51]. While in circulation, 80% and 10% Lp–PLA2 is LDL and HDL-bound respectively but negligible VLDL and Lp(a)-bound[52,53]. Atherosclerotic cells and those involved in inflammation such as mast cells, T cells, and macrophages produce Lp–PLA2 and then cleave the oxidised phosphatidylcholine fraction of oxidised LDL particles, thereby producing pro-inflammatory and proatherogenic oxidised lysophosphatidylcholine and fatty acids, which then activate inflammatory pathways in the vascular wall[54]. There is an increased upregulation of Lp–PLA2 in atherosclerotic plaques, and it is associated with plaque rupture.

4.5.Homocysteine

Increased plasma levels of homocysteine have a positive relationship with various mechanisms involved in the CVD risk that includes monocyte adhesion, endothelial cell dysfunction, and oxidation of LDL[55]. Its first identification as a potential CVD risk was in individuals with homocystinuria as a result of elevated levels of homocysteine, which some studies have demonstrated a strong association with CVD risk[56].

4.6.Glycated Hemoglobin A_{1C} (HbA_{1C})

HbA_{1C} is a long-term marker of the average concentration of blood glucose, and it is being used for screening and management of diabetes mellitus (DM). Several studies have demonstrated its strong association with CVD in HIV-positive individuals without diabetes mellitus[57,58,59,60]. Pai et al.[58] and Sander et al.[61] observed that HbA_{1C} and CRP had a positive asso-

ciation in adults without DM for whom the combination of increased HbA_{1C} and CRP levels was linked to advanced early carotid atherosclerosis development and adverse events such as stroke, myocardial infarction, and vascular death. Hyperglycaemia leads to the formation of glycated end products, which induce the generation and secretion of inflammatory cytokines and according to King et al.[62,80,81], HbA_{1C} levels can predict CRP levels in patients with established DM. Therefore, a combined effect of HbA1C and CRP potentially increases the CVD risk.

4.7.High-Sensitivity C-Reactive Protein (hsCRP)

CRP is an acute-phase reactant that is predominantly produced in the hepatocytes as a pentamer of identical subunits in response to various cytokines[63]. It is a nonspecific marker of inflammation, and one of the potent drivers of its production is interleukin (IL)-6, which is released from activated leukocytes in response to trauma or an infection. IL-6 is also released from vascular smooth muscle cells so as to respond to atherosclerosis. hsCRP directly binds to the highly atherogenic oxidised LDL cholesterol[64]. The proatherogenic effects of hsCRP involve complex mechanisms some of which include the facilitation of monocyte adhesion and transmigration into the vascular wall, which is a critical early step in the process of atherosclerosis[65,66]. hsCRP catalyses M1 macrophage polarisation, which is a trigger of pro-inflammatory response in plaque deposition and subsequently leading to macrophage infiltration of both atherosclerotic lesions and adipose tissue[67]. Association of hsCRP with CVD is a function of its strong correlation with traditional risk factors including visceral obesity, DM, smoking, and markers of inflammation[68].

4.8.Myeloperoxidase (MPO)

MPO is an enzyme associated with both oxidative stress and inflammation and is abundantly expressed in the azurophilic granules of various leukocytes subspecies that include monocytes and neutrophils[69]. Research has demonstrated that MPO and its corresponding oxidant products including chlorotyrosine and nitrotyrosine have a key role in the progression of atherosclerotic plaque and have been found at the site of plaque rupture[70]. According to Marathe et al.[70], MPO promotes several pathological events that include impaired nitric oxide bioavailability and uptake of oxidized lipid by macrophages, which facilitate plaque formation and subsequent rupture[71].

4.9.Soluble Vascular Cell Adhesion Molecule 1 (sVCAM-1)

Cook-Mills et al.[72] pointed out that one of the earliest events in the atherosclerotic process is the binding of a leukocyte to cellular adhesion molecules (CAMs). Several studies have demonstrated that increased expression of CAMs has an association with response to inflammatory cytokines that include IL-1, IL-4, interferon-gamma, tumour necrosis factor-alpha, oxidised LDL, and lipopolysaccharide[73,74]. A number of pathologic and clinical studies have demonstrated the role of sVCAM-1 in atherosclerotic plaque formation and its subsequent disruption[73,74]. sVCAM-1 are released from the surface of leukocytes and endothelial cells after cytokine activation probably by proteolytic cleavage thereby rendering them measurable in the plasma[74].

5.Problem Statement

Kenya stands at position six among African countries with a high burden of HIV with over one million people living with the virus[1]. Geographically, the epidemic is diverse in Kenya with Homa Bay County having the highest prevalence at 25.7% and Wajir County at 0.2%. Nationally, high HIV/AIDS burden in Kenya is responsible for an approximate 29% adult deaths annually, 20% of maternal mortality and 15% of children below five years of age.

With the introduction of HAART coupled with the management of HIV disease, there has been significant improvements and survival of HIV-positive individuals. As a result of such improvements, there has been a constantly increasing population of individuals living with the disease for several years. However, the high burden of illness, health care utilisation, as well as premature death in individuals living with HIV, is now HIV infection and HAART-related complications[3]. Some of these complications include an altered metabolic status that comprises of dyslipidaemia and hyperglycaemia. With increased survival, these conditions continue to contribute to other disorders that are life-threatening that are linked to atherosclerotic cardiovascular risk[4]. The impacts of HAART on cardiovascular outcome among Homa Bay County residents, for whom there is a high prevalence of new infection and access to HAART is expanding, remain largely unknown.

6.Rationale

A meta-analysis of studies done in Western countries[3] shows that HIV-infection itself and HAART increase the risk of CVD. The current Kenya National HIV treatment guidelines aim at the early initiation of ART in HIV patients. There is a paucity of local data on CVD risk in patients on HAART, which would inform strategies to minimise the risk. This study sought to provide data for Homa Bay County which has the highest national HIV prevalence with a high population of patients on HAART who could, therefore, be at risk of HAART-associated CVD.

The conventional methods used to predict CVD risk relied mostly on blood pressure and lipid profile. Studies have shown that the mechanisms involved in the pathogenesis of atherosclerotic plaque in HIV patients are complex; thus the conventional CVD risk assessment methods are unsatisfactory for this population. Therefore, it is imperative that assessment of CVD risk in HIV patients adopt the use of novel biomarkers that show early events in atherosclerosis in addition to markers of metabolic dysregulation.

7.Research Question

Does prolonged use of HAART raise the levels of serum biomarkers for risk of CVD among HIV-positive individuals receiving care in Homa Bay County referral hospital?

8.Objectives

8.1.Broad Objective

To determine the correlation between levels of serum biomarkers for risk of CVD with the duration of HAART use among HIV-positive individuals receiving care in Homa Bay County referral hospital.

8.2.Specific Objectives

1. To determine serum concentration of HbA1C, TC, LDL-C, HDL-C and MPO in HAART.
2. To calculate the proportion of the study subjects at low and high risk based on the the levels of their serum HbA1C, TC, LDL-C, HDL-C and MPO.
3. To determine the correlation between HAART duration and increased levels of the biomarkers for risk of CVD.

3.METHODOLOGY

1.Study Design

The study design was a descriptive cross-sectional study.

2.Study Area

The study was conducted in Homa-Bay County Referral Hospital and the clinical laboratories of Kenyatta National Hospital. Homa-Bay County Referral Hospital receives many patients from every part of the county. The hospital's comprehensive care clinic (CCC) operates for four days in a week. Close to fifty individuals on care attend the CCC in a single day of operation. Therefore, the study had sufficient participants within the scheduled time frame. The management of the CCC offered us a room for the study. The room was used for storing the study materials and taking of blood samples from individuals who met the inclusion criteria and consented to participate in the investigation. HbA1c was done onsite. The facilities at the KNH biochemistry and immunology laboratories were used to do analysis of TC, HDL-C, LDL-C, MPO and Lp-PLA2.

3.Study Population

The study population consisted of HIV-positive individuals who were on HAART.

4.Selection Criteria

4.1.Inclusion Criteria

1. HIV-positive patients on HAART aged 18 to 45 years
2. Agree to consent

3. HAART duration of at least 6 months

4.2.Exclusion Criteria

1. Individuals with known history of myocardial infarction/stroke
2. Those with hypertension, diabetes, and dyslipidaemia prior to HAART
3. Pregnant women
4. Cigarette Smokers
5. Severely ill patients
6. Alcoholics

5.Sample Size Determination

The study used sample size calculation formula for quantitative cross-sectional study according to Charan and Biswas[75].

$$SampleSize = \frac{(Z_{1-\alpha/2})^2 SD^2}{d^2}$$

$Z_{1-\alpha/2}$= is the standard normal variate, 1.96

SD= standard deviation of a variable taken from a previous/pilot study. This study used SD for HDL-C of 66.64±27.94 mg/L, Nsagha et al. 2015, Cameroon[76].

d= absolute error/precision set at ±5%

Substituting into the formula gave a sample size of 120

$$SampleSize = \frac{(1.96)^2(27.94)^2}{5^2}$$

Sample size (n) = 120

6.Sampling Method

The study used a systematic random sampling with an interval of two.

