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

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Abstract

Background: Human Immunodeficiency Virus (HIV) continues to be a major public health problem in both developing and developed nations. With the introduction of highly active antiretroviral therapy (HAART), a decline in morbidity and mortality from HIV has been observed. However, there is some evidence that HAART increases the risk of cardiovascular diseases (CVD) in people living with HIV. To assess this evidence, the levels of serum biomarkers for risk of CVD were measured across different HAART durations among HIV-positive individuals.

Methods: This was a descriptive cross-sectional study conducted at the Homa-Bay County Referral Hospital, Kenya. The study population consisted of male and female HIV-positive individuals on HAART between 18 and 45 years of age. Participants were enrolled into the study after consenting and meeting the recruitment criteria. 120 individuals participated in the study and provided blood samples, which were used in the biochemical analysis. Data was analyzed using descriptive statistics.

Results: The majority of the participants (67.5%) had been on HAART for more than sixty months. Most of the study participants had total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein (HDL-C), glycated hemoglobin A1c (HbA1c), lipoprotein-associated phospholipase A2 (Lp-PLA2) and myeloperoxidase (MPO) levels within the reference intervals (< 5.18 mmol/L, < 3.37 mmol/L, > 1.0 mmol/L, 21.2 ng/mL – 167, and 21.4 – 229 ng/mL respectively). The proportions that had deranged levels were as follows; 14.2% (TC), 5.8% (LDL-C), 2.5% (HDL-C), 4.2% (HbA1c), 24.2% (Lp-PLA2) and 44.9% (MPO). Most of the participants with deranged levels of serum biomarkers for risk of CVD had been on HAART for more than 60 years.

Conclusion: Our findings demonstrate a high proportion of deranged levels of some serum biomarkers are associated with increased risk of CVD in prolonged HAART. We recommend conducting prospective association studies in this population to assess the linkage between high proportions of the levels of these biomarkers to durations time of HAART.

Key Words Highly active antiretroviral therapy, serum biomarkers, cardiovascular disease, human immunodeficiency virus

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INTRODUCTION

Human immunodeficiency virus and acquired immunodeficiency syndrome (HIV/AIDS) remain a major cause of morbidity and mortality globally. In 2017, about 36.9 million people were living with HIV worldwide.[1] Kenya has the sixth highest burden of HIV in Africa, with over one million people living with the virus.[2] The high HIV/AIDS burden in Kenya is responsible for approximately 29% of adult deaths, 20% of maternal mortality and 15% of the deaths of children below five years of age annually. [2]

With the introduction of Highly Active Anti-Retroviral Therapy (HAART) coupled with effective management of opportunistic infections in HIV disease, there has been a significant improvement on morbidity and mortality from HIV infection and survival for HIV-infected individuals.[4] As a result of such improvements, there is a constantly increasing population of individuals living with the disease for several years. However, evidence suggests that a high burden of illness, health care utilization, and about 7% of deaths in individuals living with HIV, can be attributed to HAART-related metabolic complications.[4,5,6] These metabolic complications include dyslipidemia and hyperglycemia.[7, 8,9,10] With increased survival, these conditions will continue to contribute to other disorders, which are linked to atherosclerotic cardiovascular risk.[11] A survey conducted by the Kenya Ministry of Health revealed 6.1% to 8% mortality due to CVD in Kenya.[12]

