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Sub-clinical atherosclerosis in HIV-infected patients: prevalence and risk factors

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Abstract

Introduction: Antiretroviral therapy has significantly improved the prognosis of human immunodeficiency viruses (HIV) infection. Therefore, life expectancy of people living with HIV (PLHIV) has increased. However, this therapy may have some side effects. This study aimed to detect the prevalence of sub-clinical carotid and coronary atherosclerosis among asymptomatic patients living with HIV, free from known cardiovascular diseases, and to identify the factors associated with sub-clinical atherosclerosis.

Material and methods: We conducted a cross-sectional prospective study over one year (between July 2018 and June 2019). We included 75 PLHIV, followed-up in the outpatient clinic of the Infectious Diseases Department of the University Hospital Fattouma Bourguiba in Monastir, Tunisia. Cardiovascular assessment, including carotid doppler ultrasonography, electrocardiogram, exercise stress testing, and transthoracic echocardiography was proposed to all study participants.

Results: The cardiovascular assessment revealed sub-clinical atherosclerosis in 9 PLHIV (12%): carotid atherosclerosis in 9 cases and coronary artery atherosclerosis in one case. One patient had presented both carotid and coronary atherosclerosis. After multivariate regression analysis, smoking (OR = 2.6; 95% CI: 1.08-6.62%; $p = 0.03$) and age ≥ 40 years (OR = 2.3; 95% CI: 1.02-5.22%; $p = 0.04$) were found to be independent risk factors of sub-clinical atherosclerosis in PLHIV.

Conclusions: Our study revealed that sub-clinical atherosclerosis was present in 1 of 8 PLHIV. Therefore, screening for atherosclerosis using carotid ultrasound imaging, transthoracic echocardiography, and exercise stress test should be suggested for all PLHIV under 40 years and/or smokers.

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Key words: HIV, prevalence, prevention, risk factors, atherosclerosis.

Introduction

Antiretroviral therapy (ART) has significantly improved the prognosis of human immunodeficiency viruses (HIV) infection. Therefore, life expectancy of people living with HIV (PLHIV) has increased [1]. However, this prolongation of survival was associated with raised comorbidities, including cardiovascular complications [2]. This finding suggests that chronic HIV infection is involved in the development of sub-clinical atherosclerosis in PLHIV [3, 4]. On the other hand, some studies have described a correlation between ART and increased risk

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of atherosclerotic cardiovascular disease (ACVD) [5]. Therefore, identifying factors predisposing to sub-clinical atherosclerosis in this population is an essential preventive measure. To the best of our knowledge, no data concerning the prevalence of asymptomatic ACVD in PLHIV in Tunisia and all North African countries are currently available.

This study aimed to detect the prevalence of carotid and sub-clinical coronary atherosclerosis among asymptomatic PLHIV, free from known ACVD, and to identify the factors associated with sub-clinical atherosclerosis.

Material and methods

A cross-sectional prospective study between July 2018 and June 2019 was conducted at the University Hospital Fattouma Bourguiba in Monastir, Tunisia. In the research, PLHIV aged over 18 years and having no clinical symptoms suggestive of ACVD were included. Exclusion criteria were age ≤ 18 years, clinical history of ACVD, peripheral vascular disease or cerebrovascular disease, pregnancy, and contraindication to exercise stress test. Of the 170 PLHIV followed-up in our department, 75 patients were included in the study after excluding participants, who did not follow the inclusion criteria, and/or had at least one exclusion criterion and/or refused to participate in the study. The following parameters from medical records were assessed: demographic characteristics, detailed medical history including traditional ACVD risk factors, measurement of plasma glucose, triglycerides, total cholesterol, and high-density lipoprotein (HDL) cholesterol levels. Additionally, parameters related to HIV, such as HIV infection duration and its' clinical stage at diagnosis according to the Centers of Disease Control (CDC) Atlanta 1993 classification. ART, CD4+ T cell counts, nadir CD4+ T cell counts, and HIV viral load were also recorded. A questionnaire was proposed to patients, evaluating the presence of smoking, physical inactivity, and family history of ACVD. In addition, all subjects underwent physical examination, including evaluation of body mass index (BMI) and blood pressure (BP).

