

# Evaluation of Biochemical Markers of Cardiac Risk in ART-Treated HIV-Infected Adults in Rwanda

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## Research

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# Abstract

## Background

Life expectancy of people living with HIV infection has improved due to access to antiretroviral therapy (ART) in Rwanda like other African countries. However, both HIV infection and use of ART are associated with cardiovascular disease (CVD) risks, due to adverse changes in some biochemical markers, causing dyslipidemia and other metabolic imbalances. The CVD risk associated with metabolic biomarkers that may affect cardiac function with use of the ART, has not been well characterized in Rwanda. We evaluated the association between the use and duration of ART and abnormal changes in biochemical markers of CVD risk among HIV infected adults in Rwanda.

## Methods

Participants were enrolled from HIV Clinics Public Health Centers in a cross-sectional study in Kigali. A total of 150 participants between 18-45 years included 30 HIV-Uninfected (HIV-) and 120 HIV-infected (HIV+) adults. Among the HIV+ adults, 40 participants were ART-naïve. Data were collected on health-related behaviors and biochemical markers of CVD risk. We compared changes in CVD-related biochemical markers between HIV-, HIV+ ART-naïve and HIV+ on ART treatment groups.

## Results

Majority of participants were women (60%), and HIV- were younger ( $35\pm 6$  vs.  $31\pm 6$  years). We observed differences in levels of cholesterol and triglycerides in HIV+ ART-treated and HIV+ ART-naïve groups. Total cholesterol and triglycerides were associated with use of ART. Serum triglycerides were lower in HIV+ ART-naïve compared to HIV+ on ART treatment ( $61.20\pm 18.30$  mg/dl vs.  $85.00\pm 38.30$  mg/dl;  $p < 0.01$ ). While total cholesterol was higher in HIV+ on ART than HIV+ ART-naïve ( $136.00\pm 45.00$  mg/dl vs.  $119.00\pm 36.00$  mg/dl;  $p < 0.04$ ), HDL-C was associated with longer exposure to ART ( $68.70\pm 30.00$  mg/dl vs.  $54.90\pm 25.70$  mg/dl;  $p = 0.02$ ) among HIV+ on ART for 0-6 months and 7-12 months respectively.

## Conclusion

Changes in serum total cholesterol and triglycerides were associated with use of ART. Although these changes were within the upper limits of normal ranges, our findings suggest early increases in both biochemical biomarkers of cardiac risk associated. These findings underscore the need for early evaluation of lipid profiles as biomarkers of cardiovascular disease risk, to effectively monitor how ART may contribute to cardiovascular disease and deter treatment programs in African countries.

## Background

The number of people living with HIV infection continues to grow, and was estimated globally at 37.6 million [30.2 million–45.0 million] in 2020 by UNAIDS [1], with approximately 1.5 million newly infected by the year 2020, and 1 million deaths of HIV-related illnesses each year according to WHO [2]. The burden of the HIV epidemic in Sub-Saharan Africa (SSA) has been reported to be greater than anywhere else in the world, with an estimated 69% (23.5 million) of the global burden [3]. In Rwanda, population-based HIV impact assessment suggests a sustained general HIV prevalence of 3.0% among adults between 15 and 64 years over the last 4 years [4]. The prevalence is said to be much higher in females (3.7%) compared with males (2.2%), and higher in urban (4.8%) than rural areas (2.5%) [5].

Globally, there has been remarkable success in the scale up of HIV treatment, and by June 2020, 26 million people with HIV infection (HIV+) were receiving highly active antiretroviral therapy (HAART) worldwide [2]. Although the use of antiretroviral therapy (ART) has resulted in improved immunologic and virologic outcome and improved the wellbeing for HIV-infected individuals (HIV+), use of ART, particularly the first-generation antiretroviral medications has been associated with metabolic derangements [3],[5]. In particular, the extended use of Protease inhibitors (PIs) and nucleoside reverse transcriptase inhibitors (NRTIs) have been implicated as causes of metabolic disorders such as dyslipidaemia, hypertension and type 2 diabetes mellitus (T2D), which are risk factors for cardiovascular disease (CVD) [6],[7] such as coronary heart disease that results in myocardial infarction, heart failure, hypertension, stroke and other derangements [8].

The aetiologies of CVD are not always well known, however acute events of heart attacks and strokes are mainly caused by a blockage that prevents blood from flowing to the heart or brain. The most common reason for this is a build-up of fatty deposits on the inner walls of the blood vessels that supply the heart or brain [9]. This fatty deposit is due to elevated levels of cholesterol, triglycerides and/or high blood pressure [10]. Atherosclerosis develops following accumulation of LDL-C, the 'bad cholesterol,' in the lining of the blood vessels, which subsequently leads to its oxidization resulting in inflammation [11]. This inflammatory response is associated with white blood cells surrounding the LDL and the formation of a fibrous plaque [9],[11].

