Cardiovascular Disease among HIV-positive and HIV-negative Kenyan Adults

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A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2020

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Program Authorized to Offer Degree:

Global Health

University of Washington

Abstract

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Over half of the people living with HIV (PLHIV) reside in Eastern and Southern Africa where cardiovascular related mortality has continued to increase in the past decade. Data from high income countries estimates that PLHIV have shown an estimated 2-fold increased risk of cardiovascular disease (CVD) compared to HIV-negative individuals. This may be the result of traditional risk factors, including smoking, hypertension, unhealthy diets and lack of physical activity. It may also be as result of inflammation due to HIV or the use of antiretroviral therapy (ART) that has been associated with dyslipidemia, insulin resistance, and glucose intolerance, which frequently manifest as metabolic syndrome (MetS), a significant predictor of CVD. Despite increasing concerns of elevated risk of CVD among PLHIV, there is limited data on CVD risk factors, metabolic syndrome, the role of inflammation and the cost of CVD screening in sub-Saharan Africa (SSA). Data comparing people living with and without HIV infection are especially

limited. To fill the knowledge gap, we conducted a study that compares cardiovascular risk among HIV-positive and HIV-negative Kenyan adults in Western Kenya. This dissertation compares the burden of cardiovascular disease risk factors among HIV-positive and HIV negative adults, examines the relationship between inflammation and these risk factors, and determines the cost of introducing CVD screening in health settings in Kenya.

The first aim was to determine the prevalence and predictors of MetS and its individual components (hypertension, diabetes, elevated triglycerides, low HDL cholesterol, and abdominal obesity) and to compare risk profiles using the atherosclerotic CVD (ASCVD) risk score among 300 HIV-positive and 300 HIV-negative Kenyan adults in Western Kenya. We hypothesized that HIV-positive adults would have a higher prevalence of MetS and a higher ASCVD risk profile compared to HIV-negative individuals. The second aim was to compare immune activation and inflammatory markers that have been associated with increased CVD risk among HIV-positive and HIV-negative Kenyan adults in Western Kenya. We hypothesized that inflammatory markers, including interleukin-1 beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and high-sensitivity C-reactive protein (hsCRP) would be higher among HIV-positive individuals when compared to HIV-negative individuals. The third aim was to estimate the incremental costs of integrating cardiovascular disease screening into on-going outpatient and HIV services in Kisumu County Hospital in Kenya.

In the first aim, we conducted a cross-sectional study among adults ≥30 years of age with and without HIV infection seeking care at Kisumu County Hospital. Participants completed a health questionnaire and vital signs, anthropomorphic measurements, and blood for fasting blood glucose and lipids were obtained. MetS was defined using 2009 Consensus Criteria and the 10-year Atherosclerotic CVD (ASCVD) risk score was calculated using the Pooled Cohort Equation as

outlined in the 2019 American College of Cardiology (ACC) / American Heart Association (AHA) Guideline on the Primary Prevention of Cardiovascular Disease. MetS prevalence was high in both HIV-positive and HIV-negative adults in western Kenya (1 in 20 and 1 in 10, respectively). Importantly, PLHIV were less likely to have MetS and had lower ASCVD risk scores than HIV-negative participants.

In the second aim, using a multiplex immunoassay, we measured IL-1β, IL-6, TNF-α, and hsCRP concentrations among the same participants. We found higher levels of inflammatory markers (IL-1β, IL-6, TNF-α, and hsCRP) among PLHIV compared to HIV-negative participants. The majority of PLHIV were virally suppressed and higher concentrations among PLHIV persisted even after adjusting for traditional risk CVD factors, including dyslipidemia, obesity, diabetes and smoking.

In the third aim, using a societal perspective, we conducted a micro costing study using time-in-motion measurements and semi-structured questionnaires to assess the direct medical costs, direct non-medical costs and indirect costs of CVD screening. The incremental cost of CVD screening for diabetes, hypertension and dyslipidemia was estimated at $35 per person and this was reduced by 40% to $21 if we adopted a high-risk screening scenario. Laboratory supplies were the main cost driver, accounting for 74% of this cost.

Together these findings emphasize the need for CVD risk assessment among people living with and without HIV infection and the importance of further longitudinal studies to determine those risk factors that are most predictive of CVD events. We found CVD screening to be feasible, but the cost could be further reduced through global negotiations for more affordable laboratory testing options. We anticipate that this dissertation work will inform future studies of CVD among PLHIV and in pushing forward the policy initiatives on CVD screening in countries of sub-Saharan Africa.

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ACKNOWLEDGEMENTS

First, I would like to express my sincere gratitude to my dissertation committee led by the Chair, Carey Farquhar, and members, Stephanie Page, Carol Levin and James Hughes, for their mentorship, instruction, support and encouragement during my doctoral studies. I am especially grateful to my chair, Carey Farquhar for providing opportunities to conduct research and grow as a research scientist and for her career and life advice. I would like to thank the faculty and staff at the School of Public Health especially the Department of Global Health for their quality instruction and dedication to my success in this doctoral program. I would also like to thank David Katz (UW-WHO HIV testing guidelines review project) and Rachel Nugent (RTI International) for providing me with opportunities outside the University of Washington that directly impacted HIV and non-communicable diseases policy both locally in my home country Kenya and globally.

I would like to acknowledge the time and efforts of the CVD study team in Kenya including Jerusha Nyabiage, Sellah Owino, Dinah Nasimiyu, Irene Mboya, Michael Kitoi, the Kisumu County Hospital staff, KEMRI staff, UW-Kenya administrative staff and the coinvestigators for this study. I am especially thankful to each of the study participants without whom this research would not have been possible.

I am grateful for the funding for this study by the National Institutes of Health (R21TW010459) and the Fogarty International Center (D43 TW009580) who provided funding for my training through the International AIDS and Research Training Program (IARTP), without which my graduate training and this research would not have been possible.

Special thanks go to my UW peers, family and friends in Kenya and Seattle for your encouragement and walking this journey with me.

The highest praise goes to God for giving me life, strength and purpose throughout my training. I acknowledge God as my source, and he is Ebenezer.

DEDICATION

I dedicate this dissertation to my late mother Jane. She showed me how to be strong and thrive even in adversity. She taught me to always try and never give up trying. She nurtured me in the way of the Lord and ensured I had the opportunity to succeed academically.

To my daughter Esther who watched me through this doctoral program. Supporting me in every way and her discipline to allow me to study. She is my cheerleader and my entertainer making me laugh, which I needed many times.

I dedicate this to all those who have been affected by hypertension, diabetes or cardiovascular disease, including my parents, siblings and all who have struggled to access affordable care and treatment for the same. I am committed to advocating for cardiovascular disease screening and care and will champion for affordable health care for all.

To my dad, Jonathan, my husband Jonathan, my brother Peter and sisters Winnie, Christine and Caroline for all your support and the sacrifices you have made for me to emerge as I am today.

CHAPTER 1. Introduction

1. Cardiovascular disease and HIV burden: Cardiovascular disease (CVD) is a leading cause of mortality and morbidity globally with over 17 million deaths reported in 2016.1 Over 1 million deaths were attributable to CVD in SSA, constituting 6% of all global CVD-related deaths. 1 In contrast to marked reductions observed in high-income countries, age standardized death rates from CVD have remained stable, and perhaps increased, in sub-Saharan Africa (SSA) since 1990.[1,2] This may be attributable to the high HIV burden in the region, with over half of the people living with HIV (PLHIV) in the world residing in East and Southern Africa.[3] The impact of increasing morbidity and mortality is likely to be greater now that PLHIV are living longer, thus placing them at high risk of metabolic diseases and cardiovascular disease[4].

Several studies that compared HIV-positive and HIV-negative patients in US populations, have shown an increased incidence of myocardial infarction and stroke in HIV patients ranging from 1.4 to 6.76 fold increase.[5-8] These studies also showed an increase in the prevalence of traditional risk factors such as hypertension, diabetes mellitus, and dyslipidemia. However, recent studies in SSA provide conflicting evidence of similar or decreased risk of CVD risk in PLHIV as compared to the general population.[9,10] These findings could be explained by a large proportion of PLHIV having a lower prevalence of traditional risk factors such as hypertension and obesity. While there is evidence that CVD appears to be more common in the setting of HIV infection, much of this data is derived from high-income countries with paucity of data in SSA.[11-16] In addition, where data exists, most of these studies concentrated on either the general population or PLHIV separately.[17] A few studies that occurred before immediate ART initiation, compared ART-naïve and ART-experienced PLHIV and very few compare HIV-positive and negative individuals.[18-22]

2. Pathophysiology of CVD in HIV

Evidence from high-income countries suggest that HIV-positive individuals, whether treated or untreated, have significantly greater risk of CVD compared to individuals without HIV.[23,24] Several studies have shown an increased rate of myocardial infarction and stroke in PLHIV.[14,25] This is multifactorial and may be due to inflammation related to HIV infection itself, the use of antiretroviral therapy (ART), or higher prevalence of traditional risk factors in this population, including high systolic blood pressure, high plasma glucose and high body mass index (BMI).[26,27] [18,28-32]

Alcohol consumption of three or more drinks per day raises blood pressure, one of the most important cardiovascular risk factors, and alcohol-associated HTN is a common reversible form of elevated blood pressure.[33] It increases triglyceride levels in a dose-dependent manner and increases caloric intake that may result in obesity.[33] Cigarette smoking also increases triglyceride levels, inflammation, blood pressure and atherosclerosis.[34]

Chronic HIV is considered a state of persistent inflammation. There is evidence that increased CVD risk persists, even after controlling for traditional risk factors in PLHIV, suggesting that inflammation may be a contributing factor.[35] In an untreated HIV patient, there is a generalized immune activation involving both non-HIV specific and HIV specific immune responses. In treated individuals, ART reduces but does not eliminate inflammation as seen by higher interleukin-6 (IL-6), high sensitivity C-reactive protein (hs-CRP), D-dimer, sCD163 and soluble CD14 (sCD14) compared to HIV-negative individuals.[35] Earlier ART initiation at higher CD4 counts may further reduce immune activation markers such as sCD163, activated-T-cells, IL-6 and hs-CRP.[35,36] These markers have been associated with myocardial infarction and stroke. IL-6 and hs-CRP are independent predictors of CVD in the general population as well as in PLHIV.[37]

HIV infection and ART have been associated with adipose tissue changes and disorders of glucose and lipid metabolism leading to increased CVD risk.[27,30] ART has been associated with increased risk of myocardial infarction which may be linked to metabolic effects such as dyslipidemia, insulin resistance and endothelial

dysfunction.[38] Specific ART that have been associated with increased risk of myocardial infarction include indinavir, lopinavir-ritonavir, didanosine, and abacavir.[38] Newer classes of ART including selected entry inhibitors (Maraviroc), integrase inhibitors (Raltegravir), newer protease inhibitors (Atazanavir, Darunavir) have less cardiovascular effects.[39]

3. Impact of CVD on health systems and economic development: The impact of CVD mortality and morbidity in resource limited countries places an increased burden in the health system. Kenya is one of the lower middle-income countries where rising non-communicable disease (NCD) rates have become a particularly challenging health problem, accounting for 30% of the total burden of disease in terms of Disability-Adjusted Life Years (DALYs) lost.[4,40] CVD accounts for 11% and 5.1% of hospital admissions of deaths in Kenya.[5] Two hospital based studies in Kenya attributed CVD as the cause of death in 13% of cases and 5.1% of hospital admissions with the main contributors being myocardial infarction and cardiomyopathy.[41,42] Because NCDs, including CVD, disproportionately affect working age adults, this rise in NCD related morbidity and mortality has particularly significant implications in economic growth for Kenya and other LMICs.[43] The higher burden of disease results in economic productivity losses because of presenteeism and abstenteeism.[44] In addition, the increase in people living with CVD and its complications will also lead to substantial challenges in health services, human resources, and health financing. By investing in CVD screening and treatment, some of these impacts can be averted. The Kenya investment case for NCDs reports that investing in CVD screening and treatment would avert up to 89,000 deaths through avoiding CVD events and management of risk factors such as hypertension and elevated cholesterol. [44]

CVD can be prevented by routine CVD screening and by addressing modifiable behavioral risk factors, including smoking, diet and exercise, as well as introducing novel and cost effective strategies to predict early CVD risk in resource limited settings.[45,46] The Disease Control Priorities, 3rd edition showed that population-level interventions to address the risk factors for CVD appear to have favorable cost-effectiveness ratios with diet and tobacco interventions being most cost effective.[47] Prevention of CVD was

also found to be less expensive than treatment.[47,48] However, very few studies have conducted costing and cost effectiveness studies in LMICs.[48] These are needed to provide policy makers evidence on the costs of CVD prevention programs and their potential impact.

4. Impact on policy

As countries strive to achieve the 3rd Sustainable Development Goal (SDG) to reduce premature mortality of non-communicable disease, including CVD by 2030, we need to gain a deeper understanding of the burden of CVD and CVD risk factors in SSA countries, as well as health system barriers that need to be addressed.[49] This dissertation provides much needed information on the burden of CVD risk factors among HIV-positive and HIV-negative adults and the cost of integrating CVD screening in health settings in Kenya.

CHAPTER 2. Metabolic syndrome and 10-year cardiovascular risk profile

Abstract

Introduction

To determine the prevalence and correlates of metabolic syndrome (MetS) and compare 10-year cardiovascular disease (CVD) risk among Kenyan adults with and without HIV infection.

Methods

We conducted a cross-sectional study among adults ≥30 years of age with and without HIV infection seeking care at Kisumu County Hospital. Participants completed a health questionnaire and vital signs, anthropomorphic measurements, and fasting blood were obtained. MetS was defined using 2009 Consensus Criteria and 10-year Atherosclerotic CVD (ASCVD) risk score was calculated. Chi-square, independent t-tests, Wilcoxon rank sum test and multivariable logistic regression were used to determine differences and associations between HIV and MetS, CVD risk factors and ASCVD risk score.

Results

300 people living with HIV (PLHIV) and 298 HIV-negative participants with median age 44 years were enrolled, 50% of whom were female. The prevalence of MetS was 8.9% overall, but lower among PLHIV than HIV-negative participants (6.3% vs 11.6%, respectively; p=.001). The most prevalent MetS components were elevated blood pressure, decreased high density lipoprotein, and abdominal obesity. Adjusting for covariates, PLHIV were 66% less likely to have MetS compared to HIV-negative participants (adjusted odds ratio [aOR] 0.34; 95% confidence interval [95%CI] 0.18, 0.65; p=.005). Median ASCVD risk score was also lower among PLHIV compared to HIV-negative participants (1.7% vs 3.0%, p=.002).

Conclusions

MetS prevalence was high overall, but was more common among HIV-negative than HIV-positive adults. In addition, HIV-negative adults were at greater risk for CVD as measured by the atherosclerotic CVD risk score as compared to PLHIV. These data support integration of routine CVD screening and management into health programs for adult populations in resource-limited settings, regardless of HIV status.

