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Association between selected immune markers and low ankle-brachial index in virologically suppressed HIV-infected patients on antiretroviral therapy in Nigeria

Chidozie Elochukwu Agu[1], Ikenna Kinsgley Uchendu[2], Chukwugozie Nwachukwu Okwuosa[2], Peter Uwadiegwu Achukwu[2]

[1]Prime Health Response Initiative (PHRI)-sub-recipients of Global Fund HIV Impact Project, Ilorin, Kwara State, Nigeria
[2]Department of Medical Laboratory Science, Faculty of Health Science and Technology, College of Medicine, University of Nigeria Enugu Campus, Enugu State, Nigeria

Abstract

Introduction: People living with human immunodeficiency virus (HIV) on effective antiretroviral treatment (ART) are exposed to an increased risk of cardiovascular disease, often linked to inflammation and immune activation. Ankle-brachial index (ABI) has been widely accepted as screening tool for peripheral arterial disease (PAD) and future cardiovascular events, and is inexpensive and non-invasive compared to carotid intima-media thickness measurements. This study aimed at determining the association between low ankle-brachial index and selected immune markers among virologically suppressed HIV-infected participants on ART in Kwara State, Nigeria.

Material and methods: This analytical cross-sectional study was conducted between July 2018 and December 2018. One hundred and fifty HIV-infected participants and fifty HIV non-infected age matched controls were recruited into the study. Ankle-brachial index was measured, and peripheral arterial disease was defined as ABI of < 0.9. Cryopreserved plasma was used to evaluate interleukin (IL)-6 and sCD14. Student's *t*-test and χ^2 test were used to compare continuous and categorical variables. Associations of CVD and immunologic markers with low ABI were assessed using logistic regression analysis.

Results: The study group had significantly lower mean values for ABI and significantly higher mean values of IL-6 and sCD14 compared to the control group ($p < 0.05$). The prevalence of low ABI (14.6%) was higher in the study group compared to the control group (2%). IL-6 (OR 0.992, $p = 0.087$) and sCD14 (OR 0.918, $p = 0.058$) were not associated with low ABI in the study group.

Conclusions: HIV-infected individuals on suppressive ART demonstrate increased levels of IL-6 and sCD14 compared to not infected controls. The impact of inflammation and immune activation on PAD in treated HIV-infection requires further investigation.

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Address for correspondence: Chidozie Elochukwu Agu, Prime Health Response Initiative (PHRI)-sub-recipients of Global Fund HIV Impact Project, 2nd Floor, ADOL House, 15 CIPM Road, Alausa, Ikeja, Lagos, Nigeria, phone: +2348030984682, e-mail: chidozieagu@gmail.com

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Introduction

Since 2004, more than 30 low-income and middle-income countries (LMIC) worldwide have been receiving various human immunodeficiency virus (HIV) programs [1]. Through these programs, approximately 19.5 million people living with HIV (PLHIV) have obtained antiretroviral treatment (ART) [1, 2]. As more people comply with the uptake of antiretroviral treatment in LMICs, there may be an improvement in the survival of PLHIV, which is currently observed in high-income countries [3]. On achieving the Joint United Nations Programme on HIV/AIDS (UNAIDS) ambitious 90–90–90 goals, acquired immune deficiency syndrome (AIDS)-related opportunistic diseases will drastically reduce [3], and non-communicable diseases may significantly become common [4, 5]. Furthermore, it was reported that HIV infection might increase the risk of non-communicable diseases (NCDs) due to activation of inflammatory markers and adverse events caused by certain antiretroviral drugs [4]. Elevated levels of immune activation markers and inflammation have been reported to predict an increased mortality and other adverse events in HIV infection [6].

Previously, the increased cardiovascular risk reported in HIV-patients was due to metabolic changes associated with ART as a result of the effect of viral protease inhibitors (PI) [7, 8]. The D:A:D study (data collection on adverse events of anti-HIV drugs) [8] showed a significant elevation in the incidence of acute myocardial infarction (AMI) after exposure to ART, with an elevated AMI risk of close to 30% after 6 years of treatment. HIV infection may enhance cardiovascular risk through different mechanisms, such as prolonged inflammation and immune activation, increased thrombotic activity, endothelial damage, indirect metabolic disorders, higher oxidative stress, and microbial translocation. There are several risk factors that may lead to peripheral artery disease in people living with HIV. These include the HIV infection itself, increased aging process in infected individuals, adverse effects of antiretroviral therapy, and increased occurrence of conventional cardiovascular risk factors, such as diabetes and dyslipidemia [9, 10].