The Joint United Nations Programme on HIV/AIDS (UNAIDS) global 90-90-90 strategy to end the HIV epidemic aims to ensure that 90% of HIV infected persons receive effective HAART by the year 2020.[12] If this is achieved, the number of individuals on HAART will increase in t areas with high HIV burden, along with a potential rise in HAART-related complications. Therefore, attention should be focused on minimizing HAART-induced metabolic complications, which increases the risk of developing CVD in this population. Early screening for CVD can potentially help detect the initiation of atherosclerotic plaque, necessitating better strategies to mitigate the progression of plaque.[13] The levels of lipid biomarkers are used for the biochemical assessment of cardiovascular risk.[14] The lipid biomarkers that are often evaluated for risk of CVD include total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). Assessment of CVD risk also involves an estimation of blood sugar level. Glycated hemoglobin A1c is usually used for monitoring the glycemic control in diabetic individuals, but it can provide meaningful information regarding CVD risk as well.[15] There are some reports indicating that lipid derangement as well as an elevation in inflammatory markers present in the serum of African patients on HAART. [16,17] However, these markers may not accurately predict the early stages of the atherosclerotic plaque formation because their levels are not deranged during initial phase of this process. Scientific evidence indicates that some novel biomarkers such as myeloperoxidase (MPO) and lipoprotein-associated phospholipase A2 (Lp-PLA2) can give better predictions of early cardiovascular events compared to well-established lipid markers since their concentrations are elevated at during initial stages of atherosclerosis.[16, 17,18]

The study is unique in several ways. None of these novel markers have been studied in a high burden HIV population. Additionally, this is the first time such a study has been done in Kenya. The study population was selected from the highest HIV prevalence region in Kenya, as this population is most likely to present with such metabolic complications. Moreover, the study was conducted in ambulant subjects attending their normal follow-up clinic, not on in-patients who may have transient metabolic derangements due to acute illness. Finally, the study sought to measure the biomarker levels in early HAART-treatment as well as those on prolonged HAART-treatment.

METHODS

Study setting and study population

This was a descriptive cross-sectional study. We sought ethical approval and permission to conduct the study from the Kenyatta National Hospital/University of Nairobi (KNH/UON) Ethics and Research Committee (ERC) and the administration of the Homa-Bay County Referral Hospital respectively. Recruitment of study participants was conducted in Homa-Bay County Referral Hospital, Kenya. A consent form was administered to the study participants. 120 individuals were recruited in this study. The study population consisted of HIV-positive individuals on HAART aged between 18 and 45 years. The population was then divided into three study groups: those who had been on HAART for at least 6 months, those who had been on HAART for 35 to 60 months and those who had been on HAART for more than 60 months. We excluded individuals who had known history of myocardial infarction/stroke,

hypertension, diabetes, and dyslipidemia prior to HAART. We also excluded pregnant women, cigarette smokers, severe ill patients and alcoholic individuals.

Specimen collection, handling and biochemical analysis

For the specimen collection, four milliliters of non-fasting venous blood were collected aseptically from the antecubital vein. Three milliliters of blood were put in a plain (red top) tube, and one milliliter was put in an EDTA (purple) tube. Serum samples was obtained from blood in the red top tubes and was then aliquoted into half-milliliter aliquots before storing them at -20°C until the biochemical analysis was done. HbA1c estimation was performed on the one milliliter of blood in the EDTA tube at the recruitment site using CloverA1C™ analyzer. Aliquots of serum samples were shipped to Kenyatta National Hospital, Nairobi, for analysis. The Cobas™ chemistry analyzer was used to analyze serum concentration of lipids TC, LDL-c, and HDL-c. Lp-PLA2 and MPO were analyzed by quantitative sandwich enzyme immunoassay technique (BioTechne). In this method, a monoclonal capture antibody binds the human MPO and Lp-PLA2. After a washing step, a polyclonal antibody is introduced into the microplate and binds to the bound MPO and Lp-PLA2. After addition of a substrate solution to the reaction wells, color develops in proportion to the concentration of MPO and LP-PLA2 bound in the initial step. The intensity of the color is then estimated. Abnormal HbA1C was defined as ≥ 6.5%. Abnormal lipid profile was defined as TC ≥ 5.18 millimoles per liter (mmol/L), LDL-c ≥ 3.37 mmol/L, HDL-c < 1.0 mmol/L. Abnormal Lp-PLA2 was defined as >167 nanograms per milliliter (ng/mL). Abnormal MPO was defined as > 229 ng/mL.