7.Recruitment

The patients were recruited at Comprehensive Care Clinic (CCC) of Homa Bay County Referral Hospital after consenting to participate in the study. A screening questionnaire was used to identify the individuals that met the inclusion criteria. HAART was defined as a mixture of at least three classes of antiretroviral (ARV) drugs that include protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTIs) and Non-nucleoside reverse transcriptase inhibitors (NNRTs).

8.Data Collection

A structured questionnaire was used to collect demographic data and information on the medical history of the study participants. For the specimen collection, 4 ml non-fasting venous blood was collected aseptically from the antecubital vein. 3 ml was put in a plain (red top) tube, and 1 ml was put in an EDTA (purple) tube. Blood in the red top tube was allowed to clot for 15 minutes at room temperature and then centrifuged for 10 minutes at 1,500xg in a refrigerated centrifuge. 1.5 ml serum was aspirated and then apportioned into 0.5 ml ali-

quots. The samples were then frozen at -20°C until the biochemical analysis was done. 1 ml of blood in the EDTA tube was used for point of care assay for HbA1C.

9.Specimen Transportation and Storage

The collected specimen was shipped from Homa Bay County Referral Hospital to the Biochemistry Laboratory of Kenyatta National Hospital. Transportation was done by air. During the transportation, cool box with ice packs were used to maintain low temperatures and keep samples in a frozen state. Samples were then stored at -20°C until the time of analysis in which they were allowed to thaw at room temperature.

10.Biochemical Analysis

The Cobas^{TM} chemistry analyser was used to analyse the serum concentration of lipids TC, LDL-c, and HDL-c. HbA1c was analysed using the CloverA1C^{TM} analyser. Lp-PLA2 and MPO was analysed by quantitative sandwich enzyme immunoassay technique (BioTechne). Using the US National Cholesterol Education Program guideline. Abnormal HbA1C was defined as ≥6.5%.Abnormal lipid profile was defined as TC ≥ 5.18 mmol/L, LDL-c ≥ 3.37 mmol/L, HDL-c < 1.0 mmol/L. Abnormal Lp-PLA2 was defined as >167 ng/mL. Abnormal MPO was defined as > 229 ng/mL.

11.Quality Assurance

Qualified personnel at the Homa Bay County Referral Hospital and KNH biochemistry and immunology laboratories were involved in the study during specimen collection, handling, and analysis.

Pre-analytical standardisation: blood specimen was collected aseptically and put in the correct tube. The samples were stored at the defined temperature before biochemical analysis was done.

Analytical standardisation: Internal Quality Control (IQC) check was performed before doing any analysis. The analysis was done as per the manufacturer's protocol.

Post-analytical standardisation: the principal investigator made sure that the results were reported correctly by use of participant identification number (ID).

12.Ethical Consideration

The principal investigator sought ethical approval from Kenyatta National Hospital / University of Nairobi (KNH/UON) Ethics and Research Committee. Permission to carry out the study in Homa Bay County Referral Hospital was obtained from the county health director/hospital's administrator. Participation in the study was voluntary and all the information gathered from the study subjects were kept confidential. The principal investigator was the only person who had access to the gathered data. Patients only received their results through the clinician for further diagnosis and treatment accordingly.

13.Data Management and Analysis

Data were entered into a Microsoft Excel spreadsheet and were kept inaccessible to an unauthorised person by use of passwords. For data entered in paperwork, safe and secure lockable cabinets were used to keep them from access by an unauthorised person.The optical densities for MPO and L-p-PLA2 were recorded and concentration calculated by creating a standard curve using a GraphPad prism to generate a four parameter logistic (4-PL) curve-fit. The

IBM Statistical Package for Social Sciences (SPSS) version 23 was then used for data analysis. Analysis of continuous variables was done using descriptive statistics (mean, mode, median, standard deviation and range). Chi-square test was used to test the significant association between gender and age with HAART duration. Independent association between raised levels of TC, LDL-C, HDL-C, HbA1c, Lp-PLA2 and MPO with gender, age and HAART duration was tested in a logistic regression model.

4.RESULTS

1.Introduction

The chapter displays the findings of the study. One hundred and sixty five individuals attending the Comprehensive Care Clinic at the Homa Bay County Referral Hospital were approached to take part in the study. Majority (120) met inclusion criteria and consented to participate in the study. Forty five declined to give consent indicating they did not want to give blood samples.

2.Socio-Demographic characteristics of the study participants

A summary of the socio-demographic features of the 120 study subjects are shown in Table 4.2.1 below. HAART duration of those recruited was at least six months.

TABLE 4.2 SOCIO-DEMOGRAPHIC SUMMARY OF THE STUDY SUBJECTS AT HOMA BAY COUNTY REFERRAL HOSPITAL, KENYA.

Demographic characteristics	Frequency (N)	Percent (%)	Mean (SD)	Mode	Median	Range
Gender						
Female	77	64.2				
Male	43	35.8				
Age (Years)						
18-23	7	5.8				
24-28	20	16.7				
29-33	26	21.7	34.34 (±6.545)	42	35	27 (18 - 45)
34-38	33	27.5				
39-<45	34	28.3				
Level of Education						
None	2	1.7				
Primary	67	55.8				
Secondary	37	30.8				
Post-secondary	14	11.7				
Marital Status						
Single	15	12.5				
Married	80	66.7				
Widowed	20	16.7				
Divorced	5	4.2				
Occupation						
unemployed	20	16.7				
Self-employed	78	65				
Salary-employed	22	18.3				
HAART Duration						
6 - 35	17	14.2				
36 - 60	22	18.3				
>60	81	67.5				

N - Number; **(%)** - Percentage; **SD** - Standard deviation;

2.1.Gender

More females (77) than males (43) participated in the study; giving a female to male ratio of 1.8:1.

2.2.Age

The participants' age ranged from 18 to 45 years with a mean of 34.34 (SD 6.55) years and median of 35 years. Most of the participants (55.8%) were between 34 and 45 years of age as

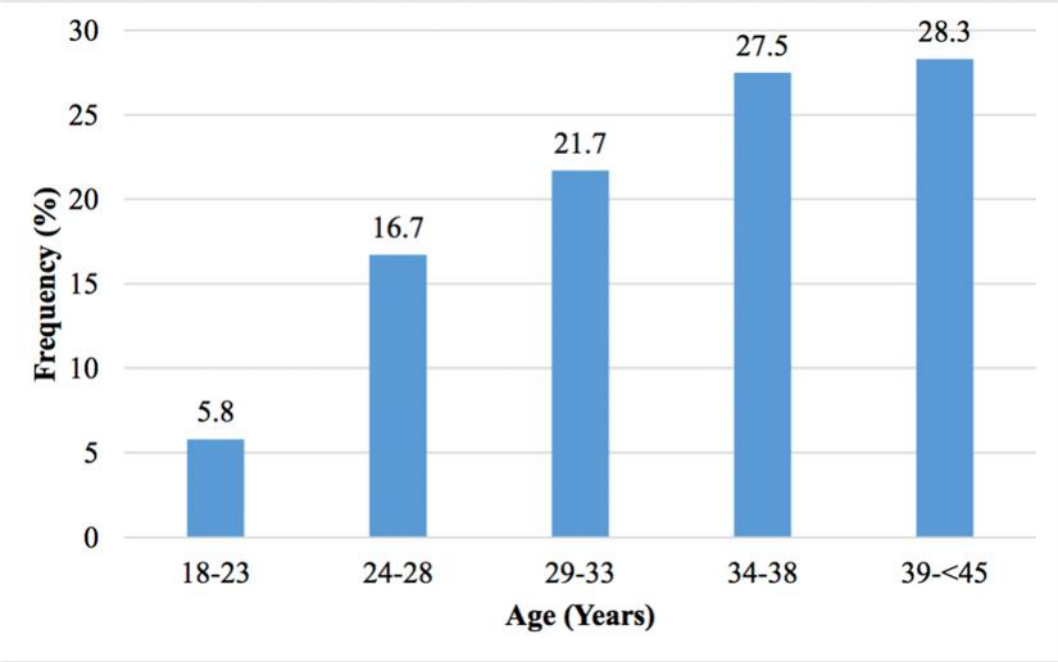

FIGURE 4.2.2 AGE DISTRIBUTION OF THE PARTICIPANTS ON HAART IN HOMA-

shown in Figure 4.2.2.