Several other markers of immune activation (sCD163, sCD14, and CD14+/CD16+ monocyte expansion) on monocytes/macrophages are associated with HIV infection. These markers were reported to be elevated during HIV infection [11]. In addition, an elevation of some activated CD8 T lymphocytes human leukocyte antigen (HLA)-DR+CD38+ was observed [11, 12].

Although the relative risk of cardiovascular disease (CVD) is increased in patients with HIV, one limitation in studying CVD in this generally young patient population has been the relatively low absolute event rate in terms of CVD-related deaths [13]. As a result, several investigators have utilized cardiac imaging and ankle–brachial index (ABI) to document the burden of sub-clinical CVDs, better understand the underlying pathophysiological mechanisms, and follow the response to treatment interventions. The cardiac imaging measurements, however, are quite expensive and not readily available. Therefore, a suitable alternative is ABI, which is a simple, inexpensive, and non-invasive diagnostic test as a powerful indicator of systemic atherosclerosis and peripheral artery disease (PAD) and a strong predictor of mortality from cardiovascular causes in the general population [14, 15]. ABI is the screening tool for PAD. An ABI ≤ 0.9 (low ABI) is diagnostic of PAD [16]. The ABI between 0.91-0.99 (borderline values) is considered significant prognostic marker in higher cardiovascular mortality. The ABI ≥ 1.3 (high ABI) suggests rigidity and non-compressibility of lower limb arteries. Abnormal ABI values in asymptomatic patients are associated with a higher incidence of CVD [17].

This study aimed at determining associations between ankle-brachial index and selected immune markers among virologically suppressed HIV-infected patients on ART in Kwara State, Nigeria.

Material and methods

Study area

The study was conducted in Kwara, a state in western Nigeria, located within the North central geopolitical zone, commonly referred to as the Middle belt, with an estimated population of 2,365,353 inhabitants [18]. The primary ethnic group is Yoruba, with significant Nupe, Bariba, and Fulani minorities. Kwara state consists of 16 local government areas, including Asa, Baruten, Edu, Ekiti, Ifelodun, Ilorin East, Ilorin South, Ilorin West, Irepodun, Isin, Kaiama, Moro, Offa, OkeEro, Oyun, and Pategi. In Nigeria, HIV programs are largely funded by the President's Emergency Plan for AIDS Relief (PEPFAR) and Global Fund, with a support from the Federal Ministry of Health and National Agency for Control of AIDS (NACA).

The research ethical approval was obtained from the Kwara State Ministry of Health Ethical Committee (MOH/KS/EU/777/252). Enrollment into the study was voluntary and informed consent was obtained from all participants.

Study design

A cross-sectional study design was adopted for this study. Informed consent was obtained from all participants before their enrollment. Socio-demographic data were collected using a structured questionnaire after which, blood samples were obtained from the participants. The study participants include HIV-positive patients attending HIV clinic of the University of Ilorin Teaching Hospital (UITH) and Sabo Oke Medical Center, Ilorin, Kwara state, and the control group consisted of non-infected healthy participants resided within the same region. The study was conducted within six months, between July 2018 and December 2018.

Sample size determination

The sample size was calculated using the sample size for comparing two proportions [19].

$n = (Z_{\alpha/2}+Z_{\beta})^2 * (p_1 (1-p_1) + p_2 (1-p_2)) / (p_1-p_2)^2$,

where $Z_{\alpha/2}$ is the critical value of the normal distribution at α/2 (e.g., for a confidence level of 95%, α is 0.05 and the critical value is 1.96), Z_{β} is the critical value of the normal distribution at β (e.g., for a power of 80%, β is 0.2 and the critical value is 0.84), and p_1 and p_2 are the expected sample proportions of the two investigated groups.

However, considering that there are several cardiovascular risk factors with respective standard deviation in different studies, the sample size was not calculated using this formula. Literature review showed that a sample size of 144 participants was used in a similar study [20], with an extra 4% included to cater for non-responders. Thus, a total of 150 individuals living with HIV who have been on antiretroviral therapy (ART) for 6 months and above were recruited into the present study.

Subject selection

A total of 200 participants, 150 virologically suppressed (viral load < 1000 copies/ ml, WHO standard for low- and middle-income countries) HIV-infected participants on antiretroviral therapy (ART) and 50 uninfected controls within the age range of 18-50 years were investigated. The study lasted for 6 months, between July 2018 and December 2018. The study group participants were recruited using random sampling technique from the Sabo Oke Medical Center Ilorin of ART treatment and care unit, and the University of Ilorin Teaching Hospital (UITH) supported by PEPFAR (President's Emergency Plan for AIDS Relief).

Inclusion criteria

HIV-infected individuals between 18-50 years presently on ART for at least six months, with viral RNA of < 1000 copies/ml.