Data management and analysis

The optical densities for MPO and L-p-PLA2 were recorded, and the concentration was calculated by creating a standard curve using a GraphPad prism to generate a four-parameter logistic (4-PL) curve-fit. The IBM Statistical Package for Social Sciences (SPSS) was then used for data analysis. Analysis of continuous variables was done using descriptive statistics (mean, median, standard deviation and range).

RESULTS

Socio-demographic characteristics of the study participants

The mean age of study participants was 34.34 ± 6.545 years with a median of 35 years. Most of the participants (55.8%) were between 34 and 45 years of age (Table 1).

TABLE I. SOCIODEMOGRAPHIC SUMMARY OF THE STUDY PARTICIPANTS

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

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

Biochemical analysis

The levels of serum biomarkers were measured and categorized as either low risk or high risk for CVD using the predefined cut-off points. Table 2 and 3 summarize the results that were obtained after performing the biochemical analysis.

TABLE II. PROPORTION AND DESCRIPTIVE STATISTICS OF LEVELS OF THE SERUM BIOMARKERS ASSESED

Laboratory Parameters	Frequency N(/%)	Mean (SD)	Range
HbA1c (%)			
Low risk (<6.5)	112 (93.3)	5.45 (±0.6579)	4.1 - 9.3 (5.4)
High risk (≥6.5)	8 (6.7)		
TC (mmol/L)			
Low risk (<5.18)	103 (85.8)	4.15 (±0.9134)	1.84 - 6.97 (4.0)
Increased risk (≥5.18)	17 (14.2)		
LDL-C (mmol/L)			
Low risk (<3.1)	109 (90.8)	2.30 (±0.5715)	1.04 - 4.21 (2.2)
Increased risk (≥3.1)	11 (9.2)		
HDL-C (mmol/L)			
Low risk (>1)	117 (97.5)	1.59 (±0.3214)	0.58 - 2.73 (1.6)
Increased risk (≤1)	3 (2.5)		
Lp-PLA2 (ng/ml)			
Low risk (<167)	91 (75.8)	172.01 (±334.8054)	0.3 - 1445.87 (52.52)
High risk (≥167)	29 (24.2)		
***MPO (ng/ml)**			
Low risk (<229)	43 (55.1)	259.84 (±191.6492)	45.02 - 603.56 (208.94)
High risk (≥229)	35 (44.9)		

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

TABLE III. PROPORTION OF LEVELS OF LIPIDS, GLUCOSE, LIPOPROTEIN-ASSOCIATED PHOSPHOLIPID A2 AND MYELPEROXIDASE ACROSS DIFFERENT HAART DURATIONS (N = 120)

		HAART Duration (Months) N (%)		
Biomarkers	**Risk Stratification**	**6 - 35**	**36 - 60**	**>60**
HbA1c (%)	Low risk (<6.5)	16(13.3)	21(17.5)	75(62.5)
	High risk (≥6.5)	**1(0.83)**	**1(0.83)**	**6(5)**
TC (mmol/L)	Low risk (<5.18)	15(12.5)	19(15.8)	69(57.5)
	High risk (≥5.18)	**2(1.7)**	**3(2.5)**	**12(10)**
LDL-C (mmol/L)	Low risk (<3.1)	17(14.2)	20(16.7)	72(60)
	High risk (≥3.1)	**0**	**2(1.7)**	**9(7.5)**
HDL-C (mmol/L)	Low risk (>1)	17(14.2)	21(17.5)	79(65.8)
	High risk (≤1)	**0**	**1(0.83)**	**2(1.7)**
Lp-PLA2 (ng/ml)	Low risk (<167)	13(10.8)	20(16.7)	58(48.3)
	High risk (≥167)	**4(3.3)**	**2(1.7)**	**23(19.2)**

N - Number.