2.3.Level of education

Only 2 of the participants (1.7%) indicated that they had not been to school. Most had attained at least the primary level of education (55.8%), and 11.7% had obtained post-

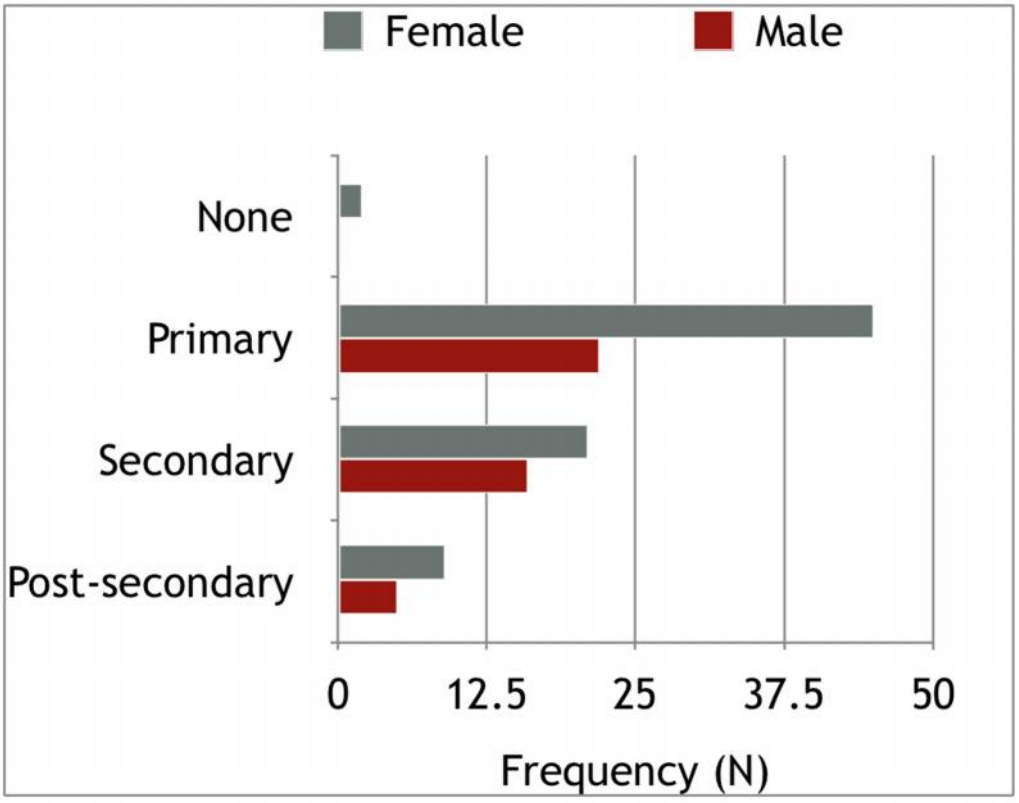

FIGURE 4.2.3 LEVEL OF EDUCATION OF STUDY PARTICIPANTS ON HAART IN

secondary education. This is shown in Figure 4.2.3.

2.4.Marital status

Majority of the subjects were married (66.7%). The widowed participants were 16% and they were all females. Fifteen participants (12.5%) were single and twelve of them were females.

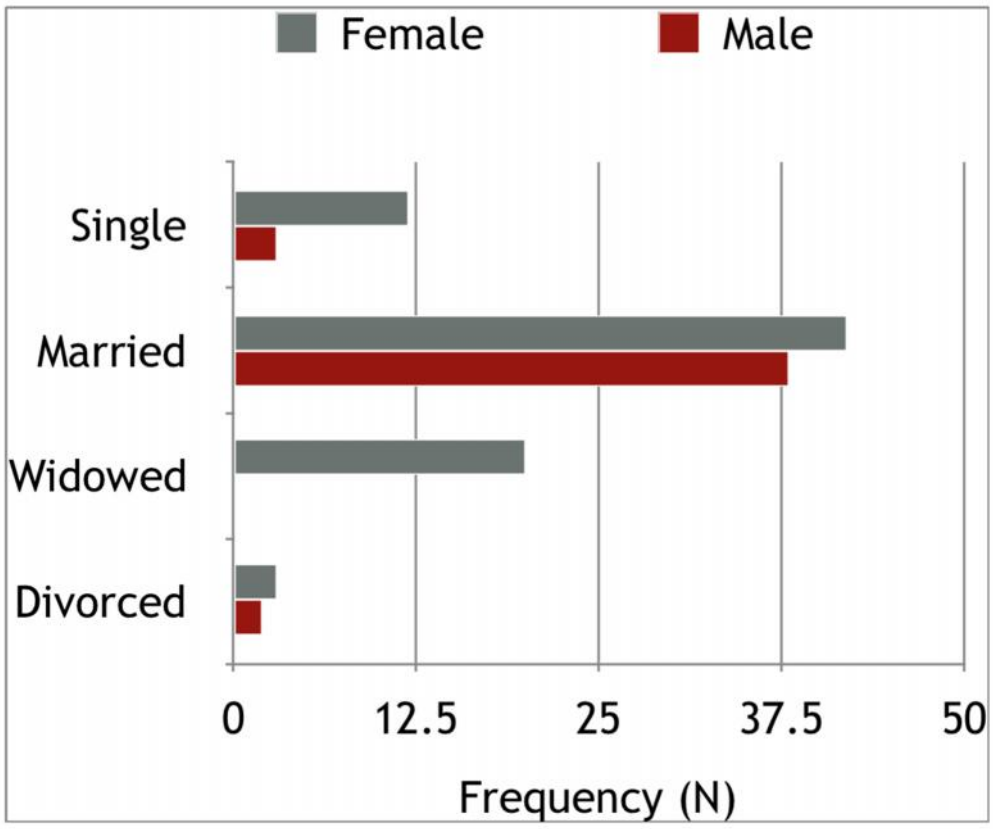

FIGURE 4.2.4 MARITAL STATUS OF THE PARTICIPANTS ON HAART IN HOMA-

This is shown in Figure 4.2.4.

2.5.Occupation

Most of the study subjects had some form of employment, the main one being self-employment in 78% subjects. Only 22% participants indicated they were in formal employ-

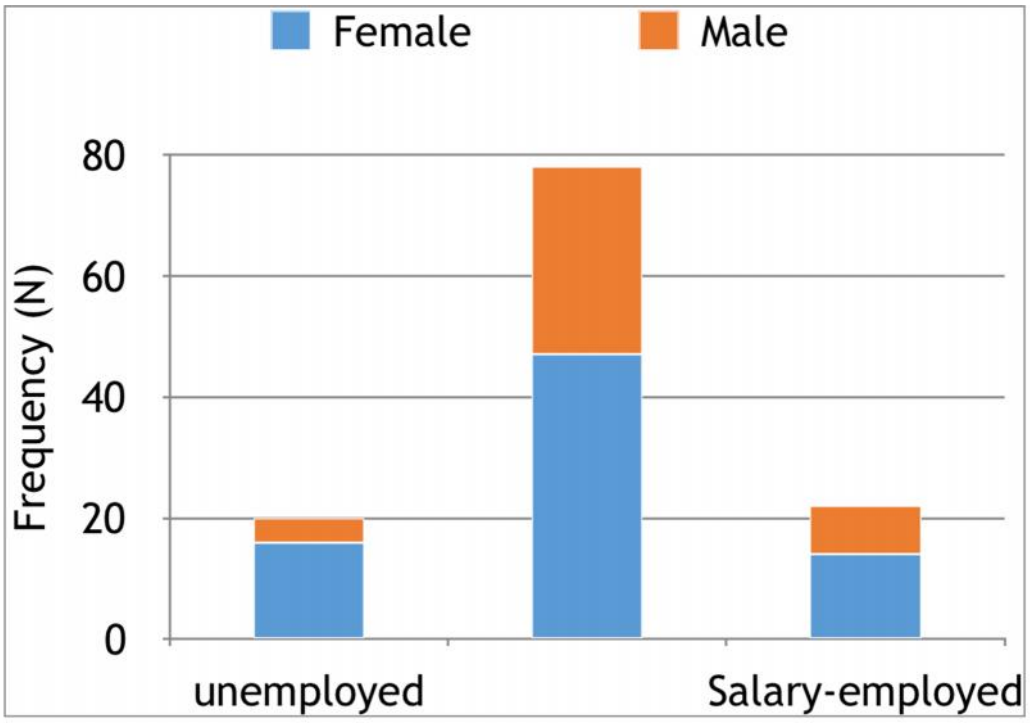

FIGURE 4.2.5 OCCUPATION OF THE PARTICIPANTS ON HAART IN HOMA-BAY

ment, (Figure 4.2.5).