Exclusion criteria

HIV-infected adults who defaulted from ART use, with a known coronary heart disease or diabetes mellitus, patients on statins for at least 6 months, and those with known co-infections, such as tuberculosis and hepatitis B and C.

Data collection tools

A detailed questionnaire interview was conducted to obtain personal data on each participant enrolled into the study, including demographic characteristics and family history of cardiovascular diseases based on the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria [21], past and current medication usage, presence of co-infections, and HIV disease characteristics (duration of HIV infection, duration of ART treatment, viral RNA count, nadir and current CD4+ T cell count, and past and current ART regimen). Medical history along with physical examination, with height, weight, waist, and hip measurements, and brachial and ankle blood pressure obtained from all the participants enrolled in the study.

Ankle-brachial index measurements

The ankle-brachial index (ABI) was determined using a hand-held continuous wave doppler device 8 MHz probe, as described by Kwiatkowska *et al.* [14] and Olalla *et al.* [22]. The examination was performed after 5 minutes of rest in the supine position. A pressure cuff, which was placed on either arm, and subsequently above the ankles was used. The probe was placed at the cubital fossa, medial malleolus, and dorsal foot for systolic blood pressure detection at the brachial artery, left posterior tibial, and dorsalis pedis artery, respectively. The cuff was inflated to 20 mm above the audible pulse signal and then slowly emptied, until the first pulse signal was detected. Ankle-brachial index value was determined by taking the higher pressure of two arteries at the ankles divided by the highest brachial systolic blood pressures. Using the formula below, ABI was calculated as:

$$ABI = \frac{\text{highest systolic blood pressure in the ankles}}{\text{highest systolic blood pressure in both arms}}$$

Interpretation of ankle-brachial index measurements: normal ABI = 1.0-1.4, borderline ABI = 0.91-0.99, PAD ≤ 0.9.

Anthropometric measurements

Anthropometric parameters, such as height (m), weight (kg), waist circumference (cm), and hip circumference (cm) were determined. Weight and height were measured with the subjects wearing light clothing and without shoes. Weight was measured in kilogram using a balanced scale, while height was measured in meters using a wall-mounted ruler with the subjects standing with feet together, and with head, shoulder, buttocks, and heels touching the wall. Waist and hip circumferences were measured to the nearest 0.1 cm using a flexible but inelastic measuring tape. Moreover, waist circumference was taken between the costal margin and the iliac crest in the mid-auxiliary line around the gluteal region [23]. Body mass index (BMI) was calculated as weight in kilogram divided by the square of the height in meters (kg/m^2), waist-to-hip (WHR) was determined by dividing the measurement of the waist (cm) by that of the hip (cm), and then used together with the waist circumference as an index of central obesity.

The following definitions were used: general obesity a BMI of ≥ 30 kg/m^2, central obesity – a waist circumference of ≥ 80 cm in women or ≥ 94 cm in men, waist-to-hip ratio of ≥ 0.90 in men or ≥ 0.85 in women. Normal weight was defined as BMI value of 18.5-24.9 kg/m^2 [21].

Sample collection

Ten milliliters (10 ml) of blood was obtained from the ante-cubital vein by venipuncture after at least a 12-hour overnight fast, and was dispensed into a plain container,

Table 1. Demographics, anthropometric parameters, and clinical characteristics of HIV-infected study participants (*N* = 150)

Parameter	
Age (years)	43.73 ± 8.74
Gender, *n* (%)	
Male	41 (27.3)
Female	109 (72.7)
Ethnicity, *n* (%)	
Yoruba	120 (80.0)
Ibo	10 (6.7)
Hausa	4 (2.7)
Other	16 (10.7)
Religion, *n* (%)	
Muslim	55 (36.7)
Christian	94 (62.7)
Other	1 (0.7)
Education, *n* (%)	
Primary	33 (22.0)
Secondary	44 (29.3)
Tertiary	62 (41.3)
None	11 (7.3)
Clinical characteristics	
SBP (mm Hg)	121.67 ± 20.96
DBP (mm Hg)	81.47 ± 7.98
BMI (kg/m^2)	25.43 ± 5.46
BMI 18.5-24. kg/m^2 (%)	78 (52)
BMI 25-29.9 kg/m^2 (%)	38 (25.3)
BMI ≥ 30 kg/m^2 (%)	34 (22.7)
WC (cm)	90.13 ± 11.52
WHR	0.88 ± 0.048
Duration of HIV infection (years)	7.73 ± 3.52
Duration on ART (years)	7.47 ± 3.42
Nadir CD4$^+$ T cell count (cells/mm^3)	263.18 ± 229.58
Current CD4$^+$ T cell count (cells/mm^3)	505.42 ± 265.85
HIV viral RNA count (copies/ml)	40.95 ± 111.61
Undetectable viremia (< 20 copies/ml)	104 (69.3)
Detectable viremia (≥ 20 copies/ml)	46 (30.7)
ART drug regimen, *n* (%)	
NRTI/NNRTI (AZT/3TC/NVP)	93 (62.0)
NRTI/NNRTI (TDF/3TC/EFV)	46 (30.7)
Protease inhibitor (LPV/r)	11 (7.3)
Family history of CVD	13 (8.6)
Diabetes	6 (4.0)
Hypertension	24 (15.9)
Lipid lowering drugs	23 (15.2)
Smoking	1 (0.7)
Metabolic syndrome	26 (17.3)