Whether the prevalence of CVD in people living with HIV on ART, is different from that in the general population, has been scarcely investigated [7]. A WHO report revealed that 17.9 million died from CVD in 2016, contributing about 31% of all deaths worldwide. Surprisingly, 82% of all CVD related deaths occurred in low and middle-income countries and up to 85% of all CVD deaths are due to heart attack and stroke [12].

There is a growing concern that HIV infection increases CVD risk [13],[14] Moreover, HIV patients are more prone to developing atherosclerosis than the general population [15],[16]. HIV infection may lead to chronic inflammation of the blood vessels, which is associated with elevated levels of LDL-C [17]. Coupled with high blood pressure, the LDL-C accumulation damages the artery walls [18]. Earlier studies have also identified an increased incidence of CVD in HIV-infected patients, exacerbated with extended use of combined antiretroviral therapy (cART) [9],[18],[19] Notwithstanding, ART has had a positive impact

on the extent of HIV-associated cardiomyopathies [7],[20]. However, the prolonged use of protease inhibitors (PIs), defined as any of the various drugs that inhibit the action of HIV protease, an enzyme necessary for replication of the HIV virus and nucleoside reverse transcriptase inhibitors (NRTIs) that are structural nucleoside analogues of DNA nucleotide which prevent reverse transcription of the HIV genome, thereby inhibiting the action of HIV-1 Reverse transcriptase (RT) and viral replication, leads to metabolic imbalances, resulting in elevated levels of LDL-C and low levels of HDL-C, which contribute to atherosclerosis, the primary cause of CVD [10],[21].

Despite a lack of sufficient data on CVD in people living with HIV in Rwanda, studies at regional level have shown that the most affected are women living with HIV at a proportion of about 58% in Sub-Saharan Africa [22]. While the information on what drives CVD in HIV infection remains limited, PIs are thought to account for 60% of metabolic changes that contribute to CVD among its users [23],[19],[24]. In the cohort study conducted by Vos et al [22] on HIV and risks of CVD in Sub-Saharan Africa, in HIV-infected patients the major cause for heart disease were traditional risk factors interacting with other mechanisms, such as pro-inflammatory effects of HIV infection, immune activation after initiation of ART and the confounding effects of different combinations of ART [18],[25]. These may predispose HIV individuals on ART to an accelerated or increased risk of CVD.

Given the paucity of data on the incidence of CVD in HIV patients on HAART in Sub-Saharan Africa, specifically in the context of potential biomarkers of disease risk, a cross-sectional study to assess biomarkers of cardiac risk in HIV+ on ART, compared to HIV+ ART-naïve and HIV-infected (HIV-) was conducted in health centers in Rwanda, involving HIV-infected individuals on ART.

## Methods

### Study Design and Participants

This was a health facility-based cross-sectional study conducted in seven health centers in urban Kigali city, Rwanda, serving as HIV infection clinics comprising Nyarugunga, Remera, Kinyinya, Masaka, Biryogo, Cornum, and Gikondo. Study participants included men and women aged between 18 and 45 years, and were either HIV-uninfected (-) or HIV-infected (+) on ART treatment or HIV+ ART-naïve. A total of 150 participants in the study included 30 HIV-uninfected individuals serving as the control group; 40 HIV-infected participants who were ART-naïve and 40 HIV-infected participants who were on WHO-recommended ART for 0-6 months, and 40 HIV-infected participants on ART for 7-12 months. Age and sex were considered during recruitment of study participants, to ensure good matches across each study group. Individuals presenting with active cardiac conditions, or significant symptoms of any heart disease such as hypertension (HTN), type 2 diabetes mellitus (T2D), or renal disease, and those with irregular ART adherence within 3 months prior to the recruitment period, were excluded from the study. Ethical approval was obtained from Institutional Research and Ethics Committee (IREC) at Moi Teaching and Referral Hospital (MTRH), Approval Number: 0003163, and from Rwanda National Ethics Committee (RNEC), FWA

Assurance No.00001973; IRB 00001497 of IORG0001100. Written informed consent was obtained from all study participants.