Evaluation of sub-clinical atherosclerosis

The following tests were performed in all enrolled patients:

- Electrocardiogram (12-lead ECG).
- Conventional two-dimensional transthoracic echocardiography (2D-TTE) was done by a physician blinded to patients' status and treatment history. The same cardiologist performed all cardiac ultrasounds. We looked for global and regional contractility abnormalities, valvular heart disease, signs of pulmonary arterial hypertension, abnormal chamber dimensions, and ejection fraction (EF). EF was expressed as a percentage greater than 55% in healthy individuals [6]. An exercise stress test (EST) was carried out in cardiology department on a bicycle, under ECG and with BP monitoring. The exercise was ended upon reaching 85% of theoretical maximum frequency according to Bruce protocol [7].
- Carotid ultrasonography imaging using color Doppler imagining combined with B-mode was performed by a radiologist, blinded to clinical and analytical data. Ultrasonography was used to assess the presence of atheromatic plaques. Severe stenosis was defined as stenosis greater than 70% in the artery lumen [8].
- Other investigations were required on a second line:
  - Coronarography was performed on one participant (1.3%) who had inferoseptal akinesia at 2D-TTE.
  - Computed tomography of supra-aortic arteries (CTA) was performed to complement carotid ultrasonography imaging in two participants (2.6%).

Statistical analysis

All analyses were performed using Statistical Package for Social Sciences (SPSS) software, version 22.0. Qualitative variables were expressed as percentages, and quantitative variables were calculated as means and standard deviations. χ^2 and Fisher exact tests were used in univariate analysis. The level of statistical significance was set at $p < 0.05$. The study was approved by the hospital's ethics committee, and written informed consent was obtained from all participants.

Results

Study population

We enrolled 75 PLHIV patients for this cross-sectional study. Demographic, clinical, HIV-related characteristics, and metabolic parameters are presented in Table 1. All the participants were asymptomatic. The mean age was 40.52 ± 9.56 years (range, 22-66 years). The study population was predominantly young, with male predominance (sex ratio = 2). Smoking (46.6%), physical inactivity (41.3%), dyslipidemia (21.3%), and obesity (12%) were the most common CVRFs. A history of high blood pressure and diabetes were noted in 5.3% and 2.6%, respectively. Sixty-three PLHIV (84%) had at least one classic CVRF, and 30 PLHIV (40%) had at least two CVRFs.

Thirty-nine patients (52%) were at stage A of HIV infection, and all the included patients were on ART. The combination of two nucleoside reverse transcriptase inhibitor (NRTI) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) was the most prescribed treatment (n = 57, 76%), followed by a combination of two NRTIs and a protease inhibitor boosted by ritonavir (PI/r) (n = 14, 18.7%). The average duration of ART was 78.7 ± 62.8 months (range, 6-228 months), with a median of 59 months.

Prevalence of sub-clinical atherosclerosis

Ultrasonography showed carotid plaque in eight cases (11%) and accelerated circulatory speeds in one patient (1%).

Table 1. Main demographic, clinical, and HIV-related characteristics (*N* = 75)

Factor	
Sex, *n* (%)	
Male	50 (67.0)
Female	25 (33.0)
Age (years)	40.52 ± 9.56
Family history of CVD, *n* (%)	1 (1.3)
Dyslipidemia, *n* (%)	16 (21.3)
Hypertension, *n* (%)	4 (5.3)
Metabolic syndrome (IDF criteria), *n* (%)	8 (10.6)
Smoking, *n* (%)	30 (46.6)
Physical inactivity, *n* (%)	31 (41.3)
BMI (kg/m^2)	25.2 ± 3.8
HIV disease-related parameters	
Years since HIV diagnosis	8 ± 6
CDC clinical stage, *n* (%)	
Stage A	39 (52.0)
Stage B	13 (17.3)
Stage C	23 (30.7)
Median nadir CD4+ T cell count (cells/mm^3)	394 ± 326
CD4 T-cell count ≥ 200/mm^3, *n* (%)	72 (96.0)
Median CD4+ T cell count (cells/mm^3)	591 ± 304.8
Current HIV RNA undetectable, *n* (%)	64 (85.3)
Protease inhibitor use history, *n* (%)	36 (48.0)
Integrase inhibitor use history, *n* (%)	4 (5.3)
Abacavir use history, *n* (%)	2 (2.7)
Current use of NRTI, *n* (%)	74 (98.7)
Current use of NNRTI, *n* (%)	57 (76.0)
Median ART duration (months)	78.7 ± 62.8
Median protease inhibitor duration (months)	66.6 ± 50.4