2.6.HAART duration

Most of the study subjects had been on HAART for more than sixty months, which represented 67.5%. For those who had been on HAART for 6 – 35 months and 36 – 60 months

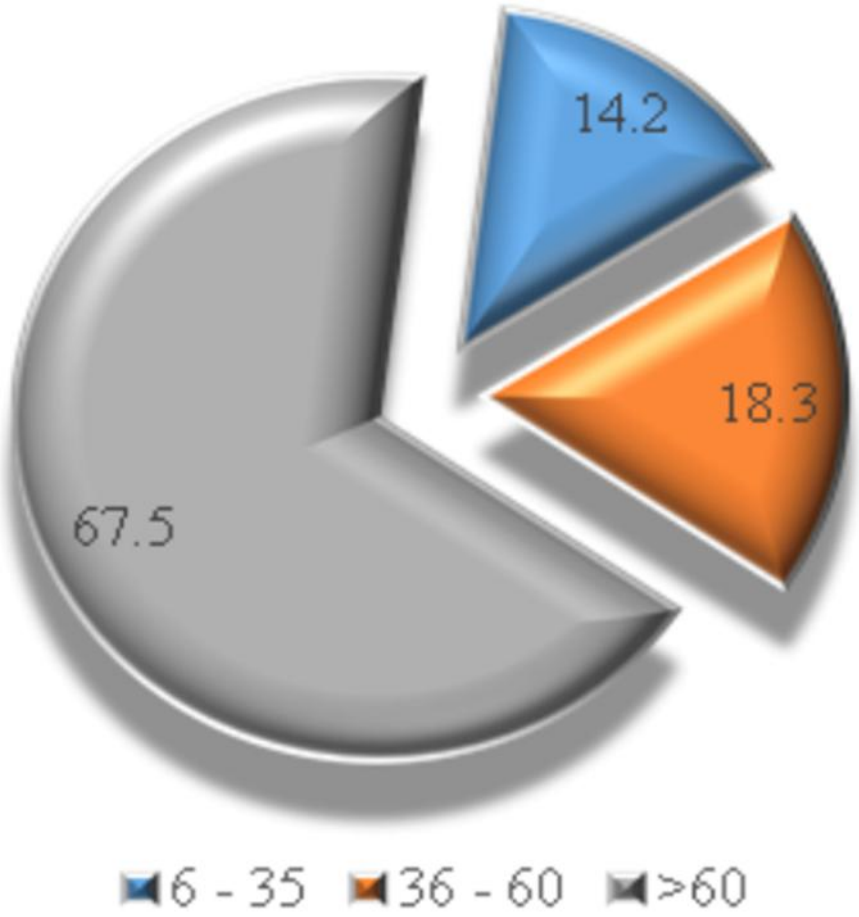

FIGURE 4.2.6 HAART DURATION AMONG THE PARTICIPANTS ON HAART IN

were 17% and 22% respectively, (Figure 4.2.6).

3.Laboratory parameters for the study participants

The levels of the laboratory parameters were measured and categorised as low risk or high risk for coronary artery disease using the predefined cut-off points. For lipid parameters, the National Cholesterol Education Program medium and high risk cut-off levels were combined and termed as increased risk as shown in Table 4.3 below.

Laboratory Parame-ters	Frequency N(/%)	Mean (SD)	Range
HbA1c (%)			
Low risk (<6.5)	112 (93.3)	5.45 (±0.66)	4.1 - 9.3 (5.4)
High risk (≥6.5)	8 (6.7)		
TC (mmol/L)			
Low risk (<5.18)	103 (85.8)	4.15 (±0.91)	1.84 - 6.97 (4.0)
Increased risk (≥5.18)	17 (14.2)		
LDL-C (mmol/L)			
Low risk (<3.1)	109 (90.8)	2.30 (±0.57)	1.04 - 4.21 (2.2)
Increased risk (≥3.1)	11 (9.2)		
HDL-C (mmol/L)			
Low risk (>1)	117 (97.5)	1.59 (±0.32)	0.58 - 2.73 (1.6)
Increased risk (≤1)	3 (2.5)		

Lp-PLA2 (ng/ml)			
Low risk (<167)	91 (75.8)	172.01 (±334.81)	0.3 - 1445.87 (52.52)
High risk (≥167)	29 (24.2)		
***MPO (ng/ml)**			
Low risk (<229)	43 (55.1)	259.84 (±191.65)	45.02 - 603.56 (208.94)
High risk (≥229)	35 (44.9)		

N - Number; **%** - Percentage; **SD** - Standard deviation;
*- had 78 subjects

TABLE 4.3 LABORATORY PARAMETERS FOR THE STUDY PARTICIPANTS AT HOMA BAY COUNTY REFERRAL HOSPITAL, KENYA. (N=120).

3.1.HbA1c

The HbA1c for the study participants ranged from 4.1 to 9.3 with a mean HbA1c level of 5.449 % (±0.66) and median of 5.4%. It was found that 8 of the study participants (6.7%) had an elevated level of HbA1c and they were classified as having high risk.

The median HbA1c levels demonstrated varied across the different occupational status for both female and male. The salary-employed group exhibit a higher median.

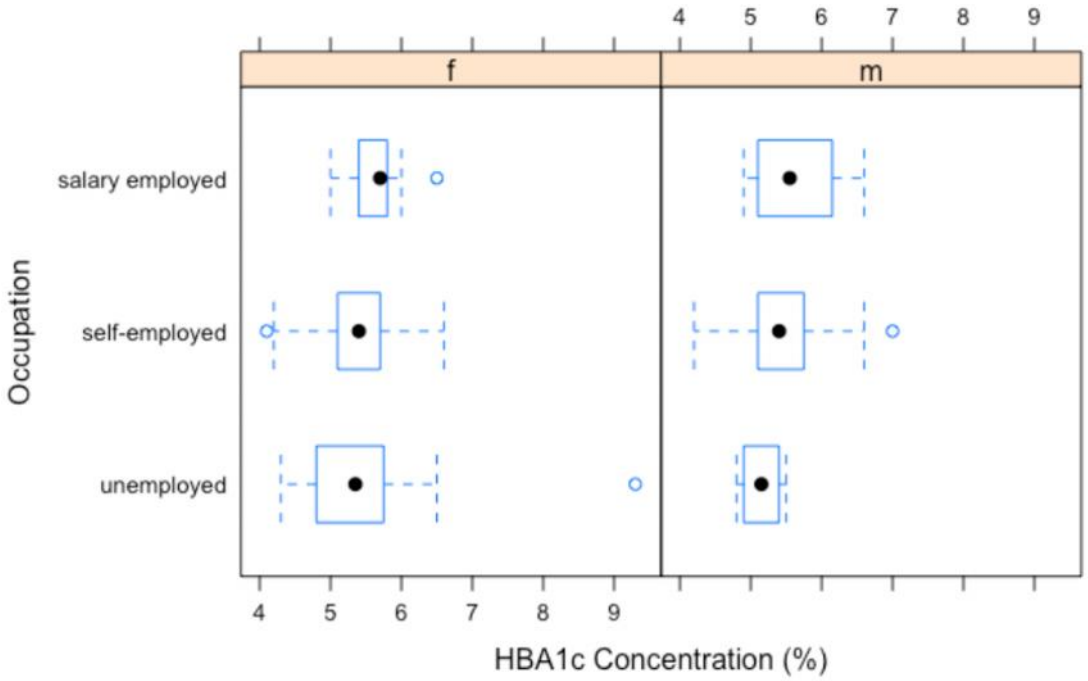

FIGURE 4.3.1 MEDIAN HBA1C CONCENTRATION ACROSS DIFFERENT OCCUPA-

3.2.Total Cholesterol

In this study, the total Cholesterol ranged from 1.84 to 6.97 mmol/L with a mean of 4.15 mmol/L (±0.91) and median 4.05 mmol/L. The number of participants having elevated TC values was 17 (14.2%) and were classified as having increased risk.

The median TC concentration exhibited almost a similar pattern for both self-employed and the unemployed groups.