Data are presented as mean ± standard deviation or numbers (percentages), PAD – peripheral artery disease, ABI – ankle-brachial index, SBP – systolic blood pressure, DBP – diastolic blood pressure, BMI – body mass index, WC – waist circumference, WHR – waist to hip ratio, CVD – cardiovascular disease, NRTI – nucleoside reverse transcriptase inhibitor, NNRTI – non-nucleoside reverse transcriptase inhibitor, AZT/3TC/NVP – zidovudine/ lamivudine/ nevirapine, TDF/3TC/EFV – tenofovir/lamivudine/efavirenz, LPV/r – lopinavir/ritonavir

allowed to clot at room temperature, spun at 5,000 r.p.m. for 5 minutes; the serum was separated and cryopreserved for analysis of immunologic markers.

Biochemical analysis

Interleukin-6 and sCD14 were determined using enzyme-linked immunosorbent assay (ELISA) [24]. The kit was obtained from Melsin Medical Co., Limited (Kuacheng District, Jilin Province, China).

Statistical analysis

The generated data was analyzed using Microsoft excel, statistical package for social science (SPSS version 20.0, California Inc., USA) and GraphPad prism (GraphPad Software, Inc., California, USA). Data was expressed as mean ± standard deviation, and data among groups was compared using one-way analysis of variance (ANOVA), followed by post-hoc analysis with Tukey's test. Correlation analysis was performed using Pearson's correlation, which was used to determine inter-variable association between various selected parameters. Values of $p < 0.05$ were considered statistically significant.

Results

The study participants were further divided into 3 groups based on duration on ART (1-5, 6-9, and ≥ 10 years), ART drug regimen (AZT/ 3TC/ NVP, TDF/ 3TC/ EFV, and LPV/r), and ankle-brachial index measurements (normal ABI, 1-1.3; borderline ABI, 0.9-0.99; and low ABI, < 0.9).

Table 1 demonstrates the sociodemographic characteristics, and anthropometric and clinical parameters of the HIV-infected study group. Of the 150 virologically suppressed HIV-infected study participants recruited into the study, 109 (72.7%) were females and 41 (27.3%) were males, with a mean age of 43.73 ± 8.74 years. Furthermore, 120 (80.0%) of the study participants were Yorubas, 10 (6.7%) Ibos, 4 (2.7%) Hausas, while 16 (10.7%) were from other ethnic groups. 94 (62.7%) were Christians, 55 (36.7%) were Muslims, and 1 (0.7%) was of other religion. The study participants level of education revealed that 11 (7.3%) had no formal education, 33 (22.0%) had primary education, 44 (29.3%) had secondary education, and 62 (41.3%) had tertiary education. Anthropometric results of the study group re-

Table 2. Comparison of cardiovascular risk factors, ankle-brachial index, interleukin-6, and sCD14 between the study and control groups using Student's *t*-test and χ^2 test

Parameter	Study group (n = 150)	Control group (n = 50)	p-value
Age (years)	43.73 ± 8.74	43.47 ± 8.87	0.714
Hypertension (%)	24 (15.9)	5 (3.3)	0.297
Family history of CVD (%)	13 (8.6)	20 (13.2)	0.001*
Diabetes mellitus (%)	6 (4.0)	1 (2.0)	0.505
Metabolic syndrome (%)	26 (17.3)	5 (10.0)	0.215
BMI (kg/m^2)	25.43 ± 5.46	25.67 ± 4.93	0.001*
Non-obese (%)	116 (76.8)	39 (25.8)	0.920
Obese (%)	24 (22.5)	11 (7.3)	0.334
WC (cm)	90.13 ± 11.52	90.58 ± 11.13	0.001*
WHR	0.87 ± 0.048	0.88 ± 0.053	0.001*
ABI	0.92 ± 0.08	0.94 ± 0.05	0.001*
Normal: 1.0-1.3 (%)	42 (27.8)	16 (10.6)	0.589
Borderline: 0.9-0.99 (%)	86 (57.0)	31 (20.5)	0.562
PAD < 0.90 (%)	22 (14.6)	3 (2.0)	0.108
SBP (mm Hg)	121.67 ± 20.96	116.40 ± 17.23	0.001*
DBP (mm Hg)	81.47 ± 7.98	76 ± 6.06	0.001*
IL-6 (pg/ml)	142.91 ± 50.17	111.88 ± 51.85	0.001*
sCD14 (ng/ml)	5.74 ± 4.83	-0.90 ± 5.53	0.001*