Introduction

Globally, 38 million people were living with HIV (PLHIV) in 2018, with over half residing in Eastern and Southern Africa.[50] One of the greatest successes in HIV has been the introduction of highly effective antiretroviral therapy (ART) which has resulted in a decrease in opportunistic infections, a decline in HIV-related mortality and increased survival among PLHIV. [36,51,52] The life expectancy of PLHIV who are virally suppressed on ART is nearly that of those who are HIV-negative.[53] Consequently, an estimated 16% of PLHIV were aged 50 and above in 2016 and is projected to increase to 21% by 2020.[54] In 2016 approximately 80% of PLHIV over the age of 50 lived in low-and middle-income countries and sub-Saharan Africa (SSA) will continue to bear the greatest burden of aging PLHIV in the future.[54] As PLHIV live longer, risk of chronic non-communicable diseases such as cardiovascular disease (CVD) increases, posing new challenges to health care systems.[55,56] In several studies in the US and Europe, PLHIV had up to a two-fold increased risk of incident atherosclerotic CVD (ASCVD) compared to those without HIV infection.[14,15,57-61] Based on this, HIV may account for nearly 25% of the CVD burden in parts of SSA, the highest population attributable fraction globally.[57]

HIV has been associated with increased risk of traditional CVD risk factors such as smoking, harmful alcohol use, reduced physical activity, unhealthy diets, hypertension and diabetes in high-income countries.[62-66] In addition to these traditional risk factors, PLHIV may be at increased CVD risk due to HIV-related inflammation and immune activation, or due to use of ART-induced dyslipidemia, insulin resistance, and glucose intolerance, especially protease inhibitors (PI).[35,58-60] Metabolic syndrome (MetS), is defined as a cluster of CVD risk factors including raised blood pressure, dyslipidemia (raised triglycerides and lowered high-density lipoprotein cholesterol [HDL-C]), hyperglycemia, and central obesity.[67] Individuals with MetS are at approximately two-fold increased risk of CVD compared to their controls.[68,69] While some studies in SSA have demonstrated increased diagnosis of MetS among PLHIV, [70-73] results have been inconsistent. [74]

Since MetS is a significant predictor of CVD, it can be used to identify patients who may benefit from interventions to reduce CVD risk.[75-79] Risk scores can also be used to predict future CVD events. The Atherosclerotic CVD (ASCVD) risk score, is a multivariable race and sex-specific risk factor algorithm incorporating age, total and HDL-C, systolic blood pressure, current smoking status and history of diabetes and antihypertensive treatment.[80] The ASCVD risk score is a well validated tool, incorporating longitudinal data from across ethnic groups and continents.[81]

Despite increasing concerns of elevated risk of CVD among PLHIV, there is limited data on CVD risk factors, MetS and CVD risk scores in SSA, a region with considerable HIV burden. We sought to define the prevalence and correlates of MetS, and its individual components, and to compare the 10- year ASCVD risk among Kenyan adults with and without HIV infection. Based upon data from Western countries, we hypothesized that PLHIV would have a higher prevalence of MetS and a higher ASCVD risk score as compared to HIV-negative individuals.

Methods

Study design and setting

Between September 2017 and May 2018, we conducted a cross-sectional study among HIV-positive and HIV-negative women and men from Kisumu County Hospital, a tertiary, public county referral facility located in Western Kenya where HIV prevalence is high at 16.3%.[82] CVD risk assessment is not part of the routine screening among PLHIV and HIV-negative individuals at Kisumu County Hospital.

Study procedures

Eligible participants had to be at least 30 years of age and live within a 50km radius of the hospital. In addition, PLHIV had to be engaged in care at the HIV Comprehensive Care Clinic (CCC) and taking ART for at least 6 months. PLHIV who met the inclusion criteria and provided consent were consecutively enrolled by a study nurse from the CCC while HIV-negative participants were recruited from the HIV

testing points until the sample size was reached. Human subject approval was obtained from the University of Washington Institutional Review Board and locally from the Kenyatta National Hospital (KNH)/University of Nairobi (UoN) Ethical and Scientific Review Committee. All participants provided written informed consent prior to any study procedures or data collection.

Clinical procedures: Using tablets, study nurse counselors interviewed all participants at the time of enrollment to collect data on socio-demographics, HIV disease status if HIV-positive, and CVD risk factors using the validated World Health Organization (WHO) STEPwise approach to chronic disease risk factor surveillance (STEPS) questionnaires modified to fit the Kenyan context.[83] Waist and hip circumference, weight and height were measured to determine body mass index (BMI) and waist hip ratio. Two blood pressure readings on each arm and pulse were measured and averaged. Participants were asked to return the following day after fasting 8 hours for blood draw if not already fasting.

Laboratory procedures: Fasting blood samples were collected for quantification of lipids (total cholesterol, HDL-C, low density lipoprotein cholesterol [LDL-C], triglycerides) and glucose. All samples were processed to serum, frozen and stored at the Kenya Medical Research Institute (KEMRI) Lab at -80°C. Serum lipids and glucose tests were performed at the University of Washington Research Testing Services using an automated Beckman Coulter AU5812. CD4 count and viral load testing was performed at the KEMRI laboratory in Kisumu.

Primary outcomes and dependent variables

The primary outcomes were MetS and ASCVD risk score. MetS was defined by the Consensus Criteria 2009 as any three of the following: 1) abdominal obesity (waist circumference of >88cm for women and >94cm for men); 2) triglycerides ≥150mg/dL; 3) HDL-C <50mg/dL for women and <40mg/dL for men; 4) blood pressure >130/85mmHg; 5) fasting plasma glucose ≥ 100mg/dL.[67]

For each participant without prior history of ASCVD (myocardial infarction or stroke), we calculated their 10-year ASCVD risk score using the Pooled Cohort Equation as outlined in the 2019 American College of Cardiology (ACC) / American Heart Association (AHA) Guideline on the Primary Prevention of Cardiovascular Disease.[80] We used the race and sex specific ASCVD risk score which includes sex, age, race, total cholesterol, HDL-C, systolic blood pressure and history of smoking, diabetes and treatment for hypertension. 10-year CVD risk was classified as low (<5%), borderline (5 to <7.5%), intermediate (7.5 to <20%) or high (≥20%). Subjects were considered to be at elevated risk if their predicted risk was ≥7.5%.[81]

Statistical analysis

We summarized continuous variables using mean and standard deviation for normally distributed variables and median and inter-quartile range (IQR) for non-normally distributed variables. We tested for differences in patient baseline characteristics using chi-square tests for categorical subgroups and a 2-group independent means t-test for continuous variables. We calculated the prevalence of MetS and its individual components, and determined ASCVD 10-year risk using the Pooled Cohort Equations for participants without prior atherosclerotic CVD. We used multivariable logistic regression to estimate the association between HIV and MetS and the CVD risk factors. Unadjusted and adjusted models were fit to evaluate associations with or without potential confounders including age, sex, education, current smoking, alcohol use, healthy diet (at least 5 daily servings of fruits and vegetables) and exercise (at least 150 minutes/week of moderate activity or 75 minutes/week of vigorous physical activity as per WHO recommendations). Differences in the ASCVD risk score by HIV status were assessed by the chi-square test and Wilcoxon rank sum test. We also calculated 95% confidence intervals (95%CI) and used a significance (α) level of .05. All analyses were conducted using Stata version 14.0 (StataCorp, College Station, TX).

Results

Baseline Characteristics

Between September 2017 and May 2018, we screened 610 participants, of whom 600 were eligible and participated. We excluded two participants whose data was not available during analysis. The study enrolled 300 PLHIV and 298 HIV-negative participants, with similar numbers of males and females in each group (Table 1.1). Fasting blood samples were available for 564 (94%) of the 598 participants. Median age was 45 years (IQR 39.5, 53.0) and 40 years (IQR 31, 55) for the PLHIV and HIV-negative participants, respectively. The majority of participants were married (74%) and employed (77%). Compared to the HIV-negative group, PLHIV were older (p<.001), less educated (p<.001), and more likely to be separated, divorced or widowed (p=0.002) (Table 1.1).

Among PLHIV, the median duration on ART was 8 years (IQR 4, 10) with a median CD4 count of 512 cells/mm^3 (IQR 364, 666) (Table 1.1), and 96% were virally suppressed (viral load <1000 copies/ ml), with 79% having a viral load <50 copies/ml (lower detection limit), which is considered undetectable by Kenyan national guidelines.[84] PLHIV were mostly on first line therapy (87%) (non-protease inhibitor [PI] regimen) with only 13% (39/300) on a second line PI-based regimen.

Out of the 598 participants, 71 (12%) self-reported prior history of hypertension (Table 1.2) with only 26 (37%) being on treatment. There were 10 participants who self-reported diabetes and only 6 (60%) of them were on oral hypoglycemic agents or insulin treatment. Eight out of the 10 participants who had history of stroke and heart attack were PLHIV.

Traditional anthropomorphic and behavioral risk factors

A third of all participants were overweight or obese with higher prevalence of obesity among the HIV-negative participants compared to PLHIV (42 vs 27%, respectively; p<.001). Abdominal obesity (assigned by waist circumference parameters) was also significantly higher among HIV-negative participants as

compared to PLHIV (p<.001). Only 5% of participants smoked in the past 30 days (Table 1.2), and there was no significant difference in smoking prevalence by HIV status. Alcohol consumption was also low across the entire cohort (18%) but was significantly higher among the HIV-negative participants (p=.010) (Table 1.2).

Prevalence and association of MetS and its individual components and HIV

MetS has 5 components and having 3 and more results in a diagnosis of MetS. Nearly 50% of participants presented with 1 or 2 components of MetS, while 50 participants (9%) had 3 or more individual components and were diagnosed with MetS (Figure 1.1). Of these 50, 42 (84%) presented with 3 components, 5 (10%) with 4, and 3 (6%) with all 5 components.

The prevalence of individual components of MetS was highest for low HDL-C (30%), followed by hypertension (29%), abdominal obesity (23%), hypertriglyceridemia (9%), and elevated fasting blood glucose (5%) (Table 1.2). The prevalence of hypertension was high in both PLHIV (22%) and HIV-negative (36%) subjects. Of note, PLHIV had a significantly lower prevalence of hypertension (p<.001) and abdominal obesity (p=.015) compared to HIV-negative participants (Table 1.2).

Overall, the prevalence of Mets was 8.9% (50/564). The prevalence of MetS was significantly lower among PLHIV as compared to among HIV-negative participants (Table 1.3). Eighteen (6.3%) of 287 PLHIV had MetS compared to 32 (11.6%) of 277 HIV-negative participants (p=.03) (Table 1.3).

After adjusting for age, sex, education, smoking, alcohol use, diet and physical activity, PLHIV were 66% less likely to have MetS compared to HIV-negative participants (adjusted odds ratio [aOR] 0.34; 95% confidence interval [95%CI] 0.18, 0.65; p=.001). PLHIV were also less likely to have elevated blood pressure (aOR 0.30; 95%CI 0.20, 0.47; p<.001), abdominal obesity (aOR 0.44; 95%CI 0.28, 0.70; p<.001), low HDL-C (aOR 0.61; 95%CI 0.41, 0.91; p=.02), and elevated fasting glucose (aOR 0.36; 95%CI 0.1, 0.83; p= .02) when compared to the HIV-negative participants.

HIV status and age were independently associated with MetS (Table 1.4). Older participants (>40 years) compared to younger participants (30-40 years) were more likely to have MetS. There was a 5-fold increased likelihood of MetS among those aged 40 -49 years and those 50-59 years compared to those <40 years (aOR 5.32; 95%CI 2.10, 13.45; p=0.002 and aOR 5.52 (95%CI 2.08, 14.67; p=0.002, respectively). Among the PLHIV in a model that in addition included CD4 count, viral load, ART duration and ART regimen, those with a CD4 count greater than 500 cells/mm^3 were 4.5-fold more likely to be diagnosed with MetS (aOR 4.25 95% CI 1.11- 16.28; p=0.03) compared to those with CD4 count below this threshold. Other HIV-related factors such as WHO stage, viral load, duration on ART and ART regimen were not significantly associated with MetS.

Predicted 10-year ASCVD risk score

Out of 598 participants, we could not calculate the ASCVD risk score for 300 participants in accordance with the ASCVD risk score algorithm[81]. These included 34 participants without blood samples, 10 with prior history of stroke or myocardial infarction, and 202 participants aged <40 years or >79 years. Among those aged between 40 and 79 years, the data for 53 participants was not in the range accepted for risk calculation and included 6 participants who had HDL-C <20 mg/dL or >100mg/dL, 41 had a total cholesterol <130mg/dL or >320mg/dL and 6 participants with a systolic blood pressure of <90mmHg or >200mmHg.

Among the 298 participants with complete data who had ASCVD risk scores computed, 182 (61%) were HIV-positive and 116 (39%) were HIV-negative. Of these, 137 (46%) were females and median age was 52 years (IQR 44, 58).

Overall, 3% of participants (8/298) had a high ASCVD risk score of which all were males and majority were HIV-negative (7/8). A quarter of participants (68/298) were classified as borderline and intermediate risk of ASCVD. The median ASCVD risk score was lower among PLHIV as compared to HIV-negative

participants (Median 1.7%; IQR 0.7%, 3.65% and 2.95%; IQR 1.1%, 7.15%, respectively; p=0.002) (Table 1.5). PLHIV had a lower median ASCVD score regardless of sex.

Discussion

We report findings of a large cross-sectional study examining prevalence and correlates of MetS and ASCVD risk scores among HIV-positive and HV-negative participants in Kenya. Overall prevalence of MetS was high in this cohort (9%), as was prevalence of its individual components. While studies in middle and high-income countries have suggested that PLHIV have higher CVD risk than those without HIV, we found that the prevalence of the MetS was higher among HIV-negative persons than PLHIV; similarly, in a subset analysis of those participants >40 years of age, 10-year ASCVD risk was significantly greater among HIV-negative persons than PLHIV.

There has been mixed evidence on the prevalence of MetS among PLHIV in Africa. A recent metanalysis in SSA found an overall prevalence of MetS of 21.5% among PLHIV compared to 12% among those without HIV infection, however there was great variation between studies that could be due to differences in populations and definitions of MetS criteria.[69] Other studies have reported results more consistent with those reported here, finding PLHIV to have lower prevalence of MetS than HIV-negative adults. Jacobson et al found that PLHIV in the Nutrition for Healthy Living study, were less likely to have MetS compared to National Health and Nutrition Examination Survey participants regardless of ART use, [85] and Nguyen et al reported no difference between the prevalence of MetS among PLHIV and the general population globally in a meta-analysis including a total of 65 studies across the five continents. [86]

We hypothesized that MetS would be more prevalent among PLHIV than among HIV-negative participants because HIV has been associated with increased risk of traditional CVD risk factors, HIV-related inflammation and use of ART-induced dyslipidemia, insulin resistance, and glucose intolerance, in high

income settings. [62-66] It is intriguing that MetS is less prevalent among PLHIV than HIV-negative adults in this study based in rural Kenya. It is intriguing that MetS is less prevalent among PLHIV than HIV-negative adults in this study based in rural Kenya. Some potential factors leading to this finding may be the following: The study only included individuals who had been on ART for 6 months or longer. This is likely to be an important contributor, reflecting the success of ART in achieving viral suppression and reducing the contribution of ART related factors to MetS. Secondly, majority of the PLHIV were on a non-PI based regimen which are less associated with cardio-metabolic disorders. Thirdly, PLHIV receive regular education on risk reduction and nutrition counseling in HIV clinics. PLHIV in this cohort were required to be enrolled in an HIV clinic that mandated clinic visits every 3 months. HIV-negative individuals do not have similar opportunities for preventative healthcare and generally have less interaction with the health system. In addition, there is a potential of survival bias as successfully treated PLHIV are likely survive to an older age and hence may be different from HIV-negative individuals or untreated PLHIV.