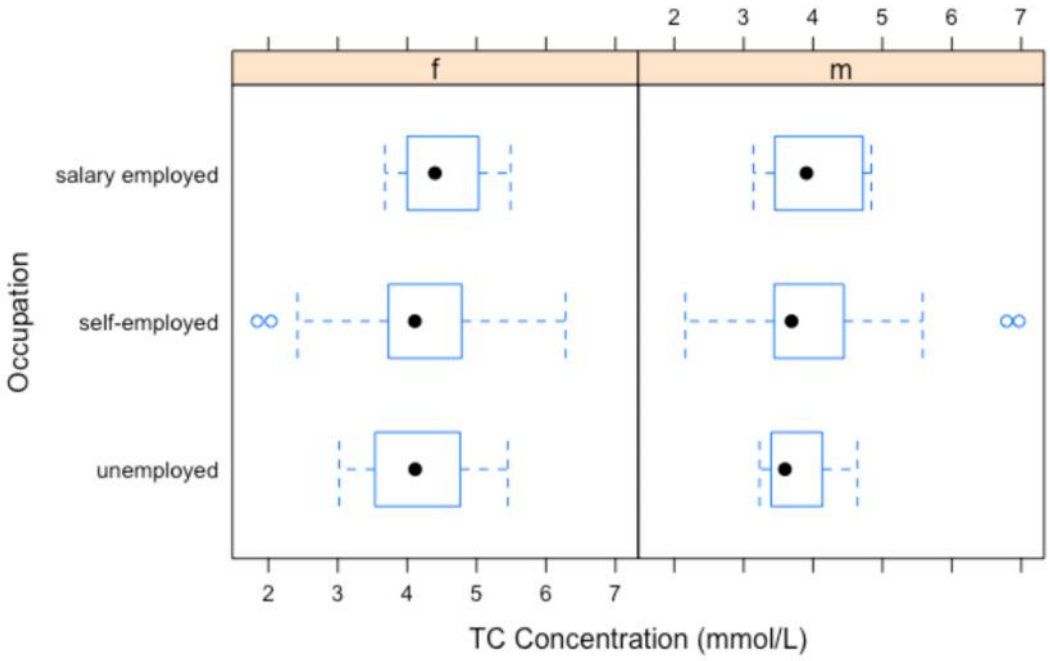

FIGURE 4.3.2 MEDIAN TC CONCENTRATION ACROSS DIFFERENT OCCUPA-

3.3.LDL- Cholesterol

The study found a mean LDL- Cholesterol level of 2.30 mmol/L (±0.57), median level of 2.22 mmol/L (range 1.04 to 4.21 mmol/L). Elevated HDL-C levels were found in 11 (9.2%) of the study participants who were classified as having increased risk.

3.4.HDL- Cholesterol

The HDL-C in this study ranged from range 1.84 to 6.97 mmol/L with a mean HDL- Cholesterol level of 4.15 mmol/L (±0.91) and median of 4.05 mmol/L. HDL-C was low in 17 (14.2%) of the study participants and they were classified as having increased risk.

3.5.Lp-PLA2

The mean value for Lp-PLA2 was found to be 172.01 ng/ml (±334.81) with a median of 52.52 ng/ml (range 0.3 to 1445.87 ng/ml). Subjects with an Elevated Lp-PLA2 values were 29 (24.2%).

3.6.MPO

The MPO levels in this study ranged from 45.02 to 603.56 ng/ml with a mean of 259.84 ng/ml (±191.65) and median of 208.94 ng/ml. Subjects with elevated MPO values were 35 (44.9%).

TABLE 4.3.1 ASSOCIATION BETWEEN HAART DURATION WITH GENDER AND AGE AT HOMA BAY COUNTY REFERRAL HOSPITAL, KENYA (N = 120).

	HAART Duration (Months) N (%)				
	6 - 35	**36-60**	**>60**	**χ 2**	***p* - Value**
Gender					
Female	11 (9.2)	12 (10)	54 (45)	1.108	0.575
Male	6 (5)	10 (8.3)	27 (22.5)		
Age (Years)					
18-23	2 (1.7)	2 (1.7)	3 (2.5)		
24-28	8 (6.7)	4 (3.3)	8 (6.7)		
29-33	3 (2.5)	6 (5)	17 (14.2)	20.944	0.007
34-38	2 (1.7)	7 (5.8)	24 (20)		
39-<45	2 (1.7)	3 (2.5)	29 (24.2)		

N - Number; **%** - percentage; **χ 2** - Chi Square Test; ***p*** - Value (Level of significance <0.05)
HAART - Highly active antiretroviral therapy

In a chi-square test, it was found out that the duration of HAART was not associated with gender (p = 0.575). However, there was a significant association between age and HAART duration (p = 0.007).

TABLE 4.3.2 ASSOCIATION OF LIPIDS, GLUCOSE, LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE 2 AND MYELOPEROXIDASE WITH GENDER, AGE AND HAART DURATION AT HOMA BAY COUNTY REFERRAL HOSPITAL, KENYA. (N = 120).

Variables	Laboratory Parameters					
	HbA1c ≥ 6.5% OR (95% CI)	TC ≥ 5.18 mmol/L OR (95% CI)	LDL-C ≥ 3.37 mmol/L OR (95% CI)	HDL-C < 1.0 mmol/L OR (95% CI)	Lp-PLA2 >167 ng/mL OR (95% CI)	*MPO > 229 ng/mL OR (95% CI)
Gender						
Female	0.49 (0.11 - 2.16)	1.21 (0.38 - 3.86)	0.65 (0.17 - 2.46)	1.73 (0.14 - 21.29)	1.26 (0.50 - 3.18)	1.12 (0.42 - 2.94)
p - value	0.343	0.752	0.531	0.669	0.632	0.837
Male	Ref	Ref	Ref	Ref	Ref	Ref
Age (Years)						
18 - 23	Ref	Ref	Ref	Ref	Ref	Ref
24 - 28	0.27 (0.03 - 1.05)	0.22 (0.01 - 4.23)	0.45 (0.03 - 1.02)	0.08 (0.02 - 1.01)	0.40 (0.06 - 2.60)	0.44 (0.03 - 5.82)
p - value	0.784	0.332	0.987	0.876	0.335	0.535
29 - 33	0.47 (0.03 - 6.77)	0.72 (0.06 - 8.18)	0.56 (0.05- 5.87)	0.12 (0.05 - 2.07)	0.324 (0.05 - 2.07)	0.21 (0.02 - 2.74)
p - value	0.578	0.079	0.632	0.785	0.233	0.236
34 - 38	0.63 (0.05 - 8.38)	1.46 (0.14 - 14.71)	0.25 (0.3 - 2.32)	2.45 (0.20 - 31.99)	0.22 (0.03 - 1.39)	0.23 (0.02 - 2.80)
p - value	0.727	0.075	0.222	0.482	0.108	0.429
39 - <45	0.33 (0.02 - 5.12)	1.57 (0.14 - 17. 29)	0.85 (0.20 - 3.64)	1.48 (0.04 - 14.56)	0.32 (0.05 - 1.97)	0.35 (0.03 - 4.48)
p - value	0.431	0.021	0.827	0.654	0.221	0.421
HAART Duration (months)						
6 - 35	Ref	Ref	Ref	Ref	Ref	Ref
36 - 60	0.41 (0.02 - 8.50)	1.41 (0.19 - 10.41)	0.23 (0.06 - 1.09	0.96 (0.41 - 6.01)	0.37 (0.06 - 2.45)	0.42 (0.08 - 2.15)
p - value	0.567	0.074	0.671	0.813	0.302	0.297
>60	0.80 (0.07 - 8.99)	1.62 (0.28 - 9.43)	0.92 (0.17 - 5.10)	2.30 (0.16 - 32.22)	1.65 (0.43 - 6.39)	0.34 (0.08 - 1.44)
p - value	0.857	0.045	0.93	0.536	0.047	0.143

HbA1c - Glycated heamoglobin; **TC** - Total cholesterol; **LDL-C** - Low density lipoprotein cholesterol; **HDL-C** - High density lipoprotein cholesterol; **Lp-PLA2** - Lipoprotein-associated phospholipase 2; **MPO** - Myeloperoxidase; * - had 78 subjects; **P**- p Value (Level of significance <0.05) **OR** - Odds ratio; **CI** - Confidence interval (95%); **HAART** - Highly active antiretroviral therapy

Using logistic regression analysis, there was no significance association between gender and raised laboratory parameters. The same was reported for the different age categories except for 39 - <45 years, which significant association with increased TC level; OR = 1.57 (CI: 0.14 – 17.29, p = 0.021). No significance association was found between raised biomarkers and HAART duration except for TC and Lp-PLA2 for HAART duration >60 months (OR = 1.62, CI: 0.28 – 9.43, p = 0.045 and OR = 1.65, CI: 0.43 – 6.39, p = 0.047 respectively).

