Rev Soc Bras Med Trop 51(3):277-283, May-June, 2018
doi: 10.1590/0037-8682-0472-2017

Major Article

Evaluation of inflammatory biomarkers, carotid intima-media thickness and cardiovascular risk in HIV-1 treatment-naive patients

***Priscila Valéria do Carmo Carvalho*[1], *Júlia Fonseca de Morais Caporali*[2], *Érica Leandro Marciano Vieira*[3], *Nathalia Sernizon Guimarães*[1], *Marise Oliveira Fonseca*[2] *and Unai Tupinambás*[1],[2]**

[1]. Programa de Pós-Graduação em Ciênicas da Saúde: Infectologia e Medicina Tropical, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil.
[2]. Departamento de Clinica Médica, Faculdade de Medicina da Universidade Federal de Minas Gerais. Belo Horizonte, MG, Brasil.
[3]. Laboratório Interdisciplinar de Investigação Médica, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil.

Abstract

Introduction: Mortality among HIV patients is 3-15 times higher than that among the general population. Currently, most deaths are due to non-infectious diseases. Chronic inflammation and adverse events due to antiretroviral therapy play crucial roles in increasing cardiovascular risk (CVR). **Methods:** This cross-sectional study aimed to evaluate carotid intima-media thickness (CIMT) and inflammatory biomarkers (D-dimer, ADAMTS13, GDF-15, sICAM-1, MPO, myoglobin, NGAL, SAA, sVCAM-1, and p-selectin) among naïve patients. **Results:** Sixty-seven participants were included: median age, 32 years; males, 82.1%; non-white, 61.1%; higher education level, 62.7%; and exposed to HIV through sexual relationship (men who have sex with men), 68.7%. The median viral load and LTCD4$^+$ value were 42,033 copies/mL and 426 cells/mm^3. The prevalence of arterial hypertension was 16.4%; those of diabetes mellitus and dyslipidemia were 3% and 70.1%, respectively. The CIMT was 494.08 (± 96.84mm). The mean vascular age was 33.2 ± 18.9 years, one year longer than the chronological age, without statistical significance. **Conclusions**: The majority of participants had a low CVR (94%). After reclassification, considering the CIMT percentiles, 13 (19.4%) patients had medium/ high CVR, while 54 (80.6%) patients had low CVR. The difference between the proportions of CVR when considering the CIMT and its corresponding percentile was statistically relevant. Body mass index was the only predictor of higher CVR (p = 0.03). No biomarker was found to predict CVR. People living with HIV have a high prevalence of dyslipidemia before ARV therapy.

Keywords: HIV. Cardiovascular risk. Biomarkers. Inflammation. Intima-media thickness.

INTRODUCTION

Infection caused by human immunodeficiency virus (HIV) is the fifth-leading cause of death among adults worldwide[1]. The policy of universalizing access to antiretroviral therapy (ART) has been determined to impact on patient survival, changing the profile of the epidemic characterized by the reduction of new cases and mortality[1]. Despite this, mortality is still 3 to 15 times higher in the population living with HIV than the general population[2-4]. Although the mortality rate can be attributed to infectious complications, more than half of these deaths are due to non-infectious causes. With the improvement of ART-associated survival, a transition between infectious and non-infectious complications was noted, increasing the importance of chronic degenerative events[5-10]. The reduction in life expectancy among people living with HIV (PLWHIV) is associated with an increased risk of a series of *non-AIDS* complications, including heart disease, cancer, liver, kidney diseases, bone diseases, and neurocognitive disorders[10,11]. HIV carriers are at 1.5 to 2 times higher risk of cardiac events than the general population[3,8,11,12-21]. This increase may be related to chronic immune-inflammatory activity and immunosenescence in HIV[3,6,9], in addition to the classic risk factors. Inflammatory biomarkers are generally elevated in PLHIV[12,22-30]. These markers have been associated with an increased risk of cardiovascular disease (CVD), opportunistic conditions, and other causes of mortality[7,12,13,22,31-35]. Persistent immune activation and inflammation associated with classical factors play an important role in the etiology of CVDs[17]. The identification of subclinical coronary artery disease, assessed by measuring the carotid intima-media thickness (CIMT), considered as a substitutive marker, may represent an early diagnostic strategy in this

Corresponding author: Prof. Unai Tupinambás
e-mail: unai@ufmg.br
Received 7 December 2017
Accepted 18 May 2018

population[18,23-26]. The CIMT of PLHIV is thicker than that in the uninfected population[25,27-29]. In this context, the present study evaluated the profiles of the inflammatory biomarkers related to CVDs and the intima-media thickness as predictors of CVD risk in human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) patients before antiretroviral therapy.