Elevated blood pressure, low HDL-C, and abdominal obesity were the most prevalent components of MetS in this cohort, findings similar to those reported by other studies in Kenya, as well as in other parts of SSA. [87,88] We also found that prevalence of these components was lower among PLHIV compared to HIV-negative individuals. Similarly a recent meta-analysis including data from nearly 30,000 individuals in SSA reported a lower level of risk factors such as hypertension, diabetes, abdominal obesity, dyslipidemia and greater BMI among PLHIV compared to HIV-negative adults.[74,89] Yet, despite PLHIV having lower systolic blood pressure, total cholesterol, HDL-C and LDL-C, there was no significant difference in the prevalence of MetS by HIV status, according to Fourie et al.[74] It is also interesting to note that rates of smoking were lower in the HIV-positive than in the HIV-negative adults in our study, contrary to findings in Western countries where there are high rates of smoking and alcohol use among PLHIV. While traditional risk factors clearly vary with local context, the fact that half of our participants had 1-2 CVD risk factors, precursors for developing MetS, suggests that a large proportion would likely benefit from interventions to deter progression to MetS.

Among PLHIV, the relationship between CD4 and MetS remains unclear. We report here that a high CD4 count was associated with increased likelihood of MetS while other HIV-related factors, including viral suppression, were not significantly associated. Similar findings were reported by Mondy et al from the United States and Bonfanti et al from Italy.[90] [91] One explanation for higher CD4 count being associated with MetS is that these participants with restored immune systems have been living with HIV and on ART longer and may be experiencing cumulative effects of the disease or drugs. Of note, we found no association with the ART drugs and MetS among PLHIV. This may be due to adoption of drugs with fewer metabolic adverse effects as part of the Kenyan national ART regimen; only a small proportion of our participants were using PI-based regimens as seen in other studies in Kenya. [92] We did not find time since HIV diagnosis or duration of ART to be associated with MetS, however this merits further investigation. Future longitudinal studies with hard clinical endpoints, such as stroke and myocardial infarction, would help to confirm these trends in MetS and ASCVD risk scores for PLHIV when compared to HIV-negative older adults in SSA.

Our study findings showed that PLHIV had lower ASCVD risk scores than those who were HIV-negative. In addition, increasing age was associated with MetS, as has been noted in several African countries.[87] Because the criteria for MetS do not include age, smoking, or total cholesterol, a combination of MetS assessment and ASCVD risk scoring could help focus on those who are at highest risk for treatment, targeting prevention among those in borderline and intermediate risk groups and potentially reducing health care costs and improving treatment outcomes.

Our study has several strengths and limitations. This is one of the largest studies in the literature that directly compares the burden of MetS and CVD risk among people with and without HIV in SSA. Our study is also different from others in that it compared PLHIV who on stable ART and mostly with undetectable HIV viral loads, to those who were HIV-negative, a comparison that is highly relevant in the current era of universal test and treat ART programs. The main study limitations are the cross-sectional study design and the fact that the study was conducted at a single facility; these may limit its generalizability. With respect

to the ASCVD risk score, since the PLHIV were older, we found a higher proportion of PLHIV having prior cardiovascular events and these individuals were excluded from analysis of ASCVD risk score. Excluding high risk participants who are HIV-positive could have influenced our ASCVD results, as could the fact that the ASCVD risk score is not validated for African populations.

Conclusion

MetS prevalence was high in both HIV-positive and negative adults in western Kenya (1 in 20 and 1 in 10, respectively). Importantly, PLHIV were less likely to have MetS and had lower ASCVD risk scores than HIV-negative participants. Together these findings emphasize the need for CVD risk assessment among people living with and without HIV infection and further longitudinal studies to determine those risk factors most highly predictive of CVD events. Early identification of persons with MetS and those at high ASCVD risk should guide decision-making regarding risk reduction strategies, such as targeting statin therapy, at the national level. This study also emphasizes the importance of advocating for additional support for integration of routine CVD screening and management into health programs in resource-limited settings, regardless of HIV status.

Acknowledgements

CF, STP, SJM developed and implemented the CVD study protocol. SJM, JH, and CF designed this analysis. JNM and SJM coordinated data collection. SJM, TT, JZ, AO analyzed the data, and SM drafted the manuscript. All authors contributed to editing of the manuscript and approved submission of the final draft for publication

We declare no conflicts of interest.

This study was funded by grants from the US National Institutes of Health (NIH), R21TW010459 and Fogarty International Center (FIC) D43 TW009580. The findings and conclusions in this manuscript are

those of the authors and do not necessarily represent the views of the Government of Kenya or the National Institutes of Health.

Table 1.1: Baseline characteristics of 598 study participants by HIV status

Variable	Total (N=598) Median (IQR) or N (%)		HIV-positive (N=300)		HIV-negative (N=298)		P value
1. Sociodemographic characteristics							
Age categories							**<.001**
Less than 40 years	213	(35.6)	75	(25.0)	138	(46.3)	
40-49 years	168	(28.1)	110	(36.7)	58	(19.5)	
50-59 years	135	(22.58)	85	(28.3)	50	(16.8)	
≥60 years	82	(13.7)	30	(10.0)	52	(17.5)	
Sex (Female)	299	(50.0)	150	(50.0)	149	(50.0)	1.00
Marital status							**.002**
Single	38	(6.4)	15	(5.0)	23	(7.7)	
Currently married	440	(73.6)	205	(68.3)	235	(78.9)	
Separated/Widowed/Divorced	120	(20.0)	80	(26.7)	40	(13.4)	
Education							**<.001**
Less primary school completed	85	(14.2)	39	(13.0)	46	(15.4)	
Primary school completed	227	(38.0)	130	(43.3)	97	(32.6)	
Secondary school completed	191	(31.9)	100	(33.3)	91	(30.5)	
More than secondary school completed	95	(15.9)	31	(10.3)	64	(21.5)	
Employment							.51
Unemployed	79	(13.2)	38	(12.7)	41	(13.8)	
Employed	519	(86.8)	257	(85.7)	262	(87.9)	
2. Characteristics of people living with HIV							

Nadir CD4 count [1]	369	(215,563)	369	(215,563)	
Time since diagnosis (years)	9	(5,11)	9	(5,11)	
Regimen					
First line regimen (non PI)[2]	260	(86.7)	260	(86.7)	
Second line regimen (PI)	39	(13.0)	39	(13.0)	
Third line regimen (PI)	1	(0.3)	1	(0.3)	
ART duration (years)	8	(4, 10)	8	(4, 10)	
Current CD4 count [3]	512	(364,666)	512	(364,666)	
Undetectable viral load (VL<50 copies/ml)[4]	235	(78.9)	235	(78.9)	
Virally suppressed (VL<1000)*	285	(95.6)	285	(95.6)	

[1] 18 participants missing nadir CD4 count data, [3, 4] 2 participants missing CD4 results and viral load result. [2] PI stands for protease inhibitors

Table 1.2. History of cardiovascular disease diagnosis and traditional risk factors by HIV status.

Variable	Total (N=598) Median (IQR) or N (%)		HIV-positive (N=300)		HIV-negative (N=298)		P value
1. History of cardiovascular disease diagnosis and treatment							
Hypertension	71	(11.9)	24	(8.0)	47	(15.8)	**.003**
Hypertension treatment	26	(36.6)	8	(33.0)	18	(38.0)	.68
Diabetes	10	(1.7)	4	(1.3)	6	(2.0)	.52
Diabetes treatment	6	(60.0)	3	(75.0)	3	(50.0)	.43
History of elevated cholesterol	6	(1.0)	2	(0.7)	4	(1.3)	.41
History of heart attack	1	(0.2)	1	(0.3)	0	(0.0)	.32
History of stroke	9	(1.5)	7	(2.3)	2	(0.7)	.09
Currently on aspirin	2	(7.7)	2	(13.3)	0	(0.0)	.21
2. Traditional risk factors							
Body mass index categories (kg/m^2)							**<.001**
Underweight (<18.5)	56	(9.4)	35	(11.7)	21	(7.1)	
Normal (18.5-24.9)	337	(56.4)	184	(61.3)	153	(51.3)	
Overweight (25-29.9)	129	(21.6)	57	(19.0)	72	(24.2)	
Obese(>30)	76	(12.7)	24	(8.0)	52	(17.5)	
Smoking							.26
Never smoked	524	(87.6)	261	(87.0)	263	(88.3)	
Ever smoked but stopped	45	(7.5)	27	(9.0)	18	(6.0)	
Currently smoking	29	(4.9)	12	(4.0)	17	(5.7)	
Alcohol use in past 12 months	110	(18.4)	43	(14.3)	67	(22.5)	**.01**

Recommended healthy diet	32	(5.35)	12	(4.0)	20	(6.7)	.59
Recommended physical activity	147	(94)	86	(95.6)	61	(92.4)	.41
Waist circumference, mean (SD)							
Female	84.8	(13.0)	82.3	(12.4)	87.3	(13.1)	**<.001**
Male	82.2	(11.0)	81.3	(9.7)	83.1	(12.2)	.16
3. Components of metabolic syndrome (MetS) criteria*							
Elevated blood pressure (BP≥135/85mmHg)	161	(28.5)	63	(22.0)	98	(35.4)	**<.001**
Abdominal obesity (Waist circumference of >88cm for women and >94cm for men)	130	(23.0)	54	(18.8)	76	(27.4)	.01
Low HDL-C (<50mg/dL for women and <40mg/dL for men)	167	(29.6)	75	(26.1)	92	(33.2)	.06
Elevated triglycerides (≥150mg/dL)	48	(8.5)	30	(10.5)	18	(6.5)	.09
Elevated fasting plasma glucose (≥100 mg/dL)	28	(5.0)	10	(3.5)	18	(6.5)	.09

*Available for 564 participants (287 HIV-positive and 277 HIV-negative). It excludes 34 participants without blood sample results (13 HIV-positive and 21 HIV-negative

Table 1.3. The association between HIV status and metabolic syndrome and its components among 564 participants

Variable	Unadjusted Odds ratio (95% CI)	P value	Adjusted Odds Ratio** (95% CI)	P value
Metabolic syndrome	0.51 (0.28, 0.93)	0.03	0.34 (0.18, 0.65)	**0.001**
Elevated blood pressure (BP≥135/85mmHg)	0.51 (0.35, 0.75)	**<0.001**	0.30 (0.20, 0.47)	**<0.001**
Abdominal obesity (Waist circumference of >88cm for women and >94cm for men)	0.61 (0.41, 0.91)	0.02	0.44 (0.28, 0.70)	**<0.001**
Low HDL-C (<50mg/dL for women and <40mg/dL for men)	0.71 (0.50, 1.02)	0.07	0.61 (0.41, 0.91)	**0.02**
Elevated triglycerides (≥150mg/dL)	1.68 (0.91, 3.08)	0.10	1.40 (0.72, 2.74)	0.31
Elevated fasting plasma glucose (≥100 mg/dL)	0.52 (0.23, 1.14)	0.11	0.36 (0.16, 0.83)	**0.02**

*Excludes 34 participants without blood sample results (13 HIV-positive and 21 HIV-negative).

**Adjusted for age, sex, education, smoking, diet, alcohol and exercise

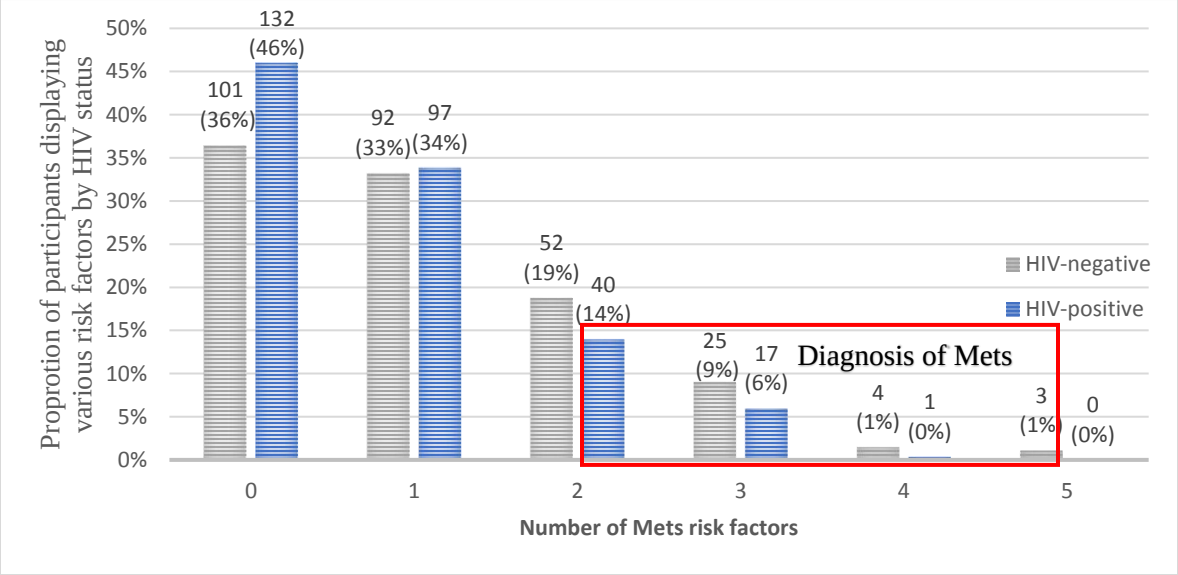
Table 1.4. Univariate and multivariate analysis of factors associated with metabolic syndrome

Variable	Univariate		Multivariate	
	OR (95% CI)	**P value**	**OR (95% CI)**	**P value**
HIV status		**.03**		**.001**
HIV-negative	Ref		Ref	
HIV-positive	0.51 (0.28, 0.93)		0.34 (0.18, 0.65)	
Age categories		**.02**		**.002**
< 40 years	Ref		Ref	
40-49 years	3.68 (1.51, 8.99)		5.32 (2.10, 13.45)	
50-59 years	3.96 (1.58, 9.92)		5.52 (2.08,14.67)	
≥ 60 years	3.41 (1.19, 9.77)		3.37 (1.06, 10.72)	
Sex		.08		.09
Female	Ref		Ref	
Male	0.58 (0.32, 1.06)		0.56 (0.29, 1.09)	
Smoking		.63		.76
Not current smoker	Ref		Ref	
Current smoker	0.85 (0.44, 1.64)		0.89 (0.44, 1.83)	
Alcohol use in past 12 months		.21		.64
No alcohol use	Ref		Ref	
Alcohol use	0.57 (0.24, 1.38)		0.79 (0.30, 2.07)	

Table 1.5. Atherosclerotic cardiovascular disease (ASCVD) risk score and risk categories stratified by HIV status and sex.

ASCVD risk	**HIV-positive males**	**HIV-negative males**	**P value**	**HIV-positive females**	**HIV-negative females**	**P value**
	Median (IQR) or N (%)			**Median (IQR) or N (%)**		
	N=102	**N=60**		**N=80**	**N=56**	
Median ASCVD risk	2.7 (1.2, 5.7)	4 (2, 14.9)	**.002**	0.9 (0.4, 2.3)	2.1 (0.8, 4.8)	**.002**
Low risk (<5%)	72 (71)	32 (53)	**.005**	76 (95)	42 (75)	**.001**
Borderline risk (≥5% to 7.5%)	13 (13)	6 (10)		1 (1)	9 (16)	
Intermediate risk (≥7.5% to <20%)	16 (16)	15 (25)		3 (4)	5 (9)	
High risk (≥20%)	1 (1)	7 (12)		0 (0)	0 (0)	

Figure 1.1. Proportion of participants displaying various number of individual components of metabolic syndrome stratified by HIV status.