*Data are presented as mean ± standard deviation or numbers (percentages). * – significant at p < 0.05, PAD – peripheral artery disease, ABI – ankle brachial index, SBP – systolic blood pressure, DBP – diastolic blood pressure, BMI – body mass index, WC – waist circumference, WHR – waist to hip ratio, CVD – cardiovascular disease, IL-6 – interleukin-6, sCD14 – soluble cluster of differentiation 14 marker*

vealed a mean body mass index (BMI) of 25.43 ± 5.46. Based on their BMI value, 78 (52%) of the study participants had normal weight, 38 (25.3%) were overweight, and 34 (22.7%) were obese. The study participants recorded mean systolic blood pressure (SBP) of 121.67 ± 20.96, diastolic blood pressure (DBP) of 81.47 ± 7.98 as well as mean of 7.73 ± 3.52 years of HIV infection, 7.47 ± 3.42 years on ART, mean nadir/ baseline CD4+ T cell count of 263.18 ± 229.58 cells/mm^3, mean current CD4+ T cell count of 505.42 ± 265.85 cells/mm^3, and mean HIV viral RNA count of 40.95 ± 111.61 copies/ml. With regards to drug regimen, 93 (61.6%) of the study participants were on the first-line fixed-dose regimen, with zidovudine/lamivudine/nevirapine (AZT/3TC/NVP), 46 (30.5%) were on tenofovir/lamivudine/efavirenz (TDF/3TC/EFV), while 11 (7.3%) were on protease inhibitor with lopinavir/ritonavir (LPV/r). Based on traditional cardiovascular risk factors evaluated, the study group recorded a prevalence rate of 13 (8.6%) for family history of cardiovascular disease, 6 (4.0%) for type 2 diabetes mellitus, 24 (15.9%) for hypertension, 26 (17.3%) for metabolic syndrome based on the IDF (the International Diabetes Association) criteria [25], 1 (0.7%) for smoking, and 23 (15.2%) were on lipid lowering drugs.

Table 2 presents the comparison of cardiovascular risk factors, and anthropometric and ankle-brachial index of the study and control groups, using Student's *t*-test and χ^2 analyses. The results revealed that diastolic blood pressure was significantly higher in the study group compared to the control group ($p < 0.05$). The control group recorded significantly higher mean levels of ankle-brachial index, WC, WHR, and BMI compared to the study group ($p < 0.05$). Based on the ankle-brachial index category, the study group recorded a higher prevalence of low ABI: 22 (14.6%) compared to the control group 3 (2%); normal ABI of 42 (27.8%) compared to 16 (10.6%) recorded in the control group and borderline ABI of 86 (57%) compared to 31 (20.5) recorded in the control group. No significant association was observed between the study and control groups based on the size of the specific sub-group, according to ABI categories ($p > 0.05$). With regards to the presence of cardiovascular risk factors, the study group recorded a higher prevalence rate of 26 (17.3%) and 6 (4%) for metabolic syndrome and diabetes mellitus compared to 5 (10%) for metabolic syndrome and 1 (2%) for diabetes mellitus recorded by the controls. No significant association was observed between the study and control groups based on the presence of metabolic syndrome and diabetes mellitus ($p > 0.05$). A significant association was observed in the prevalence of family history of CVD between the study 13 (8.6%) and control groups 20 (13.2%), $p < 0.05$, with higher prevalence rate recorded by the control group.

Comparison of anthropometric and clinical parameters based on the duration on ART in the study group are depicted in Table 3. Age, ankle-brachial index, and current CD4+

Table 3. Comparison of mean levels of interleukin-6, sCD14, clinical characteristics based on duration on antiretroviral therapy (ART) in the HIV-infected study participants using one-way ANOVA