METHODS

This observational, cross-sectional study was performed among PLWHIV before starting ART, between 2014 and 2015 in a specialized service.

Ethical considerations

The study protocol was approved by the Research Ethics Committee of the Federal University of Minas Gerais (no. 12649713.4.0000.5149).

The inclusion criteria were: age between 18 and 60 years, and both genders with a diagnosis of HIV infection without the prior use of ART. The exclusion criteria were: lack of completion of the protocol for the collection of clinical or laboratory data, pregnancy, or history of chronic non-controlled diseases.

The following information was collected: sociodemographic (age, gender, race, education, and marital status), lifestyle (smoking, alcoholism, and alcohol abuse through the CAGE questionnaire)[36], systolic and diastolic blood pressure, anthropometric data (weight and height), and biochemical parameters as fibrinogen, 25-hydroxyvitamin D, lipidogram, blood glucose, polymerase chain reaction (PCR) results, in addition to viral load (HIV-PCR), and LTCD4$^+$ lymphocyte count.

The levels of biomarkers related to CVDs were measured using the Human Cardiovascular Disease (Merck Millipore) kit according to the manufacturer's recommendations[37]. Ultrasonography of the carotid arteries was also performed by a single trained examiner, within 10 days after initiation of the treatment. The measurement of CIMT followed the current technical principles[26,37-39]. The values of intima-media thickness were expressed in terms of vascular age, based on the vascular age calculator (VAC)[38].

In the descriptive analysis, qualitative variables were summarized as absolute and relative frequencies and quantitative variables were summarized as means and standard deviations or medians and interquartile ranges. The assumption of normality for the probability distribution of the variables was verified using the Shapiro-Wilk test. Comparisons between two quantitative variables were performed using the Kruskal-Wallis test for non-normally distributed variables and analysis of variance for normally distributed variables. The tests were used to compare serum levels of the biomarkers (ADAMTS13, D-dimer, GDF-15, myoglobin, sICAM-1, MPO, p-selectin, lipocalin-2/NGAL, sVCAM-1, and SAA) among the categories of viral load and Framingham risk score. Biomarkers and viral load correlations were analyzed using Spearman's rank correlation test. Receiver operating characteristic (ROC) curve analysis was used to investigate cardiovascular risk prediction and verify the association of biomarkers with cardiovascular risk and viral load. The log-binomial model was used to evaluate whether biomarkers were predictors of cardiovascular risk. The univariate model of each marker was adjusted with the risk variable (yes or no); markers that had p-values ≤ 0.20 were considered as candidates for the multivariate model. A multivariate model was fitted with all candidates and step-by-step the markers with higher p-values were withdrawn until all significant markers remained at the 0.05 level. The quality of fit of the model was assessed by the deviance and quality measures of the model, i.e., Akaike information criterion, corrected version of the Akaike information criterion, Bayesian information criterion, and consistent Akaike information criterion. The multivariate linear regression model was used to investigate the correlation between the study variables and the thickness of the intima media. It was initiated by univariate adjustment of the simple linear regression model of each group of variables. Within each group, the candidate variables (with $p \leq 0.20$) were selected, adjusting the multivariate models for each group individually with their respective candidate variables and step-by-step, with the variables with the highest p-values being removed until they were all significant at the 0.05 level. The quality of fit was verified by residual analysis (normality, homoscedasticity, and independence). In all applied tests, significance levels of 5% were used.

RESULTS

In this study, out of 105 patients who were screened, 5 did not meet the inclusion criteria, and 9 did not undergo laboratory data collection. Among the 91 patients included in the study, 67 (73.6%) underwent carotid ultrasonography and measurement of biomarkers.

The median age of the participants was 32 (26-41) years, the majority being men who have sex with other men (MSM). Regarding comorbidities, the prevalences of hypertension, diabetes, and dyslipidemia were 16.4%, 3% and 70.1%, respectively. The median viral load was 42,033 (16,189-101,069) copies/mL and the median LTCD4$^+$ lymphocyte count was 426 (262-561) cell/mm^3. The median time between diagnosis of HIV infection and initiation of antiretroviral therapy was 4.6 months, ranging from 1 to 301.7 months, as shown in **Table 1**.

The mean CIMT was 494.08 (± 96.84mm). The mean vascular age was 33.2 ± 18.9 years, one year longer than the chronological age (32 years), but with no statistical significance. The majority of participants had a low cardiovascular risk (94%) as measured by the Framingham score (**Table 2**). According to the consensus, individuals with CIMT values greater than or equal to the 75th percentile are considered as high risk and indicate an increased CVD risk[23]. Thus, after the reclassification, considering the CIMT percentiles, 13 (19.4%) patients had medium/high cardiovascular risk, and 54 (80.6%) patients had low cardiovascular risk. Considering the CIMT measure for the estimation of cardiovascular risk was statistically significant ($p = 0.02$).