5.DISCUSSION

The decrease in the mortality and morbidity of HIV infected individuals has been the outcome of the widespread use of HAART. However, the concern now is the association of HAART with the risk of cardiovascular disease. In this study, the levels of serum biomarkers (TC, LDL-C, HbA1c, Lp-PLA2 and MPO) for risk of cardiovascular disease in patients receiving highly active antiretroviral therapy in Homa Bay county referral hospital was investigated. Most of the participants (64.2%) were females giving a female to male ratio of 1.8:1, which is in keeping with the Kenya AIDS Indicator Survey (KAIS) findings [85], which was at 1.6:1. The ratio is also comparable to what Tadewos et al.[79] reported, which was 1.9:1. Majority of the study participants (28.3) were in the age brackets of 39 - <45 years. A proportion of 77% were married and 64.2% had attained primary education. Majority (65%) were in self-employment. Most of the subjects (67.5%) had been on HAART for more than sixty months. The study found out that this finding departs from that of Abebe et al., 2014[78], Addis Ababa, Ethiopia, which reported the highest proportion of subjects (59% of the 126 participants) had only been on HAART for 25 - 41 months.

In this study, majority of the participants had TC, LDL-C, HDL-C, HbA1c, Lp-PLA2 and MPO levels within the reference interval. For the lipid and glycated haemoglobin, the proportions with derangements in these biomarkers were as follows; 14.2% (TC), 5.8% (LDL-C), 2.5% (HDL-C) and 4.2% (HbA1c). These percentages were found to be incomparable from Tadewos et al., 2014[79], in Southern Ethiopia, who reported 31% (TC), 24% (LDL-C) and 27% (HDL-C). This discrepancy can be explained by other confounding factors in Tadewos et al.[79] study such as smoking, which was included in the study. They compared the lipid profiles for HAART and pre-HAART groups.

To the best knowledge of the researchers in this study, it is the first of its kind to assess HbA1c as a marker of hyperglycaemia in this study population. Most of the studies[80, 81] done in this population mainly focused on evaluating the serum levels of fasting blood glucose. In this study, cases of hyperglycaemia were at 4.2% in the 120 participants. This is comparable percentage with findings of Abebe et al.[78], Addis Ababa Ethiopia, which reported 7.9% in 126 study participants on HAART. However, there is a discrepancy between Mbunkah et al., 2014[82] findings, South-West, Cameroon, which reported hyperglycaemia of 26.5% in the 241 study subjects, and this study. A possible explanation to this inconsistency is the effect of age as a confounder, since the researchers included elderly participants of up to 70 years of age. Old age is a risk factor to developing insulin resistance and needs to be controlled either at the design stage or using a statistical analysis such as logistic regression model[82].

The present investigation being an exploratory study, is the first of its kind to the best of the investigators' knowledge to assess MPO and Lp-PLA2 in this study population. As reported by Mangili et al.[8,] and Ross et al.[7], these two biomarkers predict the early events of the development of cardiovascular diseases. Therefore, their inclusion in the study was to identify those that were at risk of developing cardiovascular disease, which the already established markers (TC, LDL-C, HDL-C and HbA1c) could have not captured. In this study, 24.2% of the participants had raised levels above the reference interval of PLA2 of the 120 participants that were studied. Whereas, out of the 78 subjects that were assessed for serum levels of MPO, 44.9% had elevated values. Because of the exploratory nature of this study, there were no previous investigation to compare the findings with.

Among the study participants, there was no significant association between elevated TC, LDL-C, HbA1c, Lp-PLA2 and MPO levels with gender. This was found to be inconsistent

for the lipid profile in a study conducted in Thailand by Luatngoen.[84] The difference can be explained by the fact that the participants were not gender-matched in this study.

The present study also investigated the association between different age categories with raised levels of TC, LDL-C, HbA1c, Lp-PLA2 and MPO. This was found to be insignificant except for TC level for 39 - <45 age category (OR = 1.57, CI: 0.14 – 17.29, p = 0.021). The finding was in agreement with Abebe et al.[78] study, which also reported a significant association between elevated TC with > 35 years (OR = 2.30, CI: 1.29 - 4.10, p = 0.005).

Abebe et al.[78] found an association between HAART use and raised serum levels of TC, HDL-C and LDL-C; OR = 2.99 (CI: 1.74 - 5.15), p<0.0001) and OR= 1.82 (CI: 1.06 - 1.12, p = 0.02) for TC. In this study, using HAART duration for 6 - 35 months as the point of reference (base), no significance association between elevated laboratory parameters and 36 – 60 months HAART durations was found. However, the study showed a significant association between raised TC and Lp-PLA2 levels to HAART duration of >60 months (OR = 1.62, CI: 0.28 – 9.43, p = 0.045 and OR = 1.65, CI: 0.43 – 6.39, p = 0.047 respectively) and this was consistent with the findings of Abebe et al.[78] However, for other component of lipid panel (LDL-C and HDL-C), there was no significant association with prolonged HAART duration. The findings of this study were consisted with that of Nsagha et al.,[87] which reported a significant independent association between HAART duration of 42 months and more with raised serum levels of TC (aOR = 2.26, 95 % CI: 1.16 – 4.42, p = 0.017).

6.CONCLUSIONS AND RECOMMENDATIONS

1.Introduction

This section details conclusion, recommendation and limitation of the study.

2.Conclusion

Majority of the participants who had at least one derangement in the laboratory parameters being abnormal had been on HAART for more than sixty months. Dysregulated concentrations of the serum biomarkers were not significantly associated to gender. Age was significantly associated to HAART duration. Serum concentrations of TC and Lp-PLA2 showed significant association between raised serum levels with the duration of HAART.

3.Recommendation

The researchers highly recommend that a validation study should be carried out to evaluate and confirm the findings in a cohort study to aid in testing the clinical utility of both MPO and Lp-PLA2. It is also recommended that HIV negative individuals should be included as controls as well as anthropometrics parameters for the assessment of long-term effect of HAART on well-controlled cohort conditions. Carrying out the study in different regions in Kenya is also recommended.

4.Limitations

- The absence of previous studies on metabolic abnormality in HIV-positive individuals on HAART in Homa Bay setting as well as lack of HIV negative and HAART-naïve as controls to make comparison with, were deemed as potential limitations to our study.
- MPO was done only for 78 subjects due to financial constraints.

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APPENDICES

APPENDIX I (a): Informed Consent Explanation and Form

Introduction and Research Objective

My name is Kenneth Weke a postgraduate student pursuing a Master of Science degree in clinical chemistry at the department of human pathology, University of Nairobi. I am carrying out a study to determine biomarkers for risk of cardiovascular disease (CVD) among HIV-positive individuals on highly active antiretroviral therapy (HAART) in Homa-Bay County Referral Hospital, Kenya using facilities at the Kenyatta National Hospital Biochemistry Laboratory.

Benefits and Risks of the Study to You

Potential Benefits:

You will benefit by having your serum markers for risk of CVD determined, which will guide the clinician on the best treatment strategy for managing your health.

Potential Risks

There will be a minimal risk and discomfort, which will be a slight pain from the blood-drawing site.

Procedure

If you agree to take part in the study, then you will be asked to fill a questionnaire, which will take a maximum of 30 minutes to complete. I will ask a number of questions, and I will note down the responses that you will give. I will then collect a sample of blood of a volume of 4ml, which will be taken to the laboratory for analysis for biomarkers for risk of cardio-vascular disease.

Confidentiality

Names will not be required in the study since you will be identified by a study number. The questionnaire will be kept in lockable cabinets, and I will be the only person to access it. Questionnaires will be kept for one year then destroyed. Any information given to us will remain confidential and will be for your benefit. You will get your results in the usual manner during your subsequent visit.

Withdrawal from Study

Your participation in this study is voluntary. You may refuse to participate or withdraw at any time without losing the benefits to which you are entitled in this clinic.

I ……. after reading and being explained the study purpose, do hereby give informed consent to participate in the study and I am fully aware of the benefits and risks. I have not been forced to take part in this study in any way. I understand that participation in this study is completely voluntary and that I may withdraw from it at any time and without loss of any benefit or quality of management to which I am entitled. I am fully aware that the results of this study will be used for scientific purposes and may be published.

Participant's Signature: …….……. Date……..

Investigator's Signature……...……Date ……..