Parameter	1-5 years (n = 49)	6-9 years (n = 50)	≥ 10 years (n = 51)	p-value
Age (years)	41.35 ± 9.43	42.60 ± 8.61	47.14 ± 7.13	0.002*
ABI	0.93 ± 0.07	0.94 ± 0.07	0.89 ± 0.09	0.012*
BMI (kg/m^2)	25.46 ± 5.76	25.37 ± 5.61	25.45 ± 5.12	0.996
WC (cm)	88.98 ± 11.67	90.82 ± 11.76	90.55 ± 11.29	0.695
WHR	0.87 ± 0.06	0.88 ± 0.05	0.87 ± 0.037	0.505
SBP (mm Hg)	120 ± 21.51	117.60 ± 18.24	127.25 ± 22.10	0.054
DBP (mm Hg)	82.45 ± 8.55	79.60 ± 7.27	82.35 ± 7.70	0.128
Nadir CD4$^+$ T cell (cells/mm^3)	210.02 ± 223.56	271.50 ± 210.47	304.00 ± 247.26	0.122
Current CD4$^+$ T cell (cells/mm^3)	352.00 ± 219.94	560.32 ± 264.65	596.00 ± 247.22	0.001*
IL-6 (ng/ml)	124.90 ± 50.93	105.15 ± 47.84	105.97 ± 55.07	0.100
sCD14 (pg/ml)	6.35 ± 5.43	5.73 ± 4.23	5.16 ± 4.78	0.473

*Data are presented as mean ± standard deviation; * – significant at p < 0.05. PAD – peripheral artery disease, ABI – ankle-brachial index, SBP – systolic blood pressure, DBP – diastolic blood pressure, BMI – body mass index, WC – waist circumference, WHR – waist to hip ratio, IL-6 – interleukin-6, sCD14 – soluble cluster of differentiation 14 marker*

Table 4. Comparison of cardiovascular risk factors, inflammatory, and immune activation markers based on antiretroviral drug regimen in the HIV-infected study group

Parameter	AZT/3TC/NVP (n = 93)	TDF/3TC/EFV (n = 46)	LPV/r (n = 11)	p-value
BMI (kg/m^2)	25.71 ± 5.54	25.19 ± 5.18	24.04 ± 6.15	0.599
WC (cm)	90.30 ± 11.17	90.09 ± 11.49	88.82 ± 15.29	0.922
WHR	0.88 ± 0.04	0.87 ± 0.05	0.85 ± 0.06	0.041*
SBP (mm Hg)	122.58 ± 19.83	122.17 ± 24.5	111.82 ± 10.79	0.270
DBP (mm Hg)	82.15 ± 8.32	81.30 ± 7.49	76.36 ± 5.05	0.073
ABI	0.91 ± 0.09	0.94 ± 0.06	0.92 ± 0.09	0.041*
Nadir CD4+ T cells (cells/mm^3)	295.19 ± 250.38	211.20 ± 177.6	215.73 ± 210.60	0.100
Current CD4+ T cells (cells/mm^3)	549.74 ± 271.59	443.61 ± 244.56	393.27 ± 236.99	0.029*
IL-6 (ng/ml)	105.22 ± 50.99	125.90 ± 53.68	109.54 ± 43.04	0.035
sCD14 (pg/ml)	5.69 ± 4.96	6.12 ± 4.76	4.56 ± 4.15	0.624

*Data are presented as mean ± standard deviation; * – significant at p < 0.05, PAD – peripheral artery disease, ABI – ankle-brachial index, SBP – systolic blood pressure, DBP – diastolic blood pressure, BMI – body mass index, WC – waist circumference, WHR – waist to hip ratio, IL-6 – interleukin-6, sCD14 – soluble cluster of differentiation 14 marker*

T cell count significantly varied among the groups ($p < 0.05$). No significant difference was observed in BMI, WC, WHR, SBP, and DBP among the three groups ($p > 0.05$).

Table 4 shows the comparison of anthropometric parameters and clinical data of the study group based on ART regimen. WHR was significantly higher in study participants on AZT/3TC/NVP compared to those on LPV/r ($p < 0.05$), while ABI showed no significant difference between the two groups ($p > 0.05$). No significant difference was also observed in WHR and ABI in study participants on TDF/3TC/EFV regimen compared to those on LPV/r regimen ($p > 0.05$).

Comparison of anthropometric parameters and clinical data based on ankle-brachial index measurements in the study group, stratified into 3 groups (normal ABI = 1-1.3, borderline ABI = 0.9-0.99, and low ABI = < 0.9) are described in Table 5. No significant difference was observed in age, BMI, WC, WHR, nadir and current CD4+ T cell count, duration on ART, interleukin (IL)-6, and sCD14 among the 3 groups ($p > 0.05$).

Table 6 shows logistic regression analysis of risk factors associated with low ABI value in the study group. Low ABI presented associations with CD4 T cell < 200 copies/ml (OR 2.635, $p = 0.250$), WHR (OR 928.39, $p = 0.250$), hypertension (OR 2.115, $p = 0.335$), and detectable viraemia ≥ 20 copies/ml (OR 1.375, $p = 0.511$). No significant association or odd ration > 1 was observed for age, BMI, CVD family history, diabetes mellitus, protease inhibitors, and low ABI in the study group (OD ≤ 1, $p > 0.05$).