CHAPTER 3: Inflammation

Abstract

Background

Systemic inflammation independently predicts future cardiovascular events and is associated with a 2-fold increase in cardiovascular disease (CVD) risk among people living with HIV (PLHIV). We examined the association between inflammatory markers, HIV status and traditional CVD risk factors.

Methods

We conducted a cross-sectional study of Kenyan adults with and without HIV infection seeking care at Kisumu County Hospital. Using a multiplex immunoassay, we measured interleukin-1 (IL-1β), interleukin-6 (IL-6), tumor necrosis factor (TNF-α), and high-sensitivity C-reactive protein (hsCRP) concentrations. We compared inflammatory marker concentrations by HIV status using the Wilcoxon rank sum test. Multivariable linear regression was used to evaluate associations between inflammatory biomarkers and HIV status, adjusting for CVD risk factors.

Results

We enrolled 286 PLHIV and 277 HIV-negative participants. Median duration of antiretroviral therapy (ART) for PLHIV was 8 years (interquartile range [IQR] 4, 10) and 96% were virally suppressed. PLHIV had a 51% higher mean IL-6 concentration ($p<.001$), 39% higher mean IL-1β ($p=.005$), a 40% higher mean TNF-α ($p<.001$) and a 27% higher mean hsCRP ($p=.008$) compared to HIV-negative participants, independent of CVD risk factors. Male sex, older age, and obesity were associated with higher concentrations of inflammatory markers. Restricting to PLHIV, viral load of ≥1000 copies/ml was associated with higher TNF-α levels ($p=.013$).

Conclusion

We found higher levels of systemic inflammatory biomarkers among PLHIV who were virally suppressed, and this was independent of traditional CVD risk factors. Further longitudinal analyses to determine

whether these inflammatory markers predict future CVD events, and are possible therapeutic targets among PLHIV, are warranted.

Introduction

Systemic inflammation has been shown to increase cardiovascular disease (CVD) risk and independently predict future cardiovascular events such as myocardial infarction and stroke.[93-97] In addition, increased levels of biomarkers such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) have been associated with increased risk of mortality among those with preexisting CVD.[98,99] There is evidence that CVD risk remains increased even if there are significant reductions in low density lipoprotein cholesterol (LDL-C) levels, a marker of CVD risk, after use of lipid lowering statin therapy. [100] The relative contribution of the inflammatory pathway to this residual CVD risk among both people with and without HIV is unclear.[101]

In sub-Saharan Africa (SSA), which accounts for 67% of the global HIV burden, there is growing concern about rising CVD morbidity and its related mortality despite decreasing CVD death rates in high-income countries.[102] Evidence from the United States and Europe suggests that CVD risk among people living with HIV (PLHIV) is double that of the general population. [14,15,57,58,61] Higher levels of inflammatory biomarkers such as C-reactive protein (CRP) and IL-6 are associated with HIV disease progression [103] and these persist at least up to one year following initiation of antiretroviral therapy (ART) [104,105]. Persistent inflammation may contribute to the increased occurrence of subclinical atherosclerosis, myocardial infarction and stroke and increased CVD related mortality among PLHIV. [104,106-108] In SSA there is paucity of data regarding the association between inflammation and CVD risk, especially comparing people with and without HIV.

We therefore sought to determine the association between HIV status and inflammatory markers, specifically high sensitivity CRP (hsCRP), IL-1β, IL-6, and TNF-α. We measured these biomarkers, traditional CVD risk factors and HIV-specific characteristics among PLHIV and HIV-negative adults in Kisumu, Kenya. We hypothesized that PLHIV would have higher mean inflammatory biomarker levels when compared to HIV-negative study participants.

Methods

Study design and setting

Between September 2017 and May 2018, we conducted a cross-sectional study among 300 HIV-positive and 300 HIV-negative women and men from Kisumu County Hospital, a tertiary, public county referral facility located in Western Kenya where HIV prevalence is high (16.3%).[82]

Study procedures

Participants were eligible if they were at least 30 years of age and lived within a 50km radius of the hospital. PLHIV had to be engaged in care at the HIV Comprehensive Care Clinic (CCC) and taking ART for at least 6 months. PLHIV who met the inclusion criteria and provided consent were consecutively enrolled by a study nurse from the CCC while HIV-negative participants were recruited from HIV testing points until the sample size was reached. All participants provided written informed consent prior to any study procedures or data collection. Human subject approval was obtained from the University of Washington Institutional Review Board and locally from the Kenyatta National Hospital / University of Nairobi Ethical and Scientific Review Committee.

Clinical procedures: Using tablets, study nurses enrolled and interviewed all participants collecting data on sociodemographics, HIV disease status if HIV-positive, and CVD risk factors using the validated World Health Organization (WHO) STEPS questionnaires modified to fit the Kenyan context [83]. Waist and hip circumference, weight and height were measured to determine waist: hip ratio and body mass index (BMI). Two blood pressure readings on each arm and pulse were measured and averaged. Participants were asked to return the following day after fasting 8 hours for blood draw if not already fasting.

Laboratory procedures: Blood samples were collected at least 8 hours after fasting for quantification of lipids (total cholesterol, high density lipoprotein cholesterol (HDL-C), LDL-C, triglycerides), glucose and inflammatory markers including hsCRP, IL-1β, IL-6, and TNF-α as well as CD4 and viral load for PLHIV. Blood samples were processed to obtain serum, and stored at the Kenya Medical Research Institute (KEMRI) Lab at -80°C. Absolute CD4+ T-cell count, and HIV-1 RNA viral load testing was performed at the KEMRI laboratory in Kisumu. HIV RNA viral load values below 50 copies/ml were classified as

undetectable. Absolute CD4 cell counts were measured using flow cytometry. Samples for lipids, glucose and inflammatory markers were batched and shipped for testing to the University of Washington, Seattle. Serum lipids, glucose and hsCRP tests were performed at the University of Washington Research Testing Services using an automated Beckman Coulter AU5812. All samples were tested in duplicate according to manufacturer's protocols.

Cytokine assays: Serum samples for IL-1β, IL-6, and TNF-α were analyzed using Mesoscale Discovery (MSD) VPlex Proinflammatory Panel 1 (human kit). Samples with a coefficient of variation > 30% were rerun and the duplicate with the lower coefficient of variation was averaged for the analyses. If a biomarker level was below the lower limit of detection for the assay, the lower limit was used as the biomarker value. Lower limit of detection concentrations was 0.2 mg/L for hsCRP, 0.01 pg/mL for IL-1β, 0.05 pg/mL for IL-6 and 0.01 pg/mL for TNF-α.

Primary outcomes and dependent variables

The primary outcomes were inflammatory biomarker concentrations: hsCRP, IL-1β, IL-6, and TNF-α. HIV status was the exposure. Multivariable models were adjusted for age, gender, smoking status, blood pressure, body mass index (BMI), hypertension, diabetes and lipids, determined a priori and supported by literature. We assessed the presence of effect modification by metabolic syndrome and high atherosclerotic CVD risk (ASCVD).[109] Metabolic syndrome was defined by the 2009 Consensus Criteria as any three of the following: 1) abdominal obesity (waist circumference of >88cm for women and >94cm for men); 2) triglycerides ≥150mg/dL; 3) HDL-C <50mg/dL for women and <40mg/dL for men; 4) blood pressure >130/85mmHg; 5) fasting plasma glucose ≥ 100mg/dL.[67] For each participant without prior history of myocardial infarction or stroke, we calculated their 10-year ASCVD risk score using the Pooled Cohort Equation as outlined in the 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease.[80,109]

Statistical analysis

Continuous variables were summarized using mean and standard deviation for normally distributed data, and by median and inter-quartile range (IQR) for non-normally distributed variables. We compared people with and without HIV infection using a t-test for normally distributed continuous variables, Wilcoxon rank sum test for non-normally distributed continuous variables, and chi-square test for categorical variables. We restricted our analysis to hsCRP values ≤10 mg/L as hsCRP values >10mg/L are most consistent with a transient or acute phase response as would commonly occur with acute infections common in this setting. Due to the cross-sectional nature of the study, we were unable to repeat measurements after 2 weeks to rule out transient acute inflammation.

We compared inflammatory markers by HIV status using the Wilcoxon rank sum test. We created separate multivariable linear regression models to evaluate the association between (log-transformed) IL-1β, IL-6, TNF-α and hsCRP and HIV status and adjusted for CVD risk factors including age, sex, smoking, alcohol use, diet (at least 5 servings of fruits and vegetables per day), physical activity (at least 150 minutes of moderate activity or 75 minutes of vigorous activity a week), body mass index, hypertension and diabetes, and lipids. Subgroup analyses for metabolic syndrome and high ASCVD risk (score >7.5%) were carried out to assess whether associations of each biomarker with HIV status was consistent across the subgroups by including an interaction term between HIV status and the subgroup variable. In a separate model including only PLHIV, we included nadir CD4 count, viral suppression, and ART duration as covariates.

We report the exponentiated beta coefficients and their calculated 95% confidence intervals (CI) representing the fold increase/decrease in biomarker level. We used a significance (α) level of 0.05. All analyses were conducted using Stata version 14.0 (StataCorp, College Station, TX).

Results

Baseline Characteristics

Out of the 600 eligible participants, complete data was available for 564 participants, (94%): 287 PLHIV and 277 HIV-negative participants. The median age was 45 years (IQR [40, 54) for PLHIV and 40 years

(IQR 31, 54) for HIV-negative participants. PLHIV were older (p<.001), less educated (p<.001), had a lower BMI (p<.001), and had consumed less alcohol in the past 12 months (p=.007) as compared to HIV-negative participants (Table 2.1). The prevalence of metabolic syndrome, hypertension, and abdominal obesity were significantly lower among PLHIV as compared to those without HIV (Table 2.1). There were no significant differences in smoking, diet, physical activity, triglycerides, high density lipoprotein cholesterol (HDL-C) and fasting glucose levels by HIV status.

Among PLHIV, the current median CD4 count was 512 cells/mm^3 (IQR 364, 666) and the median duration on ART was 8 years (IQR 4, 10). The majority (86%) of PLHIV were on a first line regimen (two nucleoside reverse transcriptase inhibitors [NRTI] plus one non-NRTI) with only 13% on a protease inhibitor (PI) based regimen (two NRTI plus PI). Eighty percent (229/287) of PLHIV had an undetectable HIV RNA level (<50 copies/ml), 16% (46/287) had low level viremia (HIV RNA 50-1000 copies/ml) and with 4% (12/287) had a high-level viremia (HIV RNA concentration ≥1000 copies/ml) (Table 2.1).

Overall, the median IL-1β, IL-6, TNF-α, and hsCRP levels was 0.11 pg/mL (IQR 0.05, 0.30), 0.94 pg/mL (IQR 0.50, 1.83), 2.78 pg/mL (IQR 1.96, 4.02), and 1.4 mg/L (IQR 0.6, 2.8), respectively. Median IL-1β, IL-6, TNF-α, and hsCRP levels were higher among PLHIV compared to HIV-negative participants (Figure 1). PLHIV had a significantly higher median IL-1β (0.14 pg/mL vs. 0.10 pg/mL, p= .019), IL-6 (1.15 pg/mL vs. 0.80 pg/mL, p<.001), TNF-α (3.06 pg/mL vs. 2.61 pg/mL, p<.001) and hsCRP (1.5mg/L vs. 1.2 mg/L, p= .052), compared to HIV-negative participants (Figure 1).

IL-6 showed the greatest mean difference among the biomarkers examined comparing PLHIV and HIV-negative participants. After adjusting for age, sex, smoking, alcohol use, diet, physical activity, body mass index, hypertension, diabetes, and lipids**,** PLHIV had a 51% higher mean IL-6 level (p<.001), 39% higher mean IL-1β level (p=.005), a 40% higher mean TNF-α level (p<.001) and a 27% higher mean hsCRP level (p=.008) compared to HIV-negative participants (Table 2.2).

Stratification by metabolic syndrome and high ASCVD risk.

We previously reported that the prevalence of metabolic syndrome and calculated ASCVD risk among these study participants was lower among PLHIV compared the HIV-negative participants.[109] However, in this analysis, the differences in biomarkers levels between those with and without metabolic syndrome/ high ASCVD risk in HIV-positives was not significantly different from similar differences in HIV-negatives. We therefore observed no significant interaction between HIV status and metabolic syndrome or ASCVD and the inflammatory markers.

Factors associated with IL-1β, IL-6, TNF-α and hsCRP

The demographic and CVD risk factors found to be independently associated with higher IL-1β levels were male sex and obesity (BMI>30) (Table 2.3.1). A significantly higher IL-6 was associated with older age (>50 years), male sex, and lower HDL-C (Table 2.3.2). While a significantly higher TNF-α was associated with older age (>60 years) and male sex, TNF-α levels were significantly lower among participants with diabetes (Table 2.3.3). Older age (>50 years), being overweight or obese and a current smoker were associated with higher hsCRP (Table 2.3.4).

In a model restricting the analysis to participants who are PLHIV, we added HIV specific factors including the ART regimen, nadir CD4 count, current CD4 count, viral load and duration of ART use in addition to the CVD risk factors. PLHIV who had a viral load of ≥1000 copies/ml had a 114% higher TNF-α level as compared to those who had an undetectable viral load (p=.013). Apart from TNF-α, there was no association between the HIV specific risk factors and the other biomarkers.

Discussion

In this study, we found a higher level of inflammatory markers (IL-1β, IL-6, TNF-α, and hsCRP) among PLHIV, the majority of whom were virally suppressed, compared to HIV-negative participants. These higher concentrations among PLHIV persisted even after adjusting for traditional risk factors for CVD including dyslipidemia, obesity, diabetes and smoking. Our findings are among the first to examine the relationships between inflammation, HIV, and CVD risk factors in SSA and are consistent with some but

not all the literature from Europe, North America, and SSA.[104,110-114] In addition, we demonstrated that some inflammatory biomarker concentrations were even greater among PLHIV who were not virally suppressed relative to PLHIV with optimal viral suppression. Those PLHIV not achieving viral suppression (HIV RNA concentrations ≥1000 copies/ml) had twice the concentration of TNF-α compared to those PLWHV with an undetectable viral load. Higher levels of biomarkers among those not virally suppressed may indicate residual inflammation as a result of persistent viremia or a low pretreatment CD4 count [115]. This is consistent with other studies that have shown that high viral load concentrations are correlated to high levels of inflammation and increased CVD risk.[115,116]

Studies examining the relationships between HIV, inflammation and ART in SSA are limited and have reported heterogeneous results. A multi-country study including sites in Kenya, Nigeria, South Africa, Uganda, and Zambia, reported higher levels of CRP but no differences in IL-6 levels comparing HIV-positive participants on ART with HIV-negative individuals.[112] A study in South Africa comparing HIV-negative individuals and HIV- positive individuals who were ART naïve and those on ART found that mean TNF-α levels were significantly increased pre-ART among those with opportunistic infections and TNF-α remained elevated one-year post-ART. However, IL-6 levels were comparable in treated patients without opportunistic coinfection compared to those without HIV.[113] The Monitoring of Early Treatment Adherence study recruited participants from Uganda and South Africa who were starting ART in late stage disease and found that individuals starting ART had higher levels of IL-6 pretreatment but found no difference in levels of biomarkers comparing those initiating treatment with earlier- or late-stage disease one year after treatment initiation.[114] In summary, ART appears to lower inflammatory markers but whether low grade inflammation persists compared to HIV-negative individuals remains a question. In our cross-sectional study in Kenya, low grade inflammation appears to persist despite ART. The heterogeneity of results across studies may reflect context specific differences as well as reflect different underlying pathophysiology for each biomarker as suggested by Siedner et al.[114]

Consistent with previously published literature, we found an increase in hsCRP, IL-6 and TNF-α levels with increasing age especially above the age of 50 years. This is thought to be occurring due to natural aging resulting in a persistent low-grade inflammation, a process termed "inflammaging".[117] Males in our study had higher mean IL-1β, IL-6, and TNF-α concentrations compared to females. This has been reported in previous studies and may be explained by the downregulation of these inflammatory marker genes by estrogen.[118,119] We also report that higher IL-6 levels were associated with lower HDL-C and higher levels of hsCRP and IL-1β were noted among obese individuals. Obesity has been linked to low grade inflammation as adipose tissue releases cytokines that trigger production of CRP.[120] In addition, current smoking was associated with increased levels of TNF-α. Smoking cessation and obesity interventions have been shown to decrease CRP levels, and thus may provide an opportunity for reducing inflammation and CVD risk reduction in both persons with and without HIV.[121]

As reported in Chapter 2, we found that the prevalence of metabolic syndrome in the PLHIV in our study, was lower than the HIV-negative participants. Contrary to our expectation, there was no significant interaction between HIV and metabolic syndrome. Higher levels of inflammation have been seen in cardio-metabolic conditions such as metabolic syndrome and hypertension [122], although the data has not been consistent. This may be explained by genetic factors or the fact that the PLHIV in our study were virally suppressed with a lower prevalence of traditional risk factors, but this would need further investigation.