Contact Information

If you have any question regarding the study, please contact:

1. Principal Investigator
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Mobile phone: +254733617678.

3. Ethical Concern
   The Secretary,
   KNH/UoN ERC,
   P.O Box20723, KNH, Nairobi
   Tel+254-020-2726300-9 Ext44355

APPENDIX I (b): Fomu Ya Idhini

Kuanzishwa na Lengo la Utafiti

Jina langu ni Kenneth Weke mwanafunzi wa chuo kikuu cha Nairobi idara ya pathologia ya wanadamu. Nafanya shahada ya uzamili katika somo ya sayansi uwanja wa klinikol kemia. Nataka kuchunguza kiwango cha viashiria vinavyoweza hatarisha kupata ugonjwa wa moyo na mishipa kwa wale ambao wanatumia madawa ya kupunguza idadi ya virusi vya ukimwi katika hospitali ya rufaa ya jimbo la Homa-Bay. Nitatumia vifaa vya uchambuzi vinavyo patikana kataki hospital ya kitaifa ya Kenyatta.

Faida na Madhara ya Utafiti huu Kwako

Faida

Utafaidika kwa kujua viwango vya viashiria ambavyo vinawezafanya upate ugonjwa wa moyo na mishipa na hii itaongoza daktari jinsi atakavyofanya kulinda afya yako.

Uwezekano wa Hatari

Kutakua na hatari ingawa kidogo ambayo itatokana na uchungu utahisi kwenye pahali damu utakaotolowe.

Utaratibu

Ukikubali kushiriki katika utafiti huu, basi nitakuuliza ujaze hojaji nitakayokupa na hii ita-chukua muda wa dakika kumi. Nitakuuliza maswali ambayo majibu yao nitaandika chini. Baada ya hayo nitatoa damu kiwango cha 4 ml kutoka mshipa kwenye mkono wako. Nitapeleka damu kwa maabara ili nichunguze kiwango cha viashiria vya kutaharishi mtu ku-pata ungonjwa wa moyo na mishipa.

Siri

Majina hayaitajiki katika utafiti na utatambuliwa na namba ya utafiti. Stakabadhi zilizo na maswali zitawekwa chini ya ufunguo na kufuli na mpelelezi mkuu ataweza kupata huduma hiyo. Maswali yatawekwa kwa mwaka mmoja kisha kuharibiwa. Taarifa yoyote utatupea itabaki siri na itakuwa kwa faida yako. Wewe utapata matokeo yako kwa njia ya kawaida wakati wa ziara yako ijayo.

Kujitoa kutoka kwa utafiti

Kushiriki katika utafiti huu itakuwa ni kwa hiari yako na ni sehemu ya tathmini yako mara kwa mara na unaweza kujiondoa wakati wowote bila kupoteza faida ambayo una haki katika taasisi hii.

FOMU YA IDHINI:

Mimi ..... baada ya kusoma na kuelezewa madhumuni ya utafiti huu hili kutoa ridhaa ya kushiriki katika uchunguzi kikamilifu na ufahamu wa faida na hatari. Si kushinikizwa kushiriki katika utafiti huu kwa njia yoyote. Mimi naelewa ya kwamba ushiriki katika utafiti huu ni kwa hiari yangu kabisa na kwamba naweza kujiondoa wakati wowote, bila hasara ya faida yoyote au ubora wa usimamizi. Mimi nimefahamu kikamilifu kwamba matokeo ya utafiti huu yatatumika kwa ajili ya madhumuni ya kisayansi na yanaweza kuchapishwa.

Sahihi ya mshiriki .... ……Tarehe ....

Sahihi ya mkuu wa uchunguzi ..... ….Tarehe ....

<u>Mawasiliano ya Habari</u>

Kama una swali lolote kuhusu utafiti tafadhali wasiliana na:

1. Mchunguzi

   Kenneth Weke, Chuo Kikuu cha Nairobi, S.L.P 19676-00,202 Nairobi

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2. Msimamizi

   Profesa Angela Amayo

   Chuo Kikuu cha Nairobi, S.L.P 19676-00,202 Nairobi

   Nambari ya simu +254733617678.

3. Maadili

   The Secretary, KNH/UoN ERC,

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   Tel+254-020-2726300-9 Ext44355

APPENDIX II (a): Study Questionnaire

Project Title: Serum Biomarkers for Risk of Cardiovascular Disease in Patients on Highly Active Antiretroviral Therapy in Homa-Bay County Referral Hospital, Kenya

Study Number…….

A. DEMOGRAPHIC DATA

1. Gender Male ☐ Female ☐

2. Age…… (Years).

3. What is your level of education?

   None ☐ Primary ☐ Secondary ☐ Post-secondary ☐

4. What is your marital status?

   Married ☐ Single ☐ Divorced ☐ Widowed ☐

5. What is your occupation?

   Salary employed ☐ Self-employed ☐ Unemployed ☐

B. MEDICAL HISTORY

1. Which year and month were you diagnosed with HIV?

   YEAR ☐ MONTH ☐

2. Which year and month were you put on HAART?

YEAR ☐ MONTH ☐

3. HAART duration

6-35 months ☐ 36-60 months ☐ >60 months ☐

C. LABORATORY: BIOCHEMICAL ANALYSIS

Serum biomarker	Result	Units	Score (N/H)
TC		mmol/L	
LDL-C		mmol/L	
HDL-C		mmol/L	
HbA1C		%	
MPO		U/ml	
Lp-PLA2		ng/ml	

D.

APPENDIX II (b): Screening Questionare- Exclusion Criteria

1. Age between 18 to <45 years YES ☐ NO ☐
2. History of myocardial infarction/stroke YES ☐ NO ☐
3. Are you on treatment for raised blood pressure? YES ☐ NO ☐
4. Are you on treatment for raised blood sugar? YES ☐ NO ☐
5. Are you on lipid lowering treatment? YES ☐ NO ☐
6. Do you smoke cigarette? YES ☐ NO ☐
7. HAART for less than 6 months? YES ☐ NO ☐

APPENDIX II (c): Utafiti Dodoso

Lengo la Utafiti: Uchunguzi wa kiwango cha viashiria vinavyoweza hatarisha kupata ugonjwa wa moyo na mishipa kwa wale ambao wanatumia madawa ya kupunguza idadi ya virusi vya ukimwi katika hospitali ya rufaa ya jimbo la Homa-Bay

Nambari ya usajili…….

Jaza kwa kuweka alama ya ✓ kwa jibu sahihi

I. MSHIRIKI

6. Jinsia Mme ☐ Mke ☐
7. Umri…….
8. Kiwango cha elimu

   Hakuna ☐ Mshingi ☐ Upili ☐ Zaidi ya upili ☐
9. Hali ya ndoa?

   Katika ndoa ☐ Binafsi ☐ Talaka ☐ Mjane ☐
10. Uvamizi?

    Ajiriwa ☐ Mwnabiashara ☐ kazi ☐

II. HISTORIA YA MATIBABU

4. Ni tarehe gani ulipimwa na ukapatwa na virusi vya ukimwi?

   MWAKA ☐ MWEZI ☐

5. Ni tarehe gani ulianzishwa kwa madawa ya kupunguza idadi ya virusi vya ukimwi?

   MWAKA ☐ MWEZ ☐

6. Muda wa kutumia madawa ya kupunguza idadi ya virusi vya ukimwi

   Miezi 6-35 ☐ Miezi 36-60 ☐ Miezi Zaidi ya 60 ☐

APPENDIX II (d): Uchunguzi dodoso

8. Umri wa 18 to <45 NDIO ☐ LA ☐
9. Historia ya ungonjwa wa moyo? NDIO ☐ LA ☐
10. Matibabu ya ungonjwa wa sukari? NDIO ☐ LA ☐
11. Matibabu ya shinikizo la damu? NDIO ☐ LA ☐
12. Matibabu ya kupunguza mafuta kwa mwili? NDIO ☐ LA ☐
13. Unavuta sigara? NDIO ☐ LA ☐
14. Matibabu ya HAART chini ya miezi 6? NDIO ☐ LA ☐

APPENDIX III: Laboratory Methods/Principles

MPO (Bio-Techne Europe Ltd.)

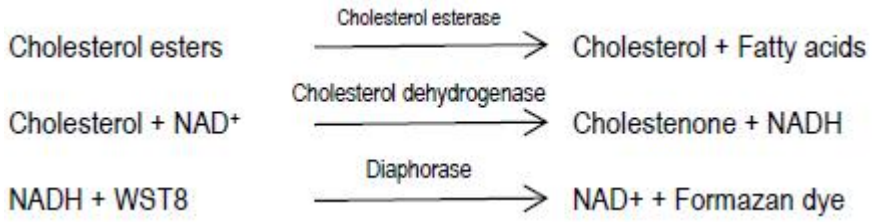
This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human MPO has been pre-coated onto a microplate. Standards and samples are pipetted into the wells, and any MPO present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human MPO is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a MPO bound in the initial step. The colour development is stopped and the intensity of the colour is measured.