Table 5. Comparison of anthropometric parameters, clinical characteristics, interleukin-6, and sCD14 based on ankle-brachial index (ABI) measurements in the study participants

Parameter	Normal ABI (1-1.3) (*n* = 42)	Borderline ABI (0.9-0.99) (*n* = 86)	PAD (> 0.9) (*n* = 22)	*p*-value
Age (years)	44.67 ± 7.85	43.43 ± 9.50	43.14 ± 7.32	0.713
BMI (kg/m^2)	25.43 ± 6.05	25.08 ± 5.31	26.77 ± 4.86	0.435
WC (cm)	89.55 ± 12.10	89.28 ± 11.38	94.55 ± 10.39	0.149
WHR	0.87 ± 0.05	0.87 ± 0.05	0.89 ± 0.04	0.468
Nadir CD4	281.00 ± 230.16	257.59 ± 242.35	250.14 ± 176.46	0.833
Current CD4	495.31 ± 248.00	507.22 ± 276.67	517.77 ± 267.30	0.946
Duration on ART (years)	6.88 ± 3.14	7.55 ± 3.45	8.32 ± 3.75	0.479
IL-6 (ng/ml)	1.09.78 ± 59.26	117.39 ± 48.29	94.35 ± 48.04	0.170
sCD14 (pg/ml)	5.96 ± 4.82	6.11 ± 4.60	3.87 ± 5.45	0.142

*Data are presented as mean ± standard deviation. *Significant at p < 0.05, ABI – ankle-brachial index, SBP – systolic blood pressure, DBP – diastolic blood pressure, BMI – body mass index, WC – waist circumference, WHR – waist to hip ratio, IL-6 – interleukin-6, sCD14 – soluble cluster of differentiation 14 marker*

Table 6. Results of logistic regression analysis: association of risk factors with low ankle-brachial index value in the study group

Parameter	OR	95% CI	*p*-value
BMI	1.052	0.971-1.138	0.214
Age	0.993	0.942-1.046	0.703
WC	1.040	1.000-1.082	0.052
WHR	928.39	0.024-36033718.68	0.205
CD4+ T cell ≥ 500 cells/mm^3	1.000	0.999-1.002	0.801
CD4+ T cell < 200 cells/mm^3	2.635	0.327-21.05	0.364
Duration of HIV	1.060	0.929-1.210	0.385
Duration on ART	1.094	0.952-1.258	0.203
IL-6 (ng/ml)	0.992	0.983-1.001	0.087
sCD14 (pg/ml)	0.918	0.840-1.003	0.058
Hypertension	2.115	0.460-9.716	0.335
Family history of CVD	0.956	0.197-4.642	0.956
MetS based on IDF	1.051	0.324-3.408	0.935
Protease inhibitor (LPV/r)	0.678	0.134-3.43	0.638
Hypertension	2.115	0.461-9.716	0.335
Diabetes mellitus	0.328	0.056-1.910	0.215
Viral load ≥ 20 copies/ml	1.375	0.532-3.55	0.511

CI – confidence interval, OR – odd ratio, BMI – body mass index, WC – waist circumference, WHR – waist hip ratio, CVD – cardiovascular disease, IL-6 – interleukin-6, sCD14 – soluble cluster of differentiation marker 14, LPV/r – lopinavir/ritonavir

Discussion

Even after a successful treatment, high-risk for cardiovascular morbidity and mortality is still a problem in HIV-infected patients due to the effects of HIV and/or ART [14, 26]. This study determined the prevalence of peripheral artery disease (PAD) in people living with HIV using ankle-brachial index measurements, and also examined inflammation and immune activation as possible predictors of PAD in the study group. The study population was relatively young and mainly composed of female participants. Moreover, the study group consisted of relatively healthy HIV-infected participants, and reflected in 22.7% obesity rate compared with 32% reported, using the 2003-2004 NHANES (National Health and Nutrition Examination Survey) data, for the general population [27].

PAD disease has been shown to be associated with future cardiovascular events particularly ischemic strokes and myocardial infarction [28]. Ankle-brachial index is considered an effective screening tool with a low ABI of < 0.9 is suggestive of PAD. A study conducted by Gutierrez [29] among HIV-infected patients revealed an association between low ABI and high carotid intima media thickness,

indicating that in HIV-infected patients, low ABI may be a surrogate marker for sub-clinical atherosclerosis. In this present study, the ankle-brachial index was significantly lower in the HIV-infected group compared to the HIV-negative control group, with 14.6% compared to 2% prevalence rate observed in the control group. This is consistent with Periard *et al*. [30], who reported a high prevalence rate (20.7%) of PAD among HIV patients on ART in a Swiss cohort. Findings from this study are also in line with the research of Beckman *et al*. [31], who reported a 19% increased risk of PAD compared to non-HIV-infected veterans.