## **Study Procedure**

A structured questionnaire was used to collect data on social demographic characteristics (gender, age, marital status, residence, social economic status). Social economic status was defined based on ubudehe categorization in Rwanda. In the Ubudehe approach, there are 5 categories (ABCD & E) defined by a set of criteria, from the poorest category (without land, facing difficulties to have food) to the richer people [26]. Middle to high category = monthly income  $\geq$  65,000 Rwandan francs (RWF) (approximately 66 USD) to  $\geq$  600,000 RWF (607 USD) and  $\geq$  780,000 RWF (791 USD) to  $\geq$  7,200,000 RWF (7,302 USD) annual income. Low Category =  $\leq$  45,000 RWF (46 USD) to  $\leq$  65,000 RWF (66 USD) per month and  $\leq$  540,000 RWF (548 USD) to  $\leq$  780,000 RWF (791 USD) annual income [27]. Lifestyle (physical activity, smoking, alcohol consumption, eating habits) and clinical data (HIV clinical stage, high blood pressure (HBP). Elevated or high blood pressure (BP) was defined as the systolic BP of 140 mmHg or higher, and/or diastolic BP of 90 mm Hg, and/or being on hypertensive medications [28]. Alcohol consumption as taking a drink that contained alcohol for the last 6 months. [29]. Health diet defined based on how often are fruits and vegetables accompanying routine meals per week and unhealthy diet, defined as an impression of usually eating meals prepared with too much oil [30]. Where as physical activity participation was defined as vigorous-intensity exercise activities that cause large increases in heart rate like running, pedal cycle or football for at least 30 minutes continuously 3 times per week [31].

Blood specimens were collected for laboratory analysis of biochemical markers (NT-pro-BNP, hs-CRP, TC, HDL-C, LDL-C, TG, and glucose). Anthropometric measurements included weight and height, and body mass index (BMI) was calculated. BMI was calculated as weight in kilograms divided by height in meters ( $\text{Kg}/\text{M}^2$ ).

## **Blood Sample Collection and Analysis**

Blood samples were collected in the morning under overnight fasting conditions of at least 8 hours. Coded blood specimens were transported to the WiWo global Indian specialized hospital laboratory in Kigali, for subsequent processing and analysis. NT-proBNP, hs-CRP, TC, HDL-C, LDL-C, TG, and glucose levels were measured. Serum dry tubes were centrifuged at 10, 000 relative centrifugal force (RCF) for 10 minutes. The centrifuged specimens with a surface lipid layer were transferred into secondary tubes for Cobas C-111 clinical lipid profile analysis (Roche Diagnostics). NT-pro BNP and hs-CRP were assayed using a Maglumi-600 automated machine. Surplus serum was stored at  $-20^{\circ}\text{C}$  and  $-40^{\circ}\text{C}$  to allow for repeat testing as needed.

## **Data Management and Analysis**

The data obtained from both the questionnaire and biochemical assays, was cleaned, coded and stored in Microsoft Access. The data were imported into Statistical packages for social sciences (SPSS) for analysis (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM

Corp). Descriptive analysis results from categorical variables were presented using frequencies and proportions, continuous variables were presented using mean and standard deviation. Chi-square test was used to compare proportions of categorical variables and the significance threshold was considered for a p-value <0.05. The mean of biochemical markers was compared using independent sample t-test and Analysis of Variance (ANOVA). Significance difference was considered for a p-value <0.05 with corresponding 95% confidence interval. The correlation between biochemical markers and ART use was assessed using linear regression with Pearson Correlation test and significance threshold set to p<0.05.

## Results

### General Characteristics of Study Participants

The majority of the participants were female (60%). The HIV-uninfected (HIV-) participants were younger than HIV-infected (HIV+) participants ( $35\pm 6$  vs.  $31\pm 6$ ), and 44% of HIV- participants were young, aged between 26 and 33 years with mean age of  $32 (\pm 6)$  years. Most participants were city dwellers (83.3% vs. 16.7%). There was no difference between the proportion of participants who were either single or married and no difference between those with low or middle to high social economic status (Table 1). Table 2 summarizes the clinical and lifestyle characteristics of study participants. Majority of participants were in HIV stage I of the WHO HIV classification compared to those in HIV Stage 2 and above (82.5% vs. 17.5%) and none were either in HIV stage 3 or Stage 4 of illnesses. In addition, there was no difference in the proportion of participants with HIV Stage 1 or HIV Stage 2 for HIV+ ART-naïve and HIV+ on ART for 6 months or 12 months. Although the majority of participants had a normal BMI of 18.5-24.9 in  $\text{Kg}/\text{M}^2$  of 70% compared with the rest of participants, the HIV+ participants group had a significantly higher proportion of those with BMI in  $\text{Kg}/\text{M}^2$  less than 18.5 (Table 2). Although there was no difference between those with normal and high Blood Pressure (BP) between HIV- and HIV+ ART-naïve or on ART, 7.3% of participants were diagnosed with high BP, and the majority had normal Blood Pressure (BP) (92.7% vs. 7.3%). The HIV- participants were less likely to smoke compared to HIV+ (100% vs. 73.3%), and were less likely to consume alcohol (50% vs. 30.8%) with the overall majority of the participants reported to have had very limited physical activity (63.3% vs. 36.7%) (Table 2).