IDF – International Diabetes Federation, BMI - body mass index, HAART – highly active antiretroviral therapy, NRTI – nucleoside reverse transcriptase inhibitor, NNRTI – non-nucleoside reverses transcriptase inhibitor

CTA showed carotid plaque in one case, and total occlusion of the left common carotid artery in the other case. In total, nine patients (12%) presented sub-clinical carotid atherosclerosis. Most of the participants had normal electrocardiogram (*n* = 74, 98.7%). Repolarization abnormalities were noted in one case (1.3%) with isolated T-wave inversion. 2D-TTE showed inferoseptal akinesia in one patient (1.3%). EF was normal in all participants, and on average it was 68.8 (range, 47-94). EST was negative in 70 patients (94.6%) and non-conclusive in 4 cases (5.4%). Chromatography was performed in one patient (1.3%) and revealed a moderate atheromatous plaque in the right coronary artery. In total, nine PLHIV (12%) presented 10 sub-clinical atherosclerotic lesions, including nine carotid lesions (12%) and one coronary lesion (1.3%). One patient had both carotid and coronary atherosclerosis.

Factors associated with sub-clinical atherosclerosis

In univariate analysis, sub-clinical atherosclerosis was significantly associated with age ≥ 40 years, smoking, hyper-LDL cholesterolemia and/ or hypercholesterolemia, classic CVRF ≥ 2, DAD score ≥ 1%, time since HIV diagnosis ≥ 10 years, stage C of HIV infection, treatment with PI/r, and ART duration ≥ 125 months (Table 2).

In multivariate regression analysis, smoking (OR = 2.6; 95% CI: 1.08-6.62%; p = 0.03) and age ≥ 40 years (OR = 2.3, 95% CI: 1.02-5.22%; p = 0.04) were found to be the independent risk factors of sub-clinical atherosclerosis in PLHIV (Table 3).

Discussion

To the best of our knowledge, this is the first assessment of sub-clinical atherosclerosis in PLHIV in Tunisia. Our study showed that asymptomatic PLHIV, who were free of ACVD, presented sub-clinical atherosclerosis in 12% of cases, including nine carotid lesions (12%) and one coronary lesion (1.3%). As expected, traditional CVRF, such as age and smoking, were significantly associated with sub-clinical atherosclerosis. Our results did not find evidence for independent association between immunovirological status of HIV infection and sub-clinical atherosclerosis. Furthermore, after multivariate regression analysis, the duration of antiretroviral treatment and PI/r exposure were not considered as independent risk factors of sub-clinical atherosclerosis.

Earlier studies showed a higher prevalence of sub-clinical atherosclerosis in PLHIV. León *et al.* [9] reported carotid atherosclerosis in 21% of cases. Another study from San Francisco included 352 PLHIV, and showed a higher prevalence (65.3%) and new carotid plaques in 58.2% of PLHIV after a mean follow-up of 3.6 years [10]. A meta-analysis of nine studies with 1,229 asymptomatic PLHIV and 1,029 sero-negative subjects showed (using cardiac computed tomography) calcified coronary plaques in 31% of cases and non-calcified plaques in 58% of PLHIV. PLHIV had a higher prevalence of non-calcified coronary plaques than HIV-negative subjects (58% vs. 17% with OR = 3.26) [11]. The differences between the studied populations could explain these different results. The cited studies had a higher incidence of CVRF than our current study. All participants in the survey conducted by León *et al.* [9] were over 35 years old, more than half were smokers, and 49% presented a family history of CVD. Moreover, antiretroviral treatment strategies used in the cited studies were different.

In agreement with our results, Hsue *et al.* [12] showed that increased atherosclerosis with HIV infection could occur without detectable viremia or definite immunodeficiency. These findings could be attributed to the immunovirological success obtained with most of the participants in our study. Sixty-four participants (85.3%) had CD4+ T cell count ≥ 200/mm^3 and 72 PLHIV (96%) had undetectable viral load. However, D'Ascenzo *et al.* [11] reported a significant associ-

Table 2. Univariate analysis of participants' baseline characteristics as determinants of atherosclerosis