The strengths of this study are that we included both HIV-positive and HIV-negative participants. The HIV-positive participants were on highly active ART, the majority having achieved viral suppression and are thus representative of the current ART program in Kenya [123]. We also measured several inflammatory markers. The limitations of this study include the cross-sectional design limiting inferences of causality. We did not measure the inflammatory markers over time to observe their trajectory. In addition, we lack data regarding CVD endpoints such as myocardial infarction or stroke to test how these biomarkers predict these events. Further longitudinal studies in SSA prospectively measuring and following inflammatory

markers and CVD events will further our understanding of the relationship between inflammation and CVD risk in PLHIV and could justify intervention studies targeting inflammation for CVD risk reduction.

Conclusion

Greater systemic inflammation was seen among PLHIV compared to HIV-negative individuals, independent of traditional CVD risk factors. Persistent inflammation among PLHIV, even those with optimal viral suppression, is a potential mechanism contributing to increased CVD risk among PLHIV. Periodic measurement of inflammatory markers may be useful for risk stratification and predicting CVD events over time, ultimately helping to identify those PLHIV at highest risk for CVD morbidity and mortality. Research is also needed to determine whether interventions that target both the metabolic and inflammatory pathways reduce CVD risk in PLHIV in SSA.

Acknowledgements

Funding

This work was supported by grants from the US National Institutes of Health (R21TW010459) and Fogarty International Center (D43 TW009580). The findings and conclusions in this manuscript are those of the authors and do not necessarily represent the views of the Government of Kenya or the National Institutes of Health.

Acknowledgements

CF, STP, SJM developed and implemented the CVD study protocol. SJM, JH, STP and CF designed this analysis. SJP, JW, AO, PM and SJM coordinated data collection. SJM, SP analyzed the data, and SM drafted the manuscript. All authors contributed to editing of the manuscript and approved submission of the final draft for publication.

We declare no conflicts of interest.

Table 2.1. Baseline characteristics of 598 study participants stratified by HIV status.

Variable	Total (N=564) Median (IQR) or N (%)		HIV-positive (N=287) Median (IQR) or N (%)		HIV-negative (N=277) Median (IQR) or N (%)		P value
3. Socio-Demographic characteristics							
Age categories							**<.001**
Less than 40 years	201	(35.6)	68	(23.7)	133	(48.0)	
40-49 years	162	(28.7)	106	(36.9)	56	(20.2)	
50-59 years	128	(22.7)	83	(28.9)	45	(16.2)	
≥60 years	73	(12.9)	30	(10.5)	43	(15.5)	
Sex (Female)	299	(50.0)	144	(50.2)	138	(49.8)	.93
Marital status							**.002**
Single	38	(6.4)	15	(5.2)	23	(8.3)	
Currently married	414	(73.4)	205	(68.3)	235	(78.9)	
Separated/Widowed/Divorced	112	(19.8)	80	(26.7)	40	(13.4)	
Education							**<.001**
< primary school completed	78	(13.8)	39	(13.6)	39	(14.1)	
Primary school completed	211	(37.4)	124	(43.2)	87	(31.4)	
Secondary school completed	183	(32.4)	95	(33.1)	88	(31.8)	
More than secondary school completed	92	(16.3)	29	(10.1)	63	(22.7)	
4. Characteristics of People living with HIV							
Nadir CD4 count [a]	365	(213,571)	365	(213,571)			
Time since diagnosis (years)	9	(5,11)	9	(5,11)			

Regimen							
First line regimen (non-PI) [b]	248	(86.4)	248	(86.4)			
Second line regimen (PI)	38	(13.2)	38	(13.2)			
Third line regimen (PI)	1	(0.3)	1	(0.3)			
ART duration (years)	8	(4, 10)	8	(4, 10)			
Current CD4 count [c]	512	(364,666)	512	(364,666)			
Viral load (VL) copies.ml) [d]							
Undetectable (VL<50)	229	(79.8)	229	(79.8)			
Low level viremia (VL 50-1000)	46	(16.0)	46	(16.0)			
Viremic (VL>1000)	12	(4.2)	13	(4.2)			
5. Traditional risk factors							
Body mass index categories (kg/m2)							**<.001**
Underweight (<18.5)	51	(9.0)	32	(11.1)	19	(6.9)	
Normal (18.5-24.9)	319	(56.6)	177	(61.7)	142	(51.3)	
Overweight (25-29.9)	119	(21.1)	54	(18.8)	65	(23.5)	
Obese (>30)	75	(13.3)	24	(8.4)	51	(18.4)	
Smoking							.17
Never smoked	494	(87.6)	249	(88.4)	245	(88.4)	
Ever Smoked but stopped	43	(7.6)	27	(9.4)	16	(5.8)	
Currently Smoker	27	(4.8)	11	(3.8)	16	(5.8)	
Alcohol use in past 12 months	105	(18.6)	41	(14.3)	64	(23.1)	**.007**
Recommended healthy diet	31	(5.5)	12	(4.2)	19	(6.9)	.16
Recommended physical activity	134	(95)	79	(95.0)	55	(95)	.92
Waist circumference, mean (SD)							

Female	84.6	(13.0)	82.3	(12.6)	87.0	(13.1)	**<.002**
Male	82.0	(11.0)	81.2	(9.8)	82.9	(12.2)	.22
6. Components of Metabolic syndrome criteria[e]							
Elevated blood pressure (BP≥135/85mmHg)	161	(28.5)	63	(22.0)	98	(35.4)	**<.001**
Abdominal obesity (Waist circumference of >88cm for women and >94cm for men)	130	(23.0)	54	(18.8)	76	(27.4)	**.01**
Low HDL-C (<50mg/dL for women and <40mg/dL for men)	167	(29.6)	75	(26.1)	92	(33.2)	.06
Elevated triglycerides (≥150mg/dL)	48	(8.5)	30	(10.5)	18	(6.5)	.09
Elevated fasting plasma glucose ≥100 mg/dL))	28	(5.0)	10	(3.5)	18	(6.5)	.09
Metabolic syndrome	50	9.0)	18	(6.3)	32	(11.6)	**.027**

[a]18 participants missing nadir CD4 count data, [b]PI stands for protease inhibitors [c,d]2 participants missing CD4 results and viral load result. [e]Excludes 34 participants without blood sample data.

Table 2.2. Exponentiated beta coefficient estimates and confidence interval; for the association between biomarkers and HIV status.

	Unadjusted			**Adjusted[a]**		
Exponentiated beta coefficients represent the fold increase in mean level of the inflammatory biomarkers comparing HIV-positive and HIV-negative participants. For example, 1.39 is interpreted as PLHIV have a 39% higher mean IL-1 level as compared to HIV-negative participants (p=.005).						
Variable	**Exp β coef**	**95% CI**	**P value**	**Exp β coef**	**95% CI**	**P value**
IL-1β (pg/mL)	1.38	1.06, 1.59	**0.010**	1.39	1.10, 1.73	**.005**
IL-6 (pg/mL)	1.46	1.21, 1.75	**<0.001**	1.51	1.23, 1.84	**<0001**
TNF-α (pg/mL)	1.45	1.22, 1.70	**<0.001**	1.40	1.16, 1.67	**<0.001**
hsCRP (mg/L)	1.22	1.02, 1.45	**0.027**	1.27	1.06, 1.52	**0.008**

[a]Adjusted for age, sex, smoking, alcohol use, diet, physical activity, body mass index, hypertension and diabetes, and lipids.

Table 2.3.1 Unadjusted and adjusted multivariable linear regression of factors associated with IL-1β

	Unadjusted		**Adjusted**	
	Beta coef. (95% CI)	P value	Beta coef. (95% CI)	P value
HIV status	0.27 (0.06, 0.47)	**.010**	0.30 (0.07, 0.52)	**.011**
Age (years)				
30-39	Reference		Reference	
40-49	0.24 (-0.11,0.49)	.061	0.10 (-0.17, 0.37)	.473
50-59	0.03 (-0.25, 0.31)	.837	-0.11 (-0.42, 0.20)	.487
≥60	0.04 (-0.29, 0.38)	.796	-0.06 (-0.42, 0.30)	.746
Sex				
Female	Reference		Reference	
Male	0.32 (0.13, 0.53)	**.002**	0.37 (0.13, 0.60)	**.002**
BMI (kg/m2)				
Normal (18-24)	Reference		Reference	
Underweight (<18)	-0.16 (-0.52,0.20)	.379	-0.26 (-0.61, 0.11)	.175
Overweight (25-29)	-0.27(-0.53, -0.01)	**.039**	-0.10 (-0.37, 0.17)	.469
Obese (≥30)	0.12 (-0.18, 0.42)	.429	0.36 (0.04, 0.69)	**.030**
Current smoker	0.18 (-0.18, 0.42)	.459	0.08 (-0.41, 0.57)	.741
Alcohol use in past 12 months	0.13 (-0.13, 0.39)	.322	0.01 (-0.28, 0.28)	.984
Hypertension	-0.13 (-0.38, 0.12)	.306	0.11 (-0.14, 0.38	.373
Diabetes	0.01 (-0.46, 0.48)	.964	0.03 (-0.44, 0.51)	.887
LDL>130mg/dL	-0.34(-0.64, -0.05)	**.024**	-0.42 (-0.96,0.11)	.120
HDL<50 mg/dL for males and <40 mg/dL for females	-0.15 (-0.37, 0.71)	.183	-0.08 (-0.32, 0.16)	.526

Total cholesterol >200 mg/dL	-0.21 (-0.48, 0.07)	.139	0.04 (-0.46, 0.54)	.871
Triglycerides >150 mg/dL	-0.01 (-0.38, 0.36)	.969	-0.13 (-0.52, 0.27)	.524

Table 2.3.2. Unadjusted and adjusted multivariable linear regression of factors associated with IL-6.

	Unadjusted		**Adjusted**	
	Beta coef. (95% CI)	P value	Beta coef. (95% CI)	P value
HIV status	0.38 (0.19, 0.56)	**<.001**	0.41 (0.21, 0.61)	**<.001**
Age (years)				
30-39	Reference		Reference	
40-49	0.33 (0.10,0.56)	**.005**	0.17 (-0.06, 0.42)	.163
50-59	0.48 (0.23, 0.73)	**<.001**	0.28 (-0.01, 0.55)	**.051**
≥60	0.71 (0.41, 1.01)	**<.001**	0.59 (0.27, 0.92)	**<.001**
Sex				
Female	Reference		Reference	
Male	0.36 (0.18, 0.55)	**<.001**	0.34 (0.13, 0.55)	**.001**
BMI (kg/m2)				
Normal (18-24)	Reference		Reference	
Underweight (<18)	-0.16 (-0.50,0.18)	.355	-0.22 (-0.55, 0.10)	.183
Overweight (25-29)	-0.24 (-0.50, -0.18)	**.048**	-0.10 (0.35, 0.14)	.406
Obese (≥30)	0.10 (-0.19, 0.38)	.505	0.27 (-0.04, 0.58)	.083
Current smoker	0.35 (-0.09, 0.79)	.117	0.19 (-0.26, 0.63)	.410
Alcohol use in past 12 months	0.17 (-0.07, 0.42)	.162	0.17 (-0.87, 0.42)	.198
Hypertension	-0.15 (-0.07, 0.38)	.194	0.08 (-0.15, 0.31)	.501
Diabetes	-0.31 (-0.74, 0.12)	.158	-0.33 (-0.75, 0.10)	.129
LDL>130mg/dL	-0.13 (-0.15, -0.41)	.355	-0.01 (-0.49, 0.46)	.962
HDL<50 mg/dL for males and <40 mg/dL for females	-0.05 (-0.16, 0.25)	.660	0.24 (-0.02, 0.45)	**.031**
Total cholesterol >200 mg/dL	0.16 (-0.08, 0.42)	.191	0.08 (-0.36, 0.52)	.729
Triglycerides >150 mg/dL	0.21 (-0.13, 0.55)	.225	-0.01 (-0.36, 0.34)	.973

Table 2.3.3. Unadjusted and adjusted multivariable linear regression of factors with TNF-α.