Table 1  
Socio-demographic characteristics of study participants (n=150)

<b>Variable</b>	<b>HIV- (n=30) n (%)</b>	<b>HIV+ ART-naïve (n=40) n (%)</b>	<b>HIV+ On ART 0 – 6 months (n=40) n (%)</b>	<b>HIV+ On ART 7 – 12 months (n=40) n (%)</b>	<b>Total n (%)</b>
<b>Gender</b>					
Male	14 (46.70)	17 (42.50)	15 (37.50)	14 (35.00)	60 (40.00)
Female	16 (53.30)	23 (57.50)	25 (62.50)	26 (65.00)	90 (60.00)
<b>Age in years</b> (Mean ± SD)	35±6	30±6	31±6	33±7	32±6
<b>Marital Status</b>					
Single	12 (40.00)	26 (65.00)	20 (50.00)	16 (40.00)	74 (49.40)
Married	18 (60.00)	14 (35.00)	20 (50.00)	24 (60.00)	76 50.60)
<b>Residence</b>					
Rural	2 (6.70)	11 (27.50)	4 (10.00)	8 (20.00)	25 (16.70)
Urban	28 (93.30)	29 (72.50)	36 (90.00)	32 (80.00)	125 (83.30)
<b>*Social Economic Status</b>					
Low	11 (36.70)	26 (65.00)	24 (60.00)	22 (55.00)	83 (55.40)
Middle to high	19 (68.30)	14 (35.00)	16 (40.00)	18 (45.00)	67 (44.70)
<p>*In the Ubudehe approach, there are 5 categories (ABCD &amp; E) defined by a set of criteria, from the poorest category (without land, facing difficulties to have food) to the richer people [26].</p> <p>-Middle to high category = monthly income ≥ 65,000 Rwandan francs (RWF) (approximately 66 USD) to ≥ 600,000 RWF (607 USD) and ≥ 780,000 RWF (791 USD) to ≥ 7,200,000 RWF (7,302 USD) annual income.</p> <p>-Low Category = ≤ 45,000 RWF (46 USD) to ≤ 65,000 RWF (66 USD) per month and ≤ 540,000 RWF (548 USD) to ≤ 780,000 RWF (791 USD) annual income [27].</p>					

Table 2  
Clinical and lifestyle characteristics of study participants

Variable	HIV- (n=30) n (%)	HIV+ ART-naïve (n=40) n (%)	HIV+ On ART 0 - 6 months (n=40) n (%)	HIV+ on ART 7 - 12 months (n=40) n (%)	Total n (%)
<b>WHO stage</b>					
Stage 1		36 (90.00)	32 (80.00)	31 (77.50)	99 (82.50)
Stage 2+		4 (10.00)	8 (20.00)	9 (22.50)	21 (17.50)
<b>BMI/ kg/m<sup>2</sup></b>					
<18.5	1 (3.30)	5 (12.50)	4 (10.00)	3 (7.50)	13 (8.70)
18.5-24.9	21 (70.10)	25 (62.50)	28 (70.00)	31 (77.50)	105 (70.00)
25.0-29.9	4 (13.30)	9 (22.50)	5 (12.50)	6 (15.00)	24 (16.00)
>=30	4 (13.30)	1 (2.50)	3 (7.50)	0 (0.00)	8 (5.30)
<b>Blood pressure (BP)</b>					
Normal	29 (96.70)	39 (97.50)	34 (85.00)	37 (92.50)	139 (92.70)
High*	1 (3.30)	1 (2.50)	6 (15.00)	3 (7.50)	11 (7.30)
<b>Smoking status</b>					
Yes	0 (0.00)	14 (35.00)	8 (20.00)	10 (25.00)	32 (21.30)
No	30 (100.00)	26 (65.00)	32 (80.00)	30 (75.00)	118 (78.70)
<b>Alcohol consumption **</b>					
Yes	15 (50.00)	29 (72.50)	26 (65.00)	28 (70.00)	98 (65.30)
No	15 (50.0)	11 (27.5)	14 (35.0)	12 (30.0)	52 (34.7)