Findings from this study revealed a significantly higher mean value of IL-6 in HIV-infected study group compared to the uninfected control group. This finding is consistent with results from other studies. In SMART study, the levels of high sensitivity C-reactive protein (hsCRP) and IL-6 in HIV-infected individuals with a suppressed viral load were approximately 50% higher than matched controls from general population (from the multi-ethnic study of atherosclerosis [MESA] and the coronary artery risk development in young adults [CARDIA] studies) [32]. IL-6 is strongly associated with atherogenesis and pathogenesis of CVD. It can stimulate hepatic synthesis of acute-phase proteins, activate endothelial cells, promote lymphocyte proliferation, neutrophil migration, and macrophage differentiation [33, 34]. However, in this present study, no association was observed between IL-6 and low ABI. This is in contrast with reports from other studies examining the associations of IL-6 and markers of sub-clinical atherosclerosis, such as carotid intima media thickness (IMT) measurements. Longenecker *et al*. [35] found that IL-6 was positively associated with common CIMT in a study of 60 HIV-infected study patients with an HIV-1 RNA less than 400 copies/ml, but the association was no longer statistically significant after an adjustment for traditional cardiovascular risk factors. Recently, Siedner *et al*. [36] observed an association between plasma IL-6 levels at 6 months after ART initiation and common CIMT. However, the results of this study were consistent with reports of Mangili *et al*. [37], who reported no significant trend towards higher circulating levels of IL-6 in patients with sub-clinical atherosclerosis measured with CIMT. These findings may be secondary to the full suppression of HIV viremia in our cohort, as previous studies have demonstrated an association between inflammatory biomarkers and HIV RNA levels [38].

In this study, the mean levels of sCD14 was significantly elevated in HIV-infected study group compared to the uninfected control group. This agrees with the study of Fitch *et al*., who demonstrated significantly higher mean values of immune activation markers (sCD163 and sCD14) in a group of HIV-infected women compared to uninfected controls. CD14 is a coreceptor on innate immune cells that, together with Toll-like receptor-4 and lipopolysaccharide (LPS)-binding protein, allows these cells to scavenge circulating bacterial LPS and releases nuclear factor Kb-dependent inflammatory cytokines [39]. The soluble form of CD14 (sCD14) is shed by activated monocytes and has been proposed as a surrogate marker of LPS-induced activation [40]; although, the liver also produces sCD14 as an acute phase protein in response to IL-6. Plasma sCD14 and LPS are elevated in chronic HIV infection and are associated with poor immunologic response to ART, progression of HIV-1 and HIV-2 disease, and mortality [40]. In the general population, plasma sCD14 levels have recently been independently associated with myocardial infarction, coronary heart disease (CHD), and all-cause mortality among older (> 65 years) men and women in a cardiovascular health study (CHS) [41]. In contrast, soluble CD14 was not associated with sub-clinical vascular disease as evaluated using ABI and did not predict low ABI in the study group, which are in contrasts with previous study by Longenecker *et al*. [35], who observed an independent association between sCD14 with coronary artery calcification, sCD14 predicted the extent of sub-clinical disease in other vascular beds in a group of HIV-infected cohort. The lack of association observed in this study could be due to the cross-sectional study design used, as these reports were observed in prospective follow-up studies. Also, this study included a relatively young population (average age, 44 years), in which atherosclerotic vascular changes and burden were expected to be low. However, this study reports are consistent with the results of Fitch *et al*. [42], who demonstrated significantly higher mean values of immune activation markers (sCD163 and sCD14) in a group of HIV-infected women compared to uninfected controls.

This current study has a number of limitations as well as strengths. To our knowledge, this is the first study to investigate PAD specifically in virologically suppressed HIV-infected individuals in our population, and to relate indices of inflammation, immune activation, and traditional CVD risk factors to such PAD. Though, because this study is cross-sectional, definitive conclusions on causality cannot be made, and further longitudinal studies relating changes in inflammation, immune activation, and CVD risk factors for low ABI characteristics in HIV-infected individuals may be necessary.

Conclusions

The levels of IL-6 and sCD14 were significantly higher in the study group compared to the uninfected controls despite viral suppression, but showed no association with low ABI. The relationship between low ABI with markers of inflammation and immune activation deserves additional investigations. These results suggest that patients living with HIV on effective ART, despite achieving viral suppression, could be at a risk of developing sub-clinical CVD compared to uninfected individuals.

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Conflict of interest

The authors declare no conflict of interest with respect to the research, authorship, and/or publication of this article.

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