	Unadjusted		**Adjusted**	
	Beta coef. (95% CI)	P value	Beta coef. (95% CI)	P value
HIV status	0.37 (0.20, 0.53)	**<.001**	0.34 (0.16, 0.52)	**<.001**
Age (years)				
30-39	Reference		Reference	
40-49	0.33 (0.12,0.54)	**.002**	0.22 (-0.01, 0.45)	**.052**
50-59	0.38 (0.15, 0.60)	**.001**	0.23 (-0.02, 0.48)	.073
≥60	0.50 (-0.23, 0.77)	**<.001**	0.42 (0.13, 0.71)	**.005**
Sex				
Female	Reference		Reference	
Male	0.31 (0.14, 0.48)	**<.001**	0.27 (0.08, -0.46)	**.005**
BMI (kg/m2)				
Normal (18-24)	Reference		Reference	
Underweight (<18)	-0.19 (-0.50, 0.11)	.210	-0.26 (-0.55, 0.04)	.088
Overweight (25-29)	-0.18 (-0.39, 0.04)	.119	-0.05 (-0.27, 0.17)	.671
Obese (≥30)	-0.19 (-0.45, 0.07)	.144	-0.06 (-0.34, 0.22)	.646
Current smoker	0.13 (-0.26, 0.52)	.506	-0.02 (-0.42, 0.39)	.927
Alcohol use in past 12 months	0.12 (-0.10, 0.33)	.289	0.12 (-0.11, 0.35)	.306
Hypertension	0.03 (-0.17, 0.24)	.745	0.10 (-0.11, 0.31)	.873
Diabetes	-0.37 (-0.76, 0.02)	.063	-0.43 (-0.84, -0.03)	**.045**
LDL>130mg/dL	0.02 (-0.23, 0.27)	.894	-0.20 (-0.63, 0.23)	.359
HDL<50 mg/dL for males and <40 mg/dL for females	0.01 (-0.18, 0.19)	.938	0.16 (-0.04, 0.35)	.110
Total cholesterol >200 mg/dL	0.11 (-0.11, 0.34)	.326	0.20 (-0.20, 0.60)	.319

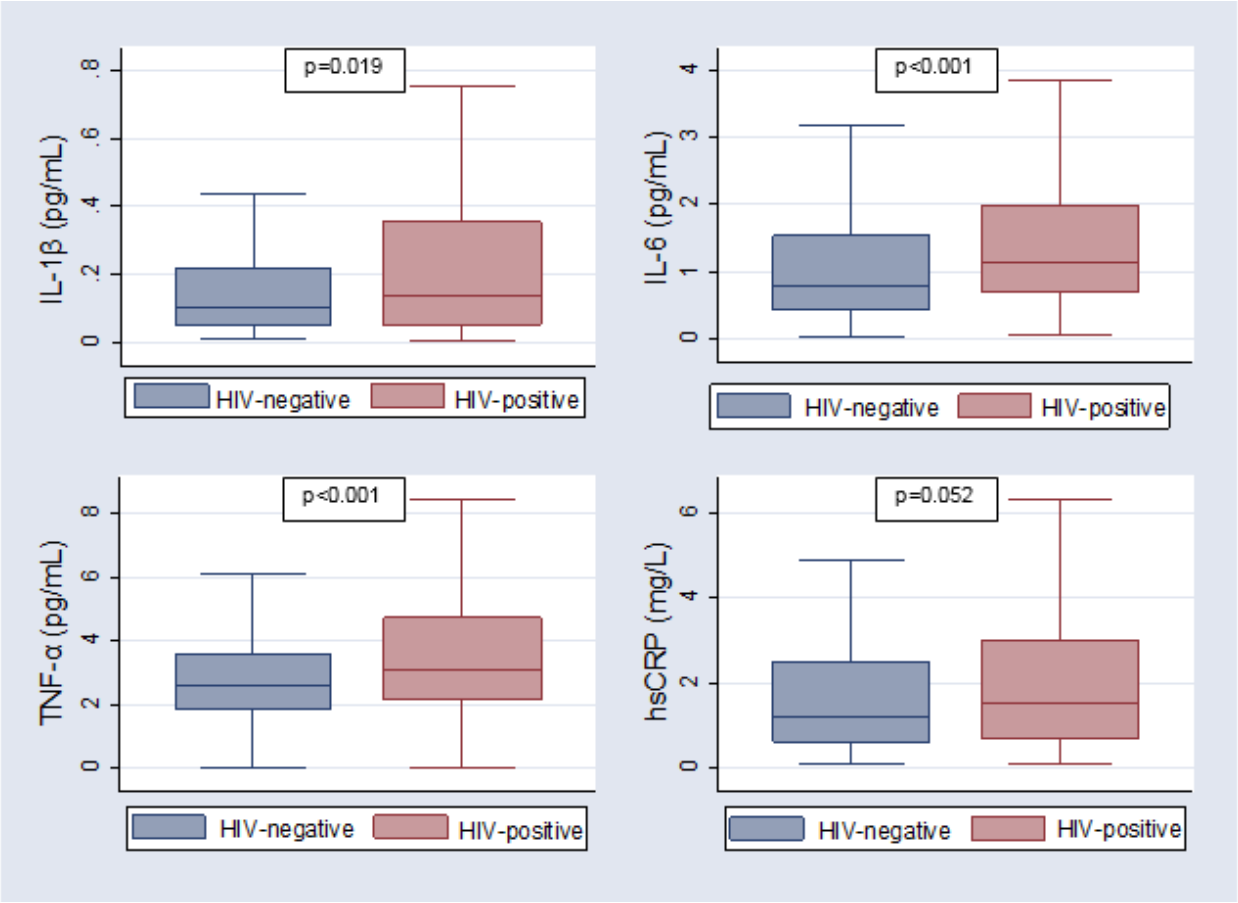
Triglycerides >150 mg/dL	0.15 (-0.15, 0.45)	.336	-0.02 (-0.34, 0.30)	.893

Table 2.3.4. Unadjusted and adjusted multivariable linear regression of factors associated with hsCRP.

	Unadjusted		**Adjusted**	
	Beta coef. (95% CI)	P value	Beta coef (95%CI)	P value
HIV status	0.20 (0.02, 0.37)	**.027**	0.24 (0.06, 0.42)	**.008**
Age (years)				
30-39	Reference		Reference	
40-49	0.28 (0.07,0.49)	**.010**	0.15 (-0.06, 0.37)	.161
50-59	0.54 (0.32, 0.77)	**<.001**	0.42 (0.18, 0.67)	**.001**
≥60	0.57 (0.28, 0.85)	**<.001**	0.59 (0.30, 0.88)	**<.001**
Sex				
Female	Reference		Reference	
Male	-0.21 (-0.38, -0.03)	**.018**	-0.06 (-0.25, -0.13)	.523
BMI (kg/m2)				
Normal (18-24)	Reference		Reference	
Underweight (<18)	-0.14 (-0.44,0.16)	.361	-0.19 (-0.48, 0.11)	.221
Overweight (25-29)	0.46 (0.25, 0.67)	**<.001**	0.43 (0.21, 0.64)	**<.001**
Obese (≥30)	0.84 (0.58, 1.11)	**<.001**	0.83 (0.56, 1.11)	**<.001**
Current smoker	0.02 (-0.19, 0.60)	.318	0.39 (0.01, 0.78)	**.050**
Alcohol use in past 12 months	-0.28 (-0.50, -0.58)	**.014**	-0.17 (-0.39, 0.05)	.131
Hypertension	0.29 (0.07, 0.50)	**.009**	-0.02 (-0.22, 0.18)	.857
Diabetes	0.08 (-0.33, 0.48)	.713	-0.04 (-0.41, 0.34)	.843
LDL>130mg/dL	0.28 (0.02, 0.53)	**.034**	-0.07 (-0.48, 0.34)	.733
HDL<50 mg/dL for males and <40 mg/dL for females	0.28 (0.08, 0.47)	**.005**	0.17 (-0.03, 0.37)	.089

Total cholesterol >200 mg/dL	0.22 (-0.01, 0.45)	**.057**	0.06 (-0.30, 0.44)	.727
Triglycerides >150 mg/dL	0.46 (0.12, 0.30)	**.003**	0.19 (-0.12, 0.50)	.228

Figure 2.1. Box plots of median and interquartile ranges of serum biomarker levels comparing HIV-positive and HIV-negative participants.



Abbreviations: IL-1β=Interleukin 1 beta, IL-6= Interleukin 6, TNF-α= Tumor necrosis factor alpha, hsCRP=high sensitivity C-reactive protein.

CHAPTER 4. Costs of CVD screening

Abstract

Background

Cardiovascular disease (CVD) screening identifies high-risk individuals early and has the potential to avert death and disability. We aimed to assess the cost of integrating screening for cardiovascular disease for primary prevention among people with and without HIV in a county referral hospital in Western Kenya.

Methods

The intervention in 2017-2018 based on CVD screening for hypertension, diabetes, and dyslipidemia was integrated into the Kisumu County Referral Hospital through the HIV clinic and outpatient department. This was done through CVD risk factor assessment using the World Health Organization STEPwise Survey questionnaire, blood pressure and anthropometric measurement, and a blood draw for fasting lipids, and fasting glucose. We conducted microcosting from a societal perspective using expenditures and activity-based costing to estimate the economic costs during program implementation. We collected cost data from expense reports and interviews with staff and participants. A time and motion assessment was used to estimate both health system and patient resource use.

Results

The incremental cost of integrating CVD screening for 598 participants from established outpatient and HIV services in one Kenyan clinic was $21,097 per year, with a cost per beneficiary screened of $35.28. The major cost drivers were laboratory testing (74%), personnel (12%) and start-up costs (5%). When laboratory testing is limited to high-risk individuals, defined as 45 years or older and BMI greater than 30, the cost was reduced by 40% to $21.36 per person screened. The cost per person diagnosed with hypertension was $131.04 and the cost per person diagnosed with diabetes was $753.46. If we are to offer CVD screening to all PLHIV using the high-risk screening, it would cost Kisumu County Hospital $462,200 against an annual budget of $ 720,000

Conclusion

While CVD screening allows early identification of persons at risk of CVD, the screening costs alone are substantial and not affordable in LMICs. To scale up this intervention, local, regional and global efforts are needed to drive down the cost of laboratory testing. Future research must also explore the best mix of non-laboratory versus laboratory-based CVD screening, as well as adoption of more innovative testing methods, such as point of care tests.

Introduction

Cardiovascular disease (CVD) is a leading cause of mortality and morbidity with over 17 million deaths reported in 2016.[1] In contrast to marked reductions observed in high-income countries and resource-limited counties, age standardized death rates from CVD have remained stable, and perhaps increased, in sub-Saharan Africa since 1990.[1,2] Ischemic heart disease and stroke accounted for 85% of all CVD deaths in 2016 [1] while high systolic blood pressure is a leading risk factor of CVD followed by a high plasma glucose and high body mass index (BMI).[26] CVD appears to be more common in the setting of HIV infection, with people living with HIV (PLHIV), whether treated or untreated, having at least a 2-fold increased risk of CVD compared to HIV-negative individuals.[23,24]

Kenya is one of the lower middle-income countries (LMICs) where rising non-communicable diseases (NCDs) including CVD rates have become a particularly challenging health problem, accounting for 60% of the total burden of disease in terms of Disability-Adjusted Life Years (DALYs) lost. Since NCDs, including CVD, disproportionately affect working age adults, this rise in NCD related morbidity and mortality has particular-rly significant economic implications for LMICs. It results in increased costs to the health system, increased out of pocket patient costs resulting in catastrophic health expenditures as well as indirect costs related to loss of productivity.[124] This increase in burden of mortality and morbidity due to CVD coupled by HIV poses an increased burden to the health system due to an increase in health needs for health services, human resources, and financing and may necessitate an organizational shift of the health system to accommodate longitudinal care. Clinical integration of CVD screening into health programs including HIV care is one strategy that can result in early identification of high-risk individuals leading to reductions in costs to the health system and the individuals.[125]

Few studies have evaluated the cost of CVD screening in SSA and more so among PLHIV.[126] In addition, several studies screened for hypertension and/or diabetes, and very few included the screening for dyslipidemia in addition to diabetes and hypertension screening. [127,128] Cost of CVD screening were variable

depending on the profile of diseases screened and the facility ownership as either public or private. A study in Nairobi including the national referral hospital and one health center, screened for hypertension and diabetes and found the cost of CVD screening varied from $4 to $36 dollars.[127] This is similar to other studies in Kenya.[128] These costs were borne by the patient and were largely unaffordable. [127] In addition to hospital based CVD screening, screening has been integrated in community based campaigns or home based testing strategies.[129] One cost effectiveness study in Kenya modelled the potential integrated HIV/NCD diagnosis and management program based on the community based SEARCH project and found it was cost effective. [130] CVD screening has been reported to be cost effective in SSA, [131] including studies that included CVD screening by community health workers.[132] To further reduce the cost of CVD screening, Gaziano et al also compared the use of laboratory based versus non-laboratory based risk assessment tools and found good correlation and hence an alternative for CVD screening.[133,134]

This analysis aims to provide the much-needed information on the incremental cost of integrating CVD screening into outpatient departments and HIV clinics in Kenya. The target audiences are policy makers, program managers at NCD and HIV programs, clinic managers and social insurance programs. The data on costs can then be used inform a budget impact analysis and economic evaluation of integration of CVD prevention that can be used by policy makers. These costs can also be used by health clinics for planning, government for budgeting and strategic planning, researchers for cost estimations, and reimbursement schemes for medical insurance coverage. The specific objective of this study is to evaluate the incremental costs of integrating CVD screening into established outpatient and HIV services in Kisumu County Hospital, Western Kenya. These data can be used to inform program and financial planning for Kenya and other countries interested in implementing a similar program.

Methods

Study design and scope

We collected cost data on CVD screening alongside a cross sectional study of 598 HIV-positive and HIV-negative adult participants from Kisumu County hospital, a large public referral facility in a high HIV prevalence region in western Kenya. During the period from June 2017 to May 2018, participants received screening for CVD for primary prevention of CVD that included collection of social, demographic and CVD risk factor information through the Kenya adapted WHO Steps Survey questionnaire, blood pressure and anthropometric measurement, and a blood draw for fasting lipids, fasting glucose, and inflammatory markers. This was in addition to their regular service at either the HIV care clinic, if HIV positive, or the outpatient department, if HIV-negative. This would allow early diagnosis and treatment of hypertension, diabetes or elevated lipids (dyslipidemia) as well as provide evaluation of risk factors that can be used to target lifestyle modifications and pharmaceutical management. We did not collect data on CVD treatment costs, as we did not follow up with participants over time. In addition, inflammatory markers that were included in CVD screening for research purposes were not included in the cost analysis, since they were not considered part of the routine CVD program. The details of the cross-sectional study on 'Cardiovascular Disease among HIV-positive and HIV-negative Kenyan Adults' are published separately.[109]

Perspective and type of costs

This micro-costing was conducted from a societal perspective and captured the incremental costs incurred by this intervention regardless of who paid for the costs. We followed the principles for best practices based on the Global Health Cost Consortium (GHCC) Reference Case for Global Health costing.[135] While this intervention is currently in the Kenya Ministry of Health Essential Package for Health, individuals still incur high out of pocket expenditures for seeking CVD screening and treatment services.[127] We collected direct and direct non-medical costs borne by both the third party payers (including government) and paid out-of-pocket by patients (e.g. registration fees, consulting fees, procedure fees, radiology fees), time costs of patients in seeking and receiving care, time costs of informal (unpaid) caregivers, transportation costs, childcare costs, lost income while seeking care, and other costs.

We used a bottom-up approach to assess the actual amount of resources to produce each service i.e. the time spent by each staff on each activity and allocated cost of CVD screening. These were divided into mutually exclusive categories of startup (microplanning, awareness raising, training and material development), recurrent (personnel, supplies, transportation/supply chain management costs, equipment, overhead, refresher training, communication), and patient costs (Table 3.1).

From these, we estimated the total incremental economic and unit costs for integrating CVD into existing clinical services. We present information on the intervention costs (cost per person screened), and the cost per by implementation phase (startup vs. recurrent) and by input cost category. We also present the average weighted costs for the HIV clinic (HIV-positive participants) and outpatient department (HIV-negative participants).

Time period

The costing captures both start up and recurrent costs collected over a period of 1 year from June 2017 to May 2018. The startup captures all costs incurred before the first screening occurred while recurrent costs are captured during the remaining implementation period.

Data sources

Costs were drawn from Ministry of Health budgets, project expense reports, and interviews with staff and participants. We estimated the opportunity costs for resource used by staff already paid by the government. A standard questionnaire was used to collect data including data on personnel time, laboratory tests radiological procedures, training, buildings, equipment, transport, supplies, operations and maintenance costs. This was accompanied with document review and observations. A patient questionnaire was administered to the participants to collect out of pocket expenditure i.e. the amount paid by the patient during the visit for any service related to the visit out-of-pocket costs. This included data on cost of registration, procedures, transportation costs, cost of childcare or dependent care, cost of accompanying

companion, and the lost income of both the patient and accompanying companion while seeking care. In addition, a time in motion study was conducted prospectively to monitor the contact time spent with the health staff and the client waiting times during the visit including routine clinic visit, screening, and laboratory testing. The prices of items were obtained from hospital invoices and price lists or published literature or international price lists. We valued staff time using Kenyan Ministry of Health salary scales, as reviewed from the payroll.