Variable	HIV- (n=30) n (%)	HIV+ ART-naïve (n=40) n (%)	HIV+ On ART 0 - 6 months (n=40) n (%)	HIV+ on ART 7 - 12 months (n=40) n (%)	Total n (%)
<b>Diet ***</b>					
Un healthy diet	22 (73.30)	31 (77.50)	26 (65.00)	27 (67.50)	106 (70.70)
Healthy diet	8 (26.7)	9 (22.5)	14 (35.0)	13 (32.50)	44 (29.30)
<b>Level of physical activity****</b>					
Adequate	9 (30.00)	24 (60.00)	12 (30.00)	10 (25.00)	55 (36.70)
Inadequate	21 (70.00)	16 (40.00)	28 (70.00)	30 (75.00)	95 (63.30)
*High blood pressure (BP): Defined as the systolic BP of 140 mmHg or higher, and/or diastolic BP of 90 mm Hg, and/or being on hypertensive medications [28].					
** Alcohol consumption: Taking a drink that contained alcohol for the last 6 months [29].					
*** Health diet: Defined based on how often are fruits and vegetables accompanying routine meals per week Unhealthy diet: Defined as an impression of usually eating meals prepared with too much oil [30].					
**** physical activity participation: Defined as vigorous-intensity exercise activities that cause large increases in heart rate like running, pedal cycle or football for at least 30 minutes continuously 3 times per week [31]					

## Biochemical characteristics of study participants

Analysis of serum biochemical markers of study participants suggest that generally the serum biochemical markers were higher among HIV- participants compared to HIV+ on ART treatment (Table 3). When assessing the trend of biochemical markers among the 3 subject groups of HIV+ participants, differences between the ART-naïve and ART-treated participants suggest elevated levels of some biomarkers which may be associated with prolonged ART use. For instance, serum levels of triglycerides were 61.20 ( $\pm$ 18.30) mg/dl among ART- naïve participants compared to 81.50 ( $\pm$ 31.90) mg/dl and 88.50 ( $\pm$ 43.90) mg/dl in ART- treated participants for 0 to 6 months and 7 to 12 months, respectively (Table 3). Similarly, plasma levels of LDL-C were 54.30 ( $\pm$ 18.60) mg/dl in ART-naïve participants compared to 59.40 ( $\pm$ 27.60) mg/dl and 57.20 ( $\pm$ 20.70) mg/dl among ART-treated participants for 0 to 6 months and 7 to 12 months, respectively. Serum levels of total cholesterol were generally higher in ART-treatment participants than ART-naïve participants but the trend did not increase with duration of ART treatment (Table 3). The

overall changes were observed in all biomarkers however; almost all the serum levels were within the cutoff values (normal range of each marker).

Table 3  
Biochemical characteristics of study participants

Variable	HIV- (n=30) mean±SD	HIV+ ART Naïve (n=40) mean±SD	HIV+ On ART 0 - 6 months (n=40) mean±SD	HIV+ On ART 7 – 12 months (n=40) mean±SD
Age	35±6	30±6	31 ±6	33±7
BMI kg/m <sup>2</sup>	23.90±3.40	23.00±3.70	23.00 ±3.60	22.30±3.40
Systolic Blood Pressure	116.20±8.10	115.45±12.60	117.98±14.00	117,70±12.90
Diastolic Blood Pressure	73.10±8.30	73.30±10.00	75.00±12.40	75.10±9.30
Fasting Blood glucose level (mg/dL)	107.00±21.00	105.00±22.00	107.00±22.00	109.00±20.00
HDL-C in mg/dl*	62.80±20.40	53.50±22.70	68.70±30.00	55.00±25.70
LDL-C in mg/dl	61.40±23.00	54.30±18.60	59.40±27.60	57.20±20.70
Total cholesterol/ mg/dl	144.60±31.40	119.00±36.60	143.90±45.90	128.10±43.30
Triglycerides/ mg/dl	97.20±48.90	61.20±18.30	81.50±31.90	88.50±43.90
High sensitivity C-reactive protein (hs-CRP) ng/ml*	61.07	79.11	39.7 0	41.3 0
NT-ProBNP in pg/ml*	137.60±82.10	92.20±100.80	105.40±148.30	93.00±81.20
CD4+ cells/mm <sup>3</sup> at enrolment	-	419.80±235.30	394.80±210.70	405.60±188.50
Viral loads in copies/ml	-	-	28.30	25.20
*Data are not normally distributed, mean rank was used as the appropriate dispersion measure.				