LP-PLA2 (Bio-Techne Europe Ltd.)

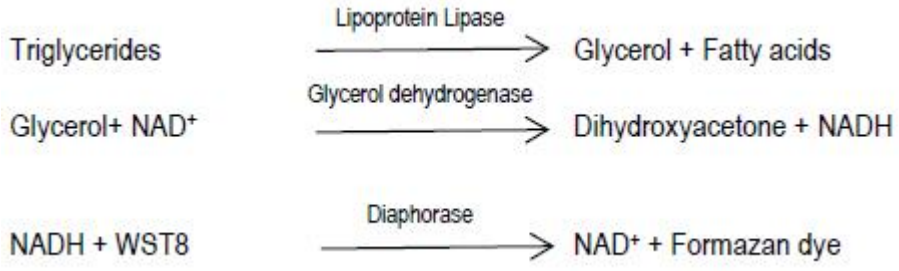
This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human PLA2G7 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells, and any PLA2G7 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human PLA2G7 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of PLA2G7 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIPID PANEL (Roche Cobas-cobas.com)

The erythrocytes of the capillary or venous blood sample are separated from the plasma by centrifugation. In the next step, the plasma sample is diluted with phosphate buffer. The HDL test uses a precipitation method with Mg2+ and phosphotungstic acid as a precipitant reagent. The components except for HDL-cholesterol are precipitated and removed. The Cobas system determines total cholesterol and HDL-cholesterol by an enzymatic method. Cholesterol esters in the sample are hydrolyzed to cholesterol and fatty acids. Cholesterol and NAD+ generate cholestenone and NADH in the presence of cholesterol dehydrogenase. WST8 is reduced to formazan dye by diaphorase and NADH through an oxidation-reduction reaction. The color intensity of formazan is measured at a specific wavelength of 460 nm and is directly proportional to the concentration of HDL-cholesterol and total cholesterol in the sample.



The triglycerides test is an enzymatic method. Triglycerides in the sample are hydrolyzed to glycerol and fatty acids by lipoprotein lipase. Glycerol and NAD+ generate dihydroxyacetone and NADH in the presence of glycerol dehydrogenase. WST8 is reduced to formazan dye by diaphorase and NADH through an oxidation-reduction reaction. The color intensity of the formazan is proportional to triglyceride concentration and calculated by measuring at a wavelength of 460 nm.



Low-density lipoprotein (calculated)

Where the concentration of triglycerides is < 400 mg/dL (4.52 mmol/L), the LDL cholesterol is calculated using the Friedewald formula.

LDL = TC - HDL – TG/5 (measured in mg/dL).8 Where the concentration of triglycerides is ≥ 400 mg/dL (4.52 mmol/L), the calculated LDL-cholesterol is not reported. The formula is also not valid for non-fasting patients and patients with Type III hyperlipoproteinemia (dysbetalipoproteinemia).

Total Cholesterol/HDL ratio and Non-high-density lipoprotein

The Cobas b 101 instrument calculates the TC/HDL ratio as well as the non-HDL cholesterol (TC - HDL) from the measured values. Where the measured values data are not available, the TC/HDL ratio or non-HDL-cholesterol values are not reported.

HBA1C (*CLOVER A1C* ™)

The *CLOVER A1c*™ *Self* system is a fully automated boronate affinity assay for the determination of the percentage of Hemoglobin A1c (HbA1c %) in whole blood(capillary and venous). The Test Cartridge is composed of a cartridge and a reagent pack containing the reagents necessary for the determination of hemoglobin A1c. A cartridge should be inserted into the analyzer cartridge holder first and later assembled with a reagent pack which was applied with 4ul blood sample at the sample collecting area. A reagent pack is composed of reaction solution and washing solution. The reaction solution contains hemolyzing agent which lyses the red blood cells, and boronate resin which binds to the cis-diols of glycated hemoglobin. The washing solution cleans the nonspecific glycated haemoglobin (except HbA1c)

The blood sample (4uL) is collected at the collection area of the reagent pack; then the reagent pack is inserted into the cartridge, where the blood is instantly lysed releasing the hemoglobin and the boronate resin binding the glycated hemoglobin. The assembled cartridge is inserted into the *CLOVER A1c™ Self* Analyzer and rotated so that the sample mixture is placed in the measurement zone of the cartridge, where the amount of total hemoglobin in the blood sample is photometrically the diffused reflectance of the optical sensor composed of LED (Light Emitting Diode) andPD (PhotoDiode)

Then, the assembled cartridge is rotated so that the washing solution washes outnonglycatedhemoglobin from the blood sample, thus the amount of glycatedhaemoglobincan be photometrically measured. The ratio of glycated Hemoglobin with respect to total hemoglobin in the blood sample is calculated.

$$\text{HbA1c\%} = A \times \left[\frac{\text{HbA1c}}{\text{Total Hemoglobin}} \times 100 \right] + B$$

Where ‘HbA1c’ and ‘Total Hemoglobin’ are the signal obtained from the *CLOVER A1c™ Self*-system, ‘A’ and ‘B’ are the slope and intercept factor to correct the value for DCCT (Diabetes Control and Complication Trial) calibration.

APPENDIX IV: PROJECT WORK PLAN AND BUDGET MATRIX

Project Title:Serum Biomarkers for Risk of Cardiovascular Disease in Patients on Highly Active Antiretroviral Therapy in Homa-Bay County Referral Hospital, Kenya

Sample Size: 120

Activity	**Date**	**Person/s Responsible**	**Item/s**	**Cost (KES)**	**Budget Source**
Journey to Homa-Bay	3/5/2017	PI	Fare	1,000	PI
Journey back to Nairobi	4/5/2017	PI	Fare	1,000	PI
Paper work	5/5/2017	PI	Stationery, printing, photocopy	5,000	PI
Purchase of sample collection products	5/5/2017	PI	a) Vacutainer 2 @ 800 per 100 box	1,600	PI
			b) Microvial 2 @ 800 per 100 box	1,600	
			c) Syringes and needles3 @ 200 per 100 box	600	

			d) Pipette tips 2 @ 1000 per 100 box	2,000	
			e) 1 litre spirit	1,000	
			f) 1 roll cotton wool	500	
			g) Gloves 4 @ 500	2,000	
Purchase of HbA_{1C} Kit	8/5/2017	PI	HbA_{1C} kit	90,000	PI
sJourney to Homa-Bay for data collection	12/5/2017	PI	Fare	1,000	PI
Data collection	15/5/2017 to 26/5/2017	PI and Research assistant	Assistance fee	20,000	PI
Sample	29/5/2017	PI and courier offi-	a. Shipping fee	1,000	PI

transportation from Homa-Bay to KNH		cials	b. Dry ice box	2,000	PI
Ordering of ELISA kits + TC, LDL-C, HDL-C reagents	15/5/2017	PI	MPO ELISA Kit 1@45,000	45,000	Well-wisher/s
			Lp-PLA2 ELISA kit 2@45,000	90,000	
			TC, LDL-C, HDL-C	40,000	PI
Clearing of the Consignment at the Airport	12 June, 2017	PI	Port and duties charges	55,000	PI
Sample analysis	23/6/2017 to 26/6/2017	PI and Lab technologist/scientist	Nil	Nil	Nil

Data analysis	5/7/2017 to 7/7/2017	PI	Nil	Nil	Nil
Presentation of results	12/7/2017	PI + audience (academic staff and postgraduate students)	Nil	Nil	Nil
Manuscript submission	28/7/2017	PI	Publication fee	10,000	PI
Compiling the book and printing for external examination	14/7/2017	PI and UoN printing office	Book printing and binding fee 5 copies @2,000	10,000	PI
TOTAL				**415,300**	

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Ref: KNH-ERC/A/101

23rd March 2017

Kenneth Omondi Weke
Reg. No. H58/83086/2015
Dept.of Human Pathology
School of Medicine
College of Health Sciences
University of Nairobi

Dear Kenneth

REVISED RESEARCH PROPOSAL: SERUM BIOMARKERS FOR RISK OF CARDIOVASCULAR DISEASE IN PATIENTS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY IN HOMA-BAY COUNTY REFERRAL HOSPITAL, KENYA (P932/12/2016)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above revised proposal. The approval period is from 23rd March 2017 – 22nd March 2018.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

"Protect to Discover"

Yours sincerely,

PROF M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Assistant Director, Health Information, KNH
The Chair, KNH-UoN ERC
The Dean, School of Medicine,UoN
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