We determined the proportion of personnel and data collection activities that were allocated to research and CVD screening activities based on staff time spent. Research time (administering informed consent, reimbursements, etc.) and other research costs (taxi costs, printing consents and study materials) were removed from the programmatic costs based on the allocation.

Capital costs, software development, and start-up cost (staff hiring, training, and community mobilization) were annualized assuming a 5-year useful life years, discounted annually at 3% and applied the appropriate annualization factor (4.58).[136,137] Where costs were collected in Kenya Shillings, they were converted to 2018 US dollars using an average exchange rate of 101.3 Kenyan Shillings per US dollar using World Bank exchange rates. Costs for supply chain costs collected in 2015 were inflated to 2018 US dollars (USD) using the Kenya GDP deflator.[138]

Health outputs and outcomes

The health outputs and outcomes of this cost data collection were obtained from the larger cross-sectional parent study.[109] These include: 1) number of people screened, 2) number of people diagnosed with cardiovascular disease (hypertension, diabetes or dyslipidemia i.e. elevated lipids). The number of beneficiaries reached was the total number of individuals who were screened for CVD in the HIV clinic (HIV-positive) and the outpatient clinic (HIV- negative).

Data Analysis

Cost Analysis

We estimated the a) the annual incremental economic costs of cardiovascular diseases screening ; b) cost per person screened; and c) cost per person diagnosed. The cost per person screened is equal to the total incremental program costs were divided by the number of persons screened. The cost per person diagnosed is equal to the total incremental program costs were divided by the number of persons diagnosed with cardiovascular disease. We also present cost shares defined as the share of resources allocated to each activity or each input as a percentage of the total cost.

First, we conducted a one-way sensitivity analysis to explore how the cost of CVD screening changes with variations in cost inputs and assumptions. We varied the cost inputs of laboratory costs between $17 to $39 based on available literature and international pricing and this is presented in a tornado diagram. We also explored the impact of introducing CVD on client load by reducing the number of patients seen per day by 12.5%. Next, we present four scenarios for options that may be more feasible and less costly to implement in a public health program. The first option screens all KCH outpatient and HIV clients using self-reported risk factor screening, blood pressure, weight, height and waist circumference measurement for all individuals, but only conducts laboratory screening for diabetes and dyslipidemia (fasting blood glucose and lipids, respectively) for high risk individuals, defined as those aged 45 years and above, and are overweight or obese and without previous history of cardiovascular disease. In a second scenario, instead of using non-laboratory based screening as in scenario 1, we only conduct laboratory testing for those with an atherosclerotic CVD risk (ASCVD) risk score of >7.5%. In scenario 3, we include a scenario of screening of all PLHIV using the high-risk criteria used in scenario 1. This is because PLHIV are considered at twice increased risk of CVD compared to the general population. The fourth scenario presents an option for future investments. It combines the baseline costs plus screening for inflammation using high sensitivity C-reactive protein. Inflammatory markers are not routinely performed as part of the current public health program, but this option explores the costs to support targets for treatment.

All analyses were conducted in Excel (version 16.5, Microsoft, Redmond, WA).

The Ethics and Research committee at the Kenyatta National Hospital and the Institutional Review Board at the University of Washington approved this study, and clients participating in structured interviews provided written informed consent.

Results

We estimated the annual incremental and unit costs of screening 598 participants in Kisumu County hospital, who received CVD screening as part of the cross sectional study, 300 people living with HIV, who were screened in the HIV clinic and 298 HIV-negative individuals, who were screened as part of outpatient department services. Out of these, 286 HIV- positive and 277 HIV-negative individuals had a blood draw for testing fasting lipids, glucose and inflammation.

Total economic and unit costs for CVD screening

We present the incremental total and unit cost of CVD screening in Table 3.2. We present the costs of CVD screening without inflammation. These costs are presented by implementation phase (startup vs. recurrent) and by input cost category.

The estimated total annualized cost of CVD screening was $21,097 for 598 participants with a cost per beneficiary screened of $35.28. The cost per person diagnosed with hypertension was $131.04 and the cost per person diagnosed with diabetes was $753.46 (Table 3.3).

Cost profile by implementation phase and cost category

Figure 1 presents the cost profile by implementation phase. Recurrent costs accounted for 91% of the CVD costs with startup cost and patients cost accounting for 5% and 4%, respectively. Figure 2 presents the cost

profile by input. Three-fourths of the economic cost is attributed to laboratory supplies, followed by 12% for personnel cost, startup (5%) and patient cost (4%).

While the service delivery personnel time and other recurrent costs were similar for HIV-positive and HIV-negative participants, the patient costs were slightly higher among HIV-negative participants ($1.31) as compared to HIV-positive participants ($1.24). This was mainly due to out of pocket payment of laboratory test and medicines that are paid by participants going through the outpatient` department. The laboratory and medicine costs for HIV-positive participants are mostly part of the HIV program. The cost profile by cost category disaggregated by HIV status is presented in figure 3.

Health workers spent an average of 18 minutes providing NCD screening to clients, regardless of HIV status (Table 3.4). The total clinical service delivery time for HIV-positive individuals in the HIV clinic was 170 minutes and this was 128 for HIV negative individuals seen at the outpatient clinic. CVD screening this accounted for 11% of the total clinical service delivery time for screening HIV positive individuals in the HIV clinic and 14% for HIV negative individuals screened in outpatient services. This is presented in Table 3.4. PLHIV had a longer care time and traveled longer distances to health facilities than HIV-negative individuals. Assuming there are no system inefficiencies, integration of CVD screening may have an impact on the number of clients that can be seen per day, reducing the client load by up to 12.5%. We explore this assumption in the section below

Sensitivity and scenario analysis

We present a sensitivity analysis varying the cost inputs of laboratory costs between $17 to $39 based on available literature and international pricing and this is presented in Figure 3.4. When we varied laboratory costs to a low cost of $17, the cost of CVD screening was reduced by 26% from $35 to $26. Startup costs and patient costs were robust to any changes in laboratory costs. We also explored the impact of introducing CVD on client load by reducing the number of patients seen per day by 12.5%.When we varied the proportion of people screened per day by 12.5%, the total cost increased by 7%.

We conducted scenario analysis for two scenarios that focused on 1) laboratory screening for high risk individuals only, 2) ASCVD risk score to identify high risk individuals, 3) scaled CVD screening for all PLHIV, and 4) baseline scenario plus screening for inflammation using high sensitivity C-reactive protein. For scenarios 1 to 3, we limited input costs of laboratory tests to high risk individuals while the scenario 4 includes screening all participants. These scenarios are presented in Table 3.5.

Scenario 1: Public health scenario that prioritizes those high-risk individuals using non-laboratory based criteria.

In this scenario, everyone receives screening for hypertension and CVD risk factors, but laboratory testing is limited to high risk individuals. By limiting the screening for diabetes and dyslipidemia to only those at least 45 years of age and are either overweight or obese with no previous history of cardiovascular disease, the total cost reduced to $12,771 with a unit cost of $21.36. This represents a reduction in costs of 40%.

Scenario 2: Public health scenario that prioritizes those high-risk individuals using the Atherosclerotic CVD risk score (ASCVD).

In this scenario, everyone receives screening for hypertension and CVD risk factors, but laboratory testing is limited to high risk individuals as defined by the ASCVD risk score. In the parent study, we estimated that 16% of participants had either an intermediate ASCV risk score of 7.5%-20% or a high risk ASVD risk score >20% that may be considered for statin therapy. By limiting the screening for diabetes and dyslipidemia to only those with an ASCVD risk score of >7.5%, the total cost reduced by 50% to $10,400 with a unit cost of $17.39.

Scenario 3: Screen all PLHIV with available resources.

Focusing on screening all PLHIV who are considered a high-risk population in KCH once annually with the available human resources, we could reach 23,944 (78.5% of the total 30,666 patients). Assuming we reach this higher number of patients, it would drive the startup cost down to $0.05 per person screened. In

addition, screening 65% of these population for diabetes and dyslipidemia, would reduce the total unit cost per person to $19.23. The total cost to screen 78.5% of PLHIV in Kisumu county hospital for CVD annually, would cost an approximate $462,200 against an annual budget of $720,000. This accounts for 64% of the hospital budget.

Scenario 4: Baseline scenario plus screening for inflammation using high sensitivity C-reactive protein only.

By including high sensitivity C-reactive to screen for inflammation, it increases the cost of screening from the baseline scenario by 50%. The total cost of CVD screening would be $31,208 and the cost per beneficiary would increase to $52.19.

Discussion

Our study empirically estimated the cost of CVD screening intervention implemented at both HIV clinic and outpatient departments in a large county referral hospital in Kisumu, Kenya. The average incremental economic costs of integrating CVD screening into HIV and outpatient services was $35.28 per person. If we limited the screening of diabetes and dyslipidemia to high risk individuals, it would reduce the cost per beneficiary by 40% to $21.36. Screening all PLHIV in KCH with available resources, would result in a unit costs of $19.23 and would cost Kisumu county hospital an estimated $462,000 which was unaffordable. CVD screening for all PLHIV would account for 64% of the annual budget for Kisumu County Hospital is $720,000. While there are global efforts underway to reduce costs of drugs for hypertension, diabetes and dyslipidemia in LMICs, this analysis demonstrates that there is a need drive down the laboratory cost of screening to allow early screening and treatment of more individuals. Without this advocacy, CVD screening is not affordable in resource limited settings. Our study therefore provides data on the need to focus on planning and gathering support for case identification and their subsequent treatment in SSA.

The World Health Organization estimates the cost of screening for CVD limiting to those with high CVD risk score of greater than 30% is $3.9 for LMICs.[139] The WHO screening cost is underestimated based on our study and available published literature. Previous studies in Kenya and SSA on CVD screening show similar costs to this current analysis. Jingi et al from Cameroon studied access to diagnostic tests for CVD and found the average cost of test including total cholesterol, HDL and triglyceride was $18.21.[140] A study in Tanzania reported the economic costs of CVD primary prevention and treatment was $71 with medication costing $55 and laboratory monitoring costing $16.[141] A study in a slum in Kenya estimated the cost of awareness and screening of hypertension using a community based approach was $17.[128]

While we estimate a cost of $21 per beneficiary screened for CVD, the affordability of this intervention will depend on the ability and willingness of the government and the funding agencies to support CVD screening. This cost can be greatly reduced by driving down the costs of laboratory testing. This could be achieved by adapting technology such as use point of care tests that reduce time taken for blood draw and eliminates the need for a return visit to get results and hence faster care. In addition, when the intervention is scaled up, this could benefit from the economies of scale to reduce costs of laboratory supplies. Health system could also adopt a non-laboratory-based screening protocol using age and other risk factors for the general population and limit laboratory testing to very high-risk individuals.[142] Further research is needed to explore the best mix of population wide screening and high risk screening as suggested by Feigin et al[143]. Other modifications to drive down cost of CVD screening include further simplification of the CVD screening process so as to utilize a task shifting approach with community health volunteers in community settings.[144,145] Cost can also be reduced through system efficiencies such as utilizing wait times through a process mapping experience. Finally, scaling up universal healthcare coverage will allow financial protection of individuals and can leverage on this platform to negotiate for lower prices.

Our study has several strengths and limitations. One strength in our study is we collected prospective patient based primary data which provides robust and reflect actual program-based costs. The main study limitations are that this study included costs from only one health facility and hence it may not be

representative of facilities in the country with difference prevalence of CVD and with different clinic volumes. However, it gives a good estimation of costs for referral facilities that cater for majority of patients diagnosed with hypertension and diabetes. Another limitation is that we did not calculate treatment cost per diagnosed case, the cost per complication averted e.g. stroke or coronary heart diseases, or labor productivity gains. NCD screening will increase health system costs through both screening and treating more individuals. In general, treatment costs for hypertension cost on average $40-60 per person per year, while average treatment costs for diabetes are more expensive at $9-43 per person per year.[44] Health systems will incur new service delivery and logistical costs associated with providing treatment. However, early screening and treatment of NCDs can reduce hospital workload of more serious CVD events which are life threatening and costly. Identifying and treating cases, before they become severe in the longer term, has the potential to be cost saving.[146] It would also reduce productivity losses associated with a healthier population due to reduced absenteeism and presenteeism at the work place.[44] The cost of treatment and labor productivity will be explored in a full economic evaluation in the future.

Future research should also explore the evaluation of innovative laboratory based versus non laboratory-based screening methods that may be more affordable. To scale up screening in SSA, there needs to be a concerted effort locally, regionally and globally to harness market forces and reduce cost of laboratory testing of diabetes and dyslipidemia to levels that are affordable to LMICs. This can be tackled to ongoing discussions to bring down costs of medicines for CVD.

Conclusion

Our study estimated the incremental economic cost of implementing CVD screening at a county referral hospital in Western Kenya. The cost of CVD screening is substantial, need to consider these in the light of growing number of deaths and disability due to CVD in Kenya. Further integration and scale up may reduce costs substantially with adoption of a mix of population based screening and high-risk laboratory-based screening. This can be achieved through national, regional and global efforts to reduce the cost of laboratory

tests as has been achieved for HIV testing including point of care rapid tests. These cost data can be used for budgeting as Kenya embraces UHC.

Acknowledgements

We would also like to express our gratitude to Kisumu county hospital administration and laboratory team for input into the study design, and the study participants

Funding

This work was supported by grants from the US National Institutes of Health (R21TW010459) and Fogarty International Center (D43 TW009580). The finding and conclusions in this manuscript are those of the authors and do not necessarily represent the views of the Government of Kenya or the National Institutes of Health.

We declare no conflict of interest.

Table 3.1. Cost categories and data sources.

Data Type	Source	Collection Methods
Provider costs- Direct Medical costs		
Start up ● Microplanning ● System development ● Training cost ● Sensitization Recurrent ● Personnel costs- Salary/time of health personnel, hiring and training ● Equipment-blood pressure machines, height and weight meters, tape measures ● Overhead costs including rent and internet ● Supplies-lab and testing consumables e.g. gloves, gowns, swabs, lab-blood collection tubes, cryovials, gloves, safety boxes, ● Laboratory testing costs- fasting glucose, fasting lipids, inflammatory markers ● Supply chain costs ● Refresher training	Expense Reports and Receipts Salary records Time in motion studies Expense Reports and Receipts Medical records, pharmacy records	Review of reports, staff interviews. Facility admin records on staff salaries Literature review Central Price Lists Ministry of Health salary scales
Patient costs		

<u>Direct medical costs</u> ● Out of pocket costs-registration, consulting fees, procedure fees, radiology fees	Patient questionnaire	Review of reports, patient and staff interviews.
<u>Direct non-medical costs</u> ● Transportation costs ● Meals ● Cost of childcare or dependent care ● Cost of accompanying companion-lost income	Patient questionnaire	Interview of patients

Table 3.6 Total economic costs and unit costs of cardiovascular disease screening (hypertension, diabetes and dyslipidemia).