ROC curve analysis showed that body mass index was the only predictor of higher cardiovascular risk ($p = 0.03$) (**Table 3**). D-dimer and p-selectin presented predictive tendencies ($p = 0.06$ and 0.06, respectively). D-dimer showed a significant correlation with viral load level, ($r = -0.389$, $p < 0.01$). P-selectin was uncorrelated to

TABLE 1: Clinical and laboratory characteristics of treatment-naïve people living with HIV, assessed in a reference center, Belo Horizonte, State of Minas Gerais, March 2014 - September 2015.

Variables	Number=67
Age	
median (interquartile range)	32 (26; 41)
Gender, n (%)	
female	12 (17.9)
male	55 (82.1)
Race, n (%)	
white	26 (39)
nonwhite	41 (61)
Education, n (%)	
illiterate	0 (0.0)
elementary/ middle school	8 (12.0)
high school	17 (25.4)
college	42 (62.7)
Marital status, n (%)	
single	54 (80.6)
married	13 (19.4)
Alcoholism	
yes	45 (67.2)
no	22 (32.8)
Alcohol abuse (CAGE), n=45	
yes	4 (8.9)
no	41 (91.1)
Tabagism	
yes	12 (17.9)
no	55 (82.1)
SBP (mmHg)	
median*	120 (110;125)
DBP (mmHg)	
median*	80 (70;80)
BP, n (%)	
normal, (SBP<120) and (DBP<80)	18 (16.9)
prehypertension (SBP 120-139) or (DBP 80-89)	38 (56.7)
hypertension (SBP >140) or (DBP >90)	11 (16.4)
Blood glucose (mg/dL), n (%)	
≤ 99 normal	65 (97.0)
> 99 abnormal	2 (3.0)
Triglycerides (mg/dL), n (%)	
< 150 normal	47 (70.1)
≥ 150 abnormal	20 (29.9
HDLc (mg/dL), n (%)	
male, n=55	
≥ 40 normal	18 (32.7)
< 40 abnormal	37 (67.3)
female, n=12	
≥ 50 normal	3 (25)
< 50 abnormal	9 (75)
Total cholesterol (mg/dL), n (%)	
< 200 normal	53 (79.1)
≥ 200 abnormal	14 (20.9)
LDLc (mg/dL) n (%)	
< 160 normal	61 (91.0)
≥ 160 abnornal	6 (9.0)
Viral load (copies/mL), n (%)	
< 50	0 (0.0)
50 –1,000	5 (7.5)
1,000–100,000	45 (67.2)
> 100,000	17 (25.4)

Continue...

TABLE 1: Continuation.

Variables	Number=67
Viral load	
median	42,033 (16,189–101,069)
CD4 count (cells/mm³) n (%)	
≤ 200	10 (14.9)
201-350	14 (20.9)
351-500	19 (28.4)
> 500	24 (35.8)
CD4	
median	426 (262–561)
Time between diagnosis and initiation of treatment (months)	
median*	4.60 (2.10;10.80)
BMI (kg/m²)	
median*	24.00 (21.67;26.85)
BMI classification, n (%)	
< 18.5 underweight	1 (1.5)
18.5–24.9 normal	46 (68.7)
≥ 25 overweight/obesity	20 (29.8)

CAGE: the CAGE questionnaire; **SBP**: systolic blood pressure; **DBP**: diastolic blood pressure; **BP**: blood pressure; **HDLc:** high density cholesterol; **LDLc:** low density cholesterols; **CD4:** lymphocytes CD4+; **BMI**: body mass index. *Interquartile range.

TABLE 2: Carotid ultrasonography findings and Framingham score of people living with HIV before antiretroviral therapy treatment in a reference center, Belo Horizonte, State of Minas Gerais, March 2014 - September 2015.

Variables	Number=67
Vascular age calculator	
mean±SD	33.21±18.87
Difference between chronological age and VAC	
mean±SD	-0.01±15.05
Mean CIMT	
mean±SD	494.08±96.84
CIMT percentile according to standard table n (%) (Stein et al., 2008)	
<2.5	0 (0.0)
2.5–10	5 (7.5)
10.1–25	8 (11.9)
25.1–50	22 (32.8)
50.1–75	21 (31.3)
75.1–90	4 (6.0)
90.1–97.5	6 (9.0)
>97.5	1 (1.5)
Framingham risk rating	
low	63 (94.0)
moderate	1 (1.5)
high	3 (4.5)
Framingham risk reclassification of individuals with CIMT >75 percentile according to the consensual recommendation (Stein et al., 2008)*	
low	54 (80.6)
moderate	9 (13.4)
high	4 (6.0)

HIV: human immunodeficiency virus; **SD**: standard deviation; **VAC:** vascular age calculator; **CIMT:** carotid intima-media thickness. * p=0.0198.