While evaluating the correlation between HIV status, use and duration of ART among HIV+ participants, and changes in biochemical markers, a positive correlation was observed between all biomarkers and the use of ART (Table 4). Differences were observed for serum triglycerides, and in serum total cholesterol with a strong significant positive correlation between triglycerides among HIV+ who were ART-naïve vs. HIV+ on ART [61.20 (±18.30) mg/dl vs. 85.00 (±38.30) mg/dl];  $p < 0.01$  and serum total cholesterol were significantly higher in HIV+ on ART than HIV+ who were ART-naïve [119.00 (± 36.00) mg/dl vs. 136.00(±45.00) mg/dl];  $p < 0.04$  (Table 4b). Comparisons between HIV+ on ART for 0-6 months vs. HIV+ on

ART for 7-12 months showed that lower HDL-C was significantly associated with longevity of exposure to ART therapy 68.70 ( $\pm 30.0$ ) mg/dl vs. 54.90 ( $\pm 25.70$ ) mg/dl];  $p=0.02$  (Table 4c).

Table 4  
a: Comparison between HIV+ and HIV- participants

Variables	HIV+ (n=120) mean±SD	HIV- (n=30) mean±SD	p-value
Age*	31±6	35±6	0.01
BMI kg/m <sup>2</sup>	23.90±3.40	22.70± 3.50	0.09
HDL-C (mg/dl)*	62.80±20.40	59.10±26.90	0.47
LDL-C (mg/dl)	61.40±23.00	56.90±22.60	0.34
Total Cholesterol (mg/dl)	144.60±31.40	130.30±43.00	0.09
CRP (ng/dl)*	79.10	61.10	0.04
Fasting Blood glucose (mg/dL)	2.60±0.50	2.60±0.50	0.87
Triglycerides(mg/dl)	77.10±34.80	97.20±48.90	0.01
Serum plasma level of NT-ProBNP in pg/ml*	69.30	100.20	<0.01

Table 4b: HIV-infected ART-naïve versus HIV-infected on ART

	HIV+ ART-naïve (n=40) mean±SD	HIV+ on ART (n=80) mean±SD	p-value
Age*	30±6	32±6	0.89
BMI	23.00±40.00	23.00±30.00	0.59
HDL-C (mg/dl)*	53.50 ± 22.70	61.80±28.60	0.70
LDL-C (mg/dl)	54.30±2.90	58.30±2.70	0.36
Total Cholesterol (mg/dl)	119.00 ± 36.00	136.00± 45.00	0.04
CRP (ng/dl)*	2.10± 0.90	1.85±0.90	0.75
Fasting Blood glucose (mg/dL)	2.50±0.50	2.60±0.50	0.58
Triglycerides(mg/dl)	61.20 ±18.30	85.00±38.30	<0.01
Serum plasma level of NT-ProBNP in pg/ml*	92.20±100.80	99.20±118.90	0.25

Table 4c: Comparison between HIV+ on ART for 0-6 months vs. HIV+ on ART for 7-12 months

	HIV+ on ART (0-6 months) (n=40) mean±SD	HIV+ on ART (7-12 months) (n=40) mean±SD	p-value
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Variables	HIV+ (n=120) mean±SD	HIV- (n=30) mean±SD	p-value
Age*	31 ±6	33±7	0.29
BMI	22.90± 3.60	22.30±3.40	0.47
HDL-C (mg/dl)*	68.70±30	54.90±25.70	0.02
LDL-C (mg/dl)	59.40± 27.60	57.20±20.70	0.68
Total Cholesterol (mg/dl)	143.90 ±45.90	128.10±6.80	0.12
Triglycerides(mg/dl)	81.50±31.90	88.5± 6.9	0.42
CRP (ng/dl)*	39.70	41.30	0.77
Fasting Blood glucose (mg/dL)	2.60±0.50	2.60±0.50	0.59
Serum plasma level of NT-ProBNP in pg/ml*	39.50	41.50	0.69

\*Data are not normally distributed, mean rank was used as the appropriate dispersion measure.

## Diet and physical lifestyle covariates in the study participants

Analysis results of diet and physical activity in study participants are not very conclusive. The physical activity classified by WHO category in low or adequate physical activity. The analysis of inflammatory biomarkers versus physical activity revealed that the inflammation is always lower in a group of participants with adequate physical activity but not statistically significant. The diet habits classified by health diet, defined based on how often are fruits and vegetables accompany routine meals per week and unhealthy diet defined as an impression of usually eating meals prepared with too much oil. The analysis of CVD risk biomarkers versus diet revealed that the HDL-C levels were lower in a group of health diet related habit but not statistically significant.