Cost category	Total Facility Cost ($) N=598	% of Total facility cost	Unit cost ($)
Start-up			
Microplanning	285.75	1%	0.48
System Development	243.87	1%	0.41
Initial Training	320.52	2%	0.54
Sensitization	274.55	1%	0.46
Sub-total start-up	***1,124.70***	***5%***	***1.88***
Recurrent			
Personnel (net costs include salaries, benefits/allowances)			
Service delivery	1,480.56	7%	2.48
Program support	1,029.84	5%	1.72
Supplies	15,569.67	74%	26.04
Equipment	214.89	1%	0.36
Transportation/Supply chain costs	161.46	1%	0.27
Communication (internet costs, mobile phone minutes)	135.04	1%	0.23
Refresher training	66.51	0%	0.11
Overhead (electricity, utilities, etc.)	550.84	3%	0.92
Sub-total recurrent	***19,208.82***	***91%***	***32.12***
Patient costs			

Patient costs	763.1	4%	1.28
Sub-total patient costs	***763.1***	***4%***	***1.28***
Total	**21,097**	**100%**	**35.28**

Table 3.3 Summary of costs of diagnosing hypertension and diabetes

Unit costs	No of beneficiaries	Cost per beneficiary ($)
Screening		
HIV-positive	300	36.28
HIV-negative	298	36.35
Diagnosis		
Hypertension	161	131.04
Diabetes	28	753.46

Figure 3.1. Cost profile by implementation phase

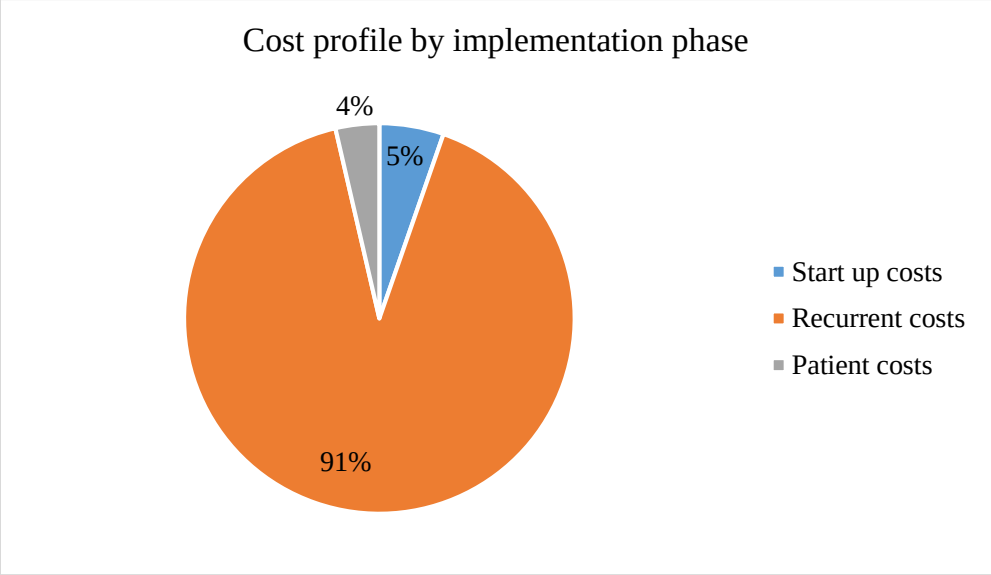


Figure 3.2 Cost profile by input cost category

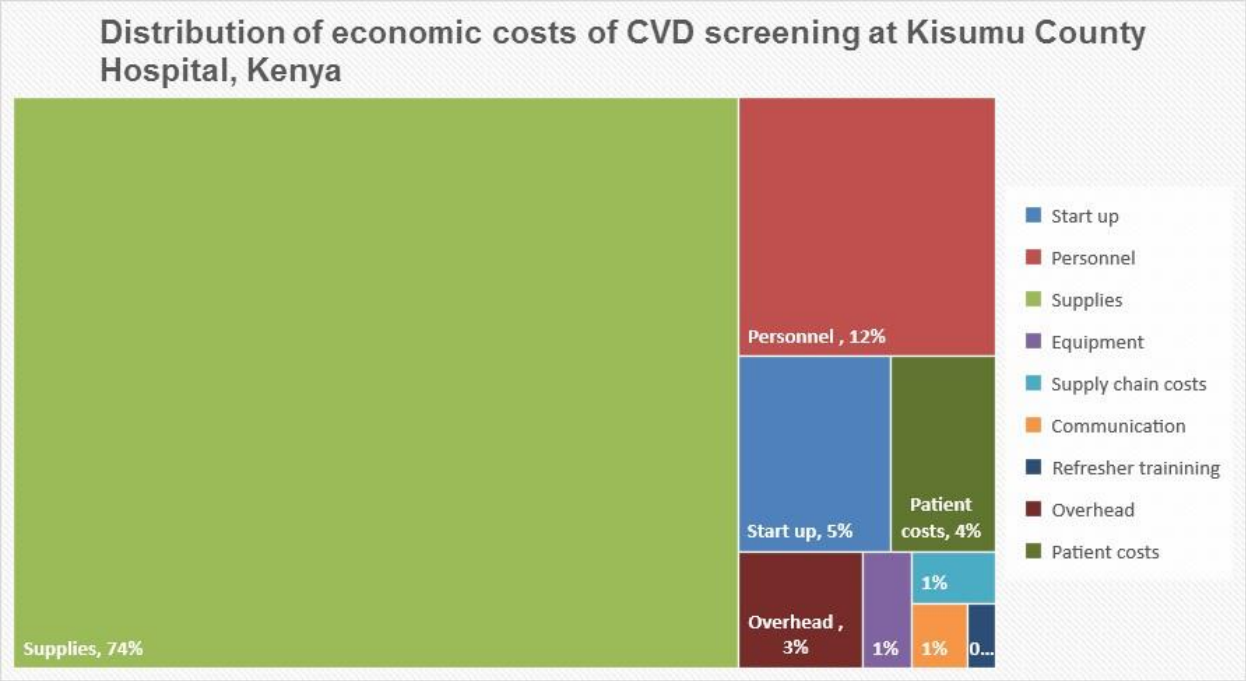


Figure 3.3 Cost profile by cost category disaggregated by HIV status

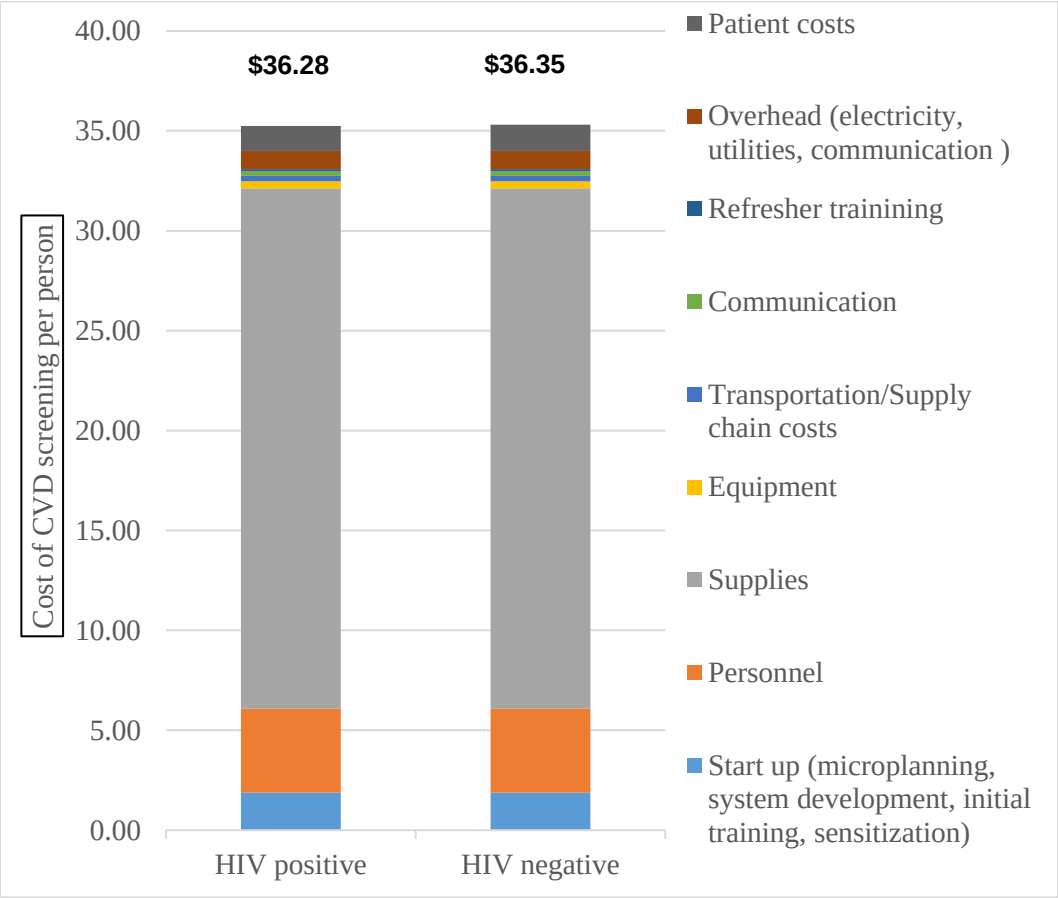


Table 3.4. Client time of CVD screening.

	HIV-positive participants	**HIV-negative participants**
	Average time (minutes)	**Average time (minutes)**
Patient time with provider		
NCD screening time		
Baseline questionnaire (data collection)	3	3
Physical measurements (Blood pressure reading, weight/height, waist and hip circumference)	8	8
Blood draw and laboratory testing	7	7
*Total care time (from entry to facility to exit)**	170	128
Patient travel		
Transit time 2-way (minutes)	100	47
Average distance to facility (kms)	13	7

*Includes waiting and consultation time

Figure 3.4 One-way sensitivity analysis

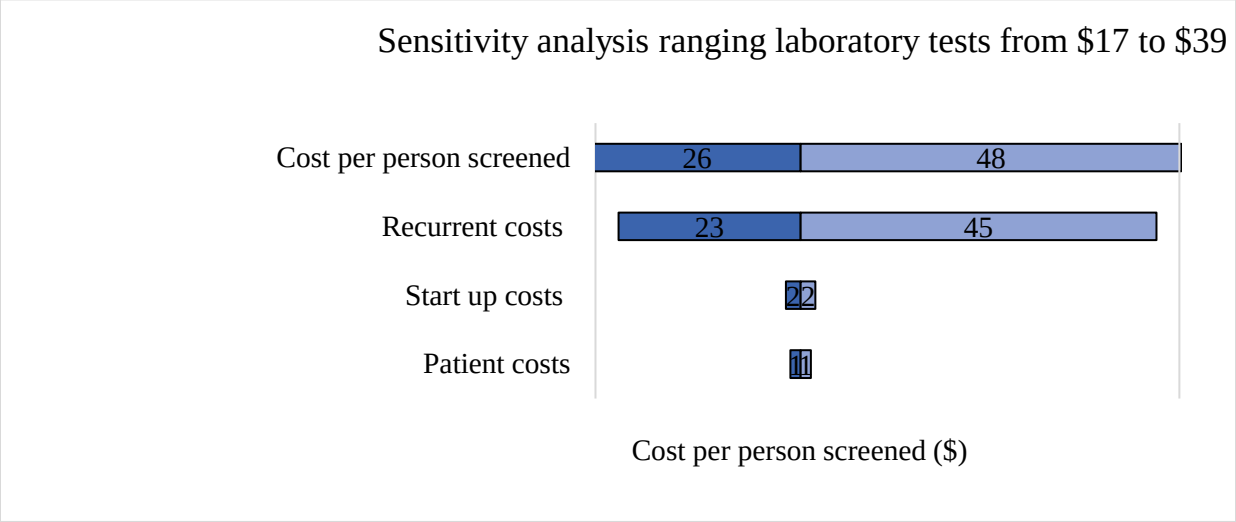


Table 3.5 Scenario analysis

Scenario	No of beneficiaries	Total cost ($)	Cost per beneficiary ($)
Baseline case scenario: Screening without inflammation	598	21,097	35.28
Scenario 1: Screening high risk individuals using age and BMI	598	12,771	21.36
Scenario 1: Screening high risk individuals using ASCVD risk score >7.5%	598	10,400	17.39
Scenario 3: Screening only PLHIV with high risk scenario	23,944	462,200	19.23
Scenario 4: Screening with inflammation (hsCRP only)	598	31,208	52.19

Chapter 5. Conclusion

This dissertation provides evidence on the burden of CVD risk factors among people with and without HIV and the incremental costs of integrating cardiovascular disease screening in Kisumu County Hospital. It describes various approaches to screening for CVD risk: 1) screening for traditional risk factors and using the metabolic syndrome criteria, 2) calculating the 10-year cardiovascular risk profile, and 3) performing laboratory assays to determine levels of inflammation. This collection of studies reveals a high burden of cardiovascular risk factors among older adults in Kisumu, including hypertension, dyslipidemia and abdominal obesity. This was true for people living with and without HIV infection. We report that while the prevalence of MetS was high in both HIV-positive and HIV- negative individuals (1 in 20 and 1 in 10 respectively), PLHIV were less likely to have metabolic syndrome than HIV negative participants. We also report that PLHIV had a lower ASCVD risk score than the HIV-negative participants. When we screened for inflammation, we found that PLHIV exhibit significantly higher levels of inflammatory markers than HIV-negative individuals, even after years of suppressive ART, suggesting a chronic, low grade inflammation. In addition, higher levels of inflammation were also associated with older age, male sex and overweight and obesity. This was also associated with high level viremia among PLHIV.

Our finding that PLHIV had a lower prevalence of MetS and lower ASCVD risk score were contrary to what we hypothesized and hence warrants further investigation. Our study findings suggest that there may be different pathways to CVD in PLHIV and HIV-negative individuals and hence the need to account for this during CVD screening. Among PLHIV, the inflammatory pathway may be contributing more to CVD pathology than the metabolic pathway, while traditional risk factors may greatly contribute to CVD risk in HIV-negative individuals. While the study answered key questions on the burden of risk factors, additional research is needed to develop and validate a tool that allows identification of high risk individuals but also can be used for population-wide screening. Further research can focus on utility and validation of non-laboratory based risk scores and their cost effectiveness for CVD screening. In addition, we may consider a risk score that incorporates inflammatory markers such as hsCRP to screen for inflammation in PLHIV.

To validate this risk assessment tool, we require data on intermediate outcomes (such myocardial infarction and stroke,) as well as long term outcomes (such as death) in people with and without HIV infection in SSA. This can be achieved through longitudinal studies, development of CVD disease registries, and using mortality data. Ideally, we would track both the metabolic and inflammatory markers over time and investigate these different pathways, as well as evaluate the use of inflammatory markers for prediction of cardiovascular events and mortality. This would require a regional collaboration to make it successful.

This dissertation also provides estimates on the costs of CVD screening in HIV clinics and outpatient clinics. Our choice of population and screening approach was driven by the profile of traditional risk factors and inflammation found in the study. We found that the costs of CVD screening for all individuals are substantial and not affordable. There is a reduction in costs when we limit laboratory based screening for high risk individuals. However, regardless of screening approach used, the greatest cost driver was the laboratory tests. Advocacy by governments, international organizations, and civil society organizations is needed to drive down the cost of laboratory testing to make it more affordable. This can be also be achieved through adoption of technology and the roll out of universal health care. Further research needs to find the best mix of population-wide screening and high-risk screening, especially in resource constrained settings. Validation of a CVD risk score that is non-laboratory based may be the most affordable approach to scale up CVD screening in LMICs.

Following this dissertation, I would like to explore the use of non-laboratory versus laboratory testing for CVD screening as well as evaluate the impact of risk reduction interventions targeting hypertension, obesity and dyslipidemia on reducing CVD risk through an implementation science study. In addition, I would like to conduct a cost effectiveness analysis comparing these various screening methods as well as the risk reduction interventions vs the standard of care. Finally, I would like to continue to explore the validation of a risk prediction tool for SSA. The work I have done to complete this dissertation positions me well to embark on studies that will address these questions.

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