DISCUSSION

The decrease in the mortality and morbidity of HIV-infected individuals is associated with the widespread use of HAART. However, the association of HAART with the risk of cardiovascular disease, as evidenced by recent research findings in Ethiopia and Cameroon, is now a cause for concern.[19, 20] In this present study, the levels of serum biomarkers (TC, LDL-C, HbA1c, Lp-PLA2 and MPO) associated with the risk of cardiovascular disease were investigated in patients receiving highly active antiretroviral therapy. The study reported a female to male ratio of 1.8:1, and was found to be comparable to the reported ratio of 1.6:1 by the Kenya AIDS Indicator Survey (KAIS) [21], and the reported 1.9:1 ratio by Tadewos et al.[19]. About 28.3% of the 120 study participants were in the age brackets of between 39 to 45 years. Most of the subjects (67.5%) had been on HAART for more than sixty months, whereas the remaining 14.2% and 18.3% of the subjects have been on HAART for 6 – 35 and 36 – 60 months HAART duration, respectively. This finding contrasts that of Abebe et al., Addis Ababa, Ethiopia, which reported the highest proportion of subjects (59% of participants) had only been on HAART for 25-41 months.[22]

In the present study, the majority of the study participants had TC, LDL-C, HDL-C, HbA1c, Lp-PLA2 and MPO levels within the reference interval. For the lipid and glycated hemoglobin biomarkers, the proportion of subjects with derangements in these levels were as follows: 14.2% (TC), 5.8% (LDL-C), 2.5% (HDL-C) and 4.2% (HbA1c). These percentages differ from Tadewos et al., in Southern Ethiopia, who reported 31% (TC), 24% (LDL-C) and 27% (HDL-C).[19] This discrepancy may be explained by unaccounted confounding factors in Tadewos et al. study such as differences in the prevalence of smoking, which is an established CVD risk marker.[19] They compared the lipid profiles for HAART and pre-HAART groups and found out that HAART use was significantly and positively associated with elevated concentrations of TC, LDL-C and triglycerides.

Most of the studies done in this population mainly focused only on evaluating the serum levels of fasting blood glucose.[22, 23] However, the incidence of hyperglycemia was also studied. In the present study, 4.2% in the 120 study participants exhibited hyperglycemia. This is comparable with findings of Abebe et al., Addis Ababa Ethiopia, which reported 7.9% in the 126 study participants on HAART which reported hyperglycemia.[18] However, the study differed with the findings of Mbunkah et al. in South-West, Cameroon, which reported hyperglycemia of 26.5% in the 241 study subjects.[20] A possible explanation for this inconsistency is age as a confounding factor, since the researchers had included subjects of up to 70 years of age.

MPO and Lp-PLA2 predict the early events of the development of cardiovascular diseases.[14] Therefore, their inclusion in the study was to identify those that were at risk of developing cardiovascular disease, which the already-established markers (TC, LDL-C, HDL-C and HbA1c) could not have captured at the early phase of atherosclerosis. The concentrations of the already-established CVD risk predictors begin to rise as the development of plaque advances. In this study, a proportion of 24.2% of the subjects had raised levels of Lp-PLA2 above the reference interval. Out of the 78 subjects that were assessed for serum levels of MPO, 44.9% had elevated values. Overall, we found that most of the study participants with deranged levels of serum biomarkers had been on HAART for more than sixty months.

LIMITATIONS

The study acknowledges the absence of pre-HAART population as a limitation. The pre-HAART population could have served as a control group and provided a better comparison. However, with the current WHO recommendation of 'test-and-treat' strategy of managing HIV infection, it is not possible to find pre-HAART population.

CONCLUSION AND RECOMMENDATION

HAART has reduced mortality and morbidity among HIV-positive individuals to a point where it is considered a manageable chronic condition. As such, care for this population should now focus on the long-term adverse effects of the drug such as CVD risk. Descriptively, in the present study, the majority of the subjects who had at least one of the serum biomarker with an abnormal value had been on HAART for more than sixty months. Therefore, we highly recommend a conducting a well-controlled cohort study to assess the association between deranged serum biomarkers for risk of CVD and prolonged HAART use.

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