## Discussion

Our analysis evaluated the association of abnormal changes in biochemical markers of cardiovascular risk among HIV-infected adults on antiretroviral therapy compared to HIV-infected adults who were antiretroviral therapy-naïve. Furthermore, we assessed whether duration of antiretroviral therapy use was associated with adverse abnormal changes in biochemical markers of cardiovascular risk for a duration of 0-6 months compared to 7 -12 months. This study did not investigate individual ART molecule, individual drug or individual therapy regimen. Our study showed that ART treated patients had significantly high levels of Total Cholesterol and Triglycerides as compared to ART-naïve patients. The proportion of patients with dyslipidaemia among our ART treated participants was higher than the rate reported in a study from Cameroon and rural Uganda [32][33]. There are suggestions that the magnitude

of first-line ART-induced lipid imbalances could vary across populations and settings. Based on LDL-C cut-off values, dyslipidemia in our study was common and higher than that reported in a study from Cameroon [32], Western India (30%) [34] and Uganda (6%) [33]. But the prevalence of high LDL-C prior to ART (5%) was almost same as reported in Uganda and Western India (4%) [35] but less than that reported in Cameroon (21%) [32]. Similar to the Cameroon study [32], our study showed no changes in HDL-C levels after ART, which is not in accordance with the findings in Western India [35], which showed significant increase in HDL-C after 18 months of treatment with first-line ART regimen.

The association between HIV infection and use of antiretroviral therapy with cardiometabolic disease and biochemical biomarkers of cardiovascular risk has been documented in several settings in developed countries [36] and in Rwanda [37]. However, to the best of our knowledge, this is the first study in Rwanda to explore the relationship between ART and biochemical biomarkers of CVD risk in patients with HIV infection in comparison with HIV-uninfected adults.

Current data suggest that both HIV infection and antiretroviral therapy are associated with, and may increase the risk of cardiovascular disease, a consequence of changes in specific biochemical biomarkers of CVD risk, mainly associated with dyslipidemia [38],[39]. Our findings are consistent with a systematic review and meta-analysis that reported that among antiretroviral therapy treated HIV-infected adults in sub-Saharan Africa, use of ART was associated with higher LDL- Cholesterol and low HDL-Cholesterol [40]. Thus, similar biochemical markers of cardiac risk were reported, and the study suggested that the differences in cardiometabolic traits between HIV-infected and uninfected individuals were associated with HIV infected and use of ART [40]. Similarly, in agreement with our findings, one study in Tanzania reported that HIV-infected adults on antiretroviral therapy who presented a cluster of biomarkers of cardiovascular risk had a higher lifetime cardiovascular risk than HIV-uninfected adults [41].

The present study was undertaken to identify whether potential candidate serum biochemical biomarkers of CVD were influenced by ART in HIV-infected adults in Rwanda. In addition, CVD risk among study participants was examined to ascertain whether ART predisposes to an overall increased risk of CVD. Notably, more than a half of the study participants were female. Although females may be presenting more frequently in HIV clinics and seeking healthcare services more than males, women between 24-55 years in sub-Saharan Africa are twice more likely to be living with HIV infection than men in the same age group. Our findings concur with those reported by global systematic review [38], and by other sub-Saharan Africa data[39].

The majority of participants were in young age range between 26 and 33 years, and the mean age was similar among all study groups. Younger age constitutes a lower risk of CVD in various studies conducted in Africa, Europe and other countries where increased age is a predictor of CVD mortality, and higher prevalence of metabolic associated diseases [42],[43]. Most study participants resided in an urban setting. Individuals living in most African urban settings are more likely to consume unhealthy foods containing high animal fats with low fiber and vitamins resulting in increased the risk of CVD. Among study participants 16% and 5.3% were overweight and obese, respectively. These figures are higher than

those reported in the previous STEP Survey in Rwanda, which reported 14.3% and 2.8% as overweight and obese, respectively [44]. Weight gain is common in HIV-infected adults who initiate ART treatment, which if not controlled may result in adipose tissue changes. Studies from developed countries indicate that excess adiposity and HIV infection contribute to metabolic complications [45]. The majority of participants over 60% lived a sedentary life style. Although this figure is higher than that reported in the STEP Survey among the general population, it is similar to that reported in Kigali in the STEP Survey, which showed that only 46.7% were highly active [45]. Most of study participants had an unhealthy diet with fat saturated rich animal foods with low fiber and vitamins. These results concur with the findings of the STEP Survey, which showed that most participants did not consume fruit and vegetables [43].