TABLE 3: Results of receiver operating characteristic curve analysis of biomarkers, BMI and VL related to cardiovascular risks.

Variable	AUC	95% CI of AUC	*p-value*
BMI	**0.69**	**0.52; 0.86**	**0.03**
VL	0.64	0.49; 0.80	0.11
sVCAM-1	0.51	0.35; 0.67	0.89
ADAMTS13	0.55	0.37; 0.73	0.60
D-Dimer	**0.33**	**0.18; 0.48**	**0.06**
GDF15	0.44	0.28; 0.61	0.53
sICAM	0.37	0.22; 0.52	0.16
MPO	0.64	0.48; 0.80	0.11
P-Selectin	**0.33**	**0.17; 0.49**	**0.06**
Lipocalin-2NGAL	0.50	0.31; 0.70	0.99
SAA	0.53	0.35; 0.71	0.74
Myoglobin	0.53	0.35; 0.72	0.71

BMI: body mass index; **VL:** viral load; **95% CI:** confidence interval 95%; **AUC:** area under the curve; **CI:** confidence interval; **sVCAM-1**: vascular adhesion molecule1; **ADAMTS13:** Disintegrin and Metalloproteinase with Thrombospondin motifs; **GDF15**: growth differentiation factor 15; **sICAM:** soluble molecule of intercellular adhesion; **MPO:** myeloperoxidase; **2NGAL:** vascular growth factors angiopoietin; **SAA**: serum amyloid A.

the intima-media thickness, and without statistical significance, i.e. β = -5.57 (95% confidence interval: -12.31-1.18), p = 0.10. There was an association between measured thickness and cardiovascular risk and age.

DISCUSSION

The majority of participants were male and MSM with a mean age of 32 years. Being a young population, there were low prevalences of arterial hypertension and diabetes mellitus. However, the prevalence of smoking was higher than that reported in Brazilian national surveys[40-43]. In addition, we found a high (70%) prevalence of dyslipidemia, mainly with high density lipoprotein cholesterol reduction, despite the young population and prior ART[44], which can be explained, among other factors by the alteration in the maturation of this lipoprotein caused by the direct action of HIV[45,46]. All efforts should be made to promote health and disease prevention in this population, aiming to reduce the classical factors of chronic-degenerative diseases, mainly smoking cessation. Regarding the immunological statuses of the patients, the majority had LTCD4+ counts above 400 cells/mm³, considered adequate and higher than those reported in another study in Brazil[46]. There was no association between biomarkers and cardiovascular risk.

There was an association between the measured thickness, the cardiovascular risk, and the age. Age showed a positive correlation with the intima-media thickness; older patients had thicker intima-media. This result corroborates findings from the literature on risk factors of mean CIMT[25].

The sample size may have been insufficient to show the relationship between biomarker changes and other classic cardiovascular risk factors, such as age, tabagism and immunological status, as evidenced in the literature[7,12,32].

In this cross-sectional study, associated factors and effects were evaluated simultaneously. Therefore, this study only allows the establishment of associations between events, and it is not possible to define causality. No sedentary lifestyle was evaluated in this population, and no association could be made between physical activity and cardiovascular risk in the described population. Another limitation of the study is the absence of a control group of healthy participants in order to compare serum levels of studied biomarkers, due to the absence of reference values in the literature.

In this study, we noted a high (70.4%) prevalence of dyslipidemia in relation (60.3%) to the general Brazilian population. This level of change was found prior to the individual's exposure to ART and tended to increase significantly with the initiation of therapy, since most ART negatively modify the user's long-term lipid profile, gradually increasing cardiovascular risk over time.

There was no association between cardiovascular biomarkers and carotid atherosclerosis, similar to other studies that did not find such an association[48]. We identified early changes in the intima-media thickness and the increase in the vascular age calculated in the majority of participants, similar to other studies[49]. This result points to the role of HIV itself in the pathogenesis of atherosclerotic disease since the population studied was young, most of them were without classical risk factors, and were all without previous ART exposure. Prospective studies are needed to confirm this tendency. The intima-media thickness was directly associated with the age of the participants, a finding that corroborates those of the current literature, and shows that age is a risk factor for the increase in intima media thickness. The intima-media thickness seems to be a good marker for subclinical arteriosclerosis in the HIV population, as it is already in the general population. We suggest that prospective studies evaluate the effectiveness of inclusion of this variable in the care protocols for PLWHIV.

Conflict of interest

The authors declare that there is no conflict of interest.

Financial support

Fundação de Amparo à Pesquisa do Estado de Minas Gerais: *Edital Universal* 2014.

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