Factor	Sub-clinical atherosclerosis		*p*-value
	(+) (*n* = 9)	(–) (*n* = 66)	
Demographic characteristics			
Age ≥ 40 years	9 (100.0%)	31 (47.0%)	**0.003**
Male gender	7 (77.7%)	43 (65.1%)	0.45
Smoking	7 (77.7%)	28 (42.4%)	**0.046**
BMI ≥ 25 kg/m^2	2 (22.2%)	34 (51.5%)	0.09
Hypertension	1 (11.1%)	3 (4.5%)	0.41
Hyper-LDL cholesterolemia and/or hypercholesterolemia	2 (22.2%)	1 (1.5%)	**0.003**
Metabolic syndrome (IDF criteria)	0 (0.0%)	8 (12.1%)	0.26
Classic CVRF ≥ 2	8 (88.7%)	22 (33.3%)	**0.001**
Time since HIV diagnosis ≥ 10 years	6 (66.6%)	21 (31.8%)	**0.041**
Stage C	6 (66.6%)	17 (25.7%)	**0.013**
Virological and immunological characteristics			
Median nadir CD4+ T cell count (cells/mm^3)	342.7 ± 337.1	401.1 ± 326.5	0.61
HIV RNA detectable	2 (22.2%)	9 (13.6%)	0.49
Therapeutic characteristics			
PI/r use history	5 (55.5%)	11 (16.6%)	**0.008**
Abacavir use history	0 (0.0%)	2 (3.0%)	0.59
INTI use history	9 (100.0%)	65 (98.5%)	0.7
ART duration ≥ 125 months	4 (44.4%)	11 (16.6%)	**0.05**

ation between CD4+ T cell count and coronary atherosclerosis. Moreover, Post *et al.* [13] demonstrated that immunosuppression is associated with atherosclerosis. Lower nadir CD4+ T cell count (OR = 0.8; 95% CI: 0.69-0.94%; $p = 0.005$) was associated with coronary stenosis in greater than 50%. The association between low CD4+ T cell count and atherosclerosis may be explained by chronic inflammation in immunosuppressed PLHIV [14, 15]. Other studies have shown an association between HIV replication and inflammation markers [16]. Direct vascular toxicity of the virus was reported [17].

Conflicting results from studies investigating the relationship between HIV infection, PI/r exposure, and atherosclerosis have been published. Gleason *et al.* [18] showed results of a survey including PLHIV in Ethiopia, and concluded that ART with lopinavir/ritonavir was associated with an increased markers of sub-clinical atherosclerosis. The association between PI/r, insulin resistance, and metabolic diseases may explain this relationship. Nevertheless, other studies have not demonstrated a clear association between PI/r and cardiovascular risk [19, 20]. Lai *et al.* [21] reported that a duration of exposure to ART for more than 36 months was a predictive of sub-clinical coronary atherosclerosis in PLHIV (OR = 1.62; 95% CI: 1.17-2.35%; $p = 0.01$). Antiretroviral treatment can improve endothelial dysfunction in the short-term. In contrast, long-term exposure may aggravate endothelial dysfunction [22, 23].

Table 3. Factors associated with sub-clinical atherosclerosis in multivariate analysis

Associated factors	Sub-clinical atherosclerosis		
	Odds ratio	95% CI	*p*-value
Age ≥ 40 years	2.3	1.02-5.22%	**0.04**
Smoking	2.6	1.08-6.62%	**0.03**

The present study demonstrates the importance of researching sub-clinical atherosclerosis. However, the relatively small population and non-randomized design of the study cause a lack of power in statistical analysis. Furthermore, the lack of comparison between PLHIV receiving ART and PLHIV not receiving ART makes it challenging to determine the role of antiretrovirals in the occurrence of atherosclerosis. Finally, stress testing as a screening test for coronary atherosclerosis is insufficient. According to the limited sensitivity of this test for detecting low-risk myocardial lesions, it can lead to underestimating the prevalence of coronary atherosclerosis in our study.

Conclusions

Our study revealed that sub-clinical atherosclerosis was present in 1 of 8 PLHIV. Therefore, screening for atherosclerosis

rosis using carotid ultrasound imaging, transthoracic echocardiography, and an exercise stress test should be suggested for all PLHIV under 40 years and/or smokers. Although our findings should be tentative, and replication in large cohorts is required. We also encourage preventing atherosclerosis and managing traditional cardiovascular risk factors in PLHIV. Subsequent follow-up of our patients should be considered to optimize the appropriate time for monitoring cardiovascular explorations.

Conflict of interest

The authors declare no conflict of interest.

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