Comparison of biochemical markers between HIV-infected and HIV-Uninfected adults participants suggest abnormal serum levels of HDL- cholesterol, LDL- cholesterol, total cholesterol and triglycerides. Low HDL levels were observed in ART-treated participants between 7 to 12 months compared to ART-treated participants between 0 and 6 months. HDL-C is considered a cholesterol scavenger that removes excess cholesterol from the blood. Low HDL-C constitutes a risk of hypercholesterolemia and this constitutes an increased risk of CVD. Our findings are similar to one study in Cameroon which assessed the prevalence and characteristics of lipid profile derangements associated with antiretroviral therapy [32]. In this study, it was observed that use of ART was significantly and positively associated with high total cholesterol, LDL-cholesterol and HDL-cholesterol [32]

The changes in HDL-C were also observed in a Tanzanian study [46], as well as other studies in the US and Europe [47],[48] indicating the need for routine clinical monitoring and care of HIV patients to ensure timely prevention and management of any cardiovascular disease associated derangements and risks. Hypercholesterolemia and hypertriglyceridemia were characteristic of dyslipidemia in the present study with higher serum plasma levels in ART treated patients and statistically significant. Similar findings were reported from an earlier study [49],[47] There was no statistically significant differences observed for serum plasma levels of HDL-C, LDL-C, hs-CRP, NT-ProBNP and glucose, between ART naïve and ART treated participants. There was a significant positive correlation between triglycerides ( $p < 0.01$ ), cholesterol ( $p < 0.04$ ) and use of antiretroviral therapy. Similar results were reported elsewhere [50]. In line with our findings, though many studies have failed to demonstrate that ART is the main cause of most derangements in lipid metabolism, a study by Cunha et.al,(2015) demonstrated that PI-based ART combination impairs normal lipid metabolism resulting in increased triglyceride levels and lowered HDL-C [51].

Several developed countries and low- and middle-income countries (LMICs) such as Brazil as well as Rwanda have registered tremendous HIV treatment progress in terms of immunologic and virologic outcome after access to potent ART [52][ 53] The efficacy and safety of ART has substantially improved the wellbeing of people living with HIV infection after the introduction of newer drug classes of antiretrovirals that are now available to patients and HIV care providers. Thus, constant evaluation and assessment of potential metabolic derangements is required in order to curtail potential adverse impact

of HIV treatment and functional cure towards the economic sustainability of patients living with HIV infection.

Our study has some limitations. First, it is a cross sectional study which limits temporal trends and patterns in association that would provide more evidence to causation. Second, our participants in the study were conveniently enrolled from Health Care Clinics in urban areas of the City of Kigali province limiting inclusion of diversity of participants from rural areas of Rwanda. Third, our sample size is relatively small, which makes it rather explorative and calling for a larger study to confirm our conclusions.

## Conclusions

In conclusion, our study indicated that changes in serum levels of total cholesterol and triglycerides were associated with use of ART in HIV-infected adults on treatment in Rwanda. Compared to data from developed countries, these biochemical markers of cardiac risk changes were within the upper limits of normal ranges. Therefore, these findings likely revealed early increases in both biomarkers accompanying antiretroviral therapy. In countries faced with the scourge of HIV infection, routine clinical monitoring of lipid profile and other cardiovascular risk factors should be under the treatment guidelines to be monitored in patients on antiretroviral therapy so that any adverse effects of HIV treatments can be optimally managed. Our study suggest the need for early evaluation of lipid profiles as biomarkers of cardiac risk, to effectively monitor how antiretroviral therapy may contribute to cardiovascular disease risk, and deter treatment programs in Rwanda and other African countries.

## Abbreviations

ART: Antiretroviral therapy; CAD: Coronary artery disease; CVD: Cardiovascular Diseases; HAART: Highly Active Antiretroviral Therapy; HDL-C: High density lipoproteins; cholesterol; IREC: Institutional Research and Ethics Committee; LDL-C: Low Density Lipoprotein cholesterol; RNEC: Rwanda National Ethics Committee; TC: Total cholesterol; TG: Triglycerides

## Declarations

**Ethics approval and consent to participants:** This study was approved by Institutional Research and Ethics Committee (IREC) at Moi Teaching and Referral Hospital (MTRH) (approval notice: 0003163) and by Rwanda National Ethics Committee (RNEC), FWA (Assurance No.00001973; IRB 00001497 of IORG0001100). Participants were informed of the potential risks and benefits of the study and signed an informed consent form in Kinyarwanda language before enrolment in the study. All participant data were anonymized to optimize privacy and confidentiality of responses.

**Consent for publication:** Not Applicable

**Data statement:** Data is available upon request to the corresponding author

**Competing Interests statement:** The authors declare that they have no competing interests

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**Author's Contributions:** MB, CN, AK, EM: Protocol concept, study design, laboratory analysis, data collection, data analysis interpretation and drafted initial version of manuscript. MT, JNU, RM, VD, DR, KS, AN, TC, EM: Adjudication of the data analysis and review of the whole manuscript. All authors read, contributed to and approved the final manuscript.

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