Markers of subclinical atherosclerotic disease in HIV-infected individuals

Pulin K Gupta[1]*, Manu Gupta[1], Ashok K Lal[1], Anil Taneja[2], Rajesh S Taneja[1] and Bharat B Rewari[1]

[1] Department of Medicine, Postgraduate Institute of Medical Education and Research, Dr Ram Manohar Lohia Hospital, New Delhi, India
[2] Department of Radiology, Postgraduate Institute of Medical Education and Research, Dr Ram Manohar Lohia Hospital, New Delhi, India

Abstract

Background: Wider access to antiretroviral treatment (ART) has resulted in a decline in the number of people dying due to AIDS-related causes. However, with this increased longevity, accelerated rates of cardiovascular and atherosclerotic diseases are on the rise. We hypothesised that the prevalence of atherosclerotic cardiovascular diseases is greater in HIV/AIDS patients as compared to the normal population. Thus, we aimed to study the predictors of subclinical atherosclerotic disease in HIV-infected individuals.

Methods: In total, 168 HIV-positive individuals below 45 years of age (124 [73.08%] on ART and 44 [26.2%] ART naive) along with 150 age- and sex-matched healthy controls were recruited for this cross-sectional observational study. Carotid intimal medial thickness (cIMT), a surrogate marker of atherosclerosis, was assessed by a carotid colour doppler ultrasound and a mean of four measurements (both sides) were taken. cIMT was correlated with the age of the individuals, duration and type of ART, duration of disease and the level of immunodeficiency (CD4 cell count) along with conventional cardiac risk markers.

Results: In 168 HIV-positive individuals, the mean CD4 cell count was 332.41 ±17.1 cells/mm^3. The mean cIMT of all HIV-positive individuals was 0.712 ±0.039 mm (0.596–0.840 mm) as compared to 0.616 ±0.023 mm (0.540–0.655 mm) in HIV-negative individuals (*P*<0.001). cIMT in HIV-positive individuals on ART (subgroup A) was 0.723 ±0.034 mm as compared to 0.682 ±0.038 mm in HIV-positive individuals not on ART (subgroup B) (*P*<0.01). Low CD4 cell counts, longer duration of HIV infection, exposure to ART and longer duration of ART were found to be independent predictors of a higher cIMT in HIV-positive subjects whereas age, diastolic blood pressure, low HDL, smoking and high BMI were predictors of high cIMT in HIV-negative controls. No difference was observed in cIMT among patients on different ART regimens but individuals who were on nevirapine had higher cIMT as compared to those who were on efavirenz, both non-nucleoside reverse transcriptase inhibitors (NNRTIs).

Conclusions: Individuals with HIV infection (whether on ART or ART naive) have higher cIMT, and therefore a higher atherosclerotic burden, as compared to HIV-negative individuals. HIV infection itself, along with ART, overshadows conventional cardiac risk markers as a predictor of atherosclerotic disease in these individuals.

Keywords: HIV, carotid intimal medial thickness, cluster differentiation counts, AIDS, atherosclerosis, ART

Introduction

India has the third largest number of people living with HIV/AIDS (PLWH). As per the NACO Annual Report 2011–2012, the estimated adult HIV prevalence in India was 0.32% (0.26–0.41%) in 2008 and 0.31% (0.25–0.39%) in 2009. The total number of PLWH in India is estimated to be 2.4 million (range 1.93–3.04 million) in 2009, 83% of whom are in the age group 15–49 years [1]. Wider access to ART has resulted in a decline in the number of people dying from AIDS-related causes. With this reduction in early mortality and increased longevity, there is an associated increase in the burden of comorbid chronic conditions such as the accelerated rate of cardiovascular diseases (CVD) and atherosclerotic heart disease. This increased cardiovascular risk is partly attributed to traditional risk factors for atherosclerosis, such as smoking, hypertension, diabetes, age, sex, race, and also to the HIV infection itself, which is known to cause vascular endothelial inflammation by oxidative stress, other mechanisms (including cytokine production and thrombogenic effects), and antiretroviral therapy (ART), which has been implicated in metabolic disturbances [2]. Many studies have suggested an increased risk for coronary heart disease in association with the use of ART, with some studies specifically pointing at protease inhibitors for which a temporal correlation with atherosclerotic disease has been shown

[3]. There are few studies from India regarding the association of HIV infection and ART with cardiovascular disease. To address this, we studied various factors affecting carotid intimal medial thickness (cIMT) in individuals with HIV/AIDS. cIMT is considered a surrogate marker for atherosclerotic cardiovascular disease and its proportionate increase is significantly associated with a higher rate of adverse cardiovascular events [4].

Methods

In total, 168 HIV-infected individuals and 150 healthy age- and sex-matched controls were enrolled in this cross-sectional, observational study, between April 2013 and March 2015 at the Antiretroviral Treatment Centre (ART Centre) at the Post Graduate Institute of Medical Education Research, Dr Ram Manohar Lohia Hospital, New Delhi, India.

Inclusion criteria

All HIV-positive individuals in the age group 18–45 years and age- and sex-matched HIV-negative healthy volunteers were included in this study (Table 1).

Exclusion criteria

Subjects with

- Type 2 diabetes mellitus, hypertension, rheumatological diseases and chronic kidney disease;

- Aspirin/statin therapy;

*Corresponding author: Pulin K Gupta, Room No-103, Academic Block, PGIMER, Dr RML Hospital, Baba Kharak Singh Marg, New Delhi-110001, India
Email: guptapulin@yahoo.com

Table 1. Characteristics of HIV-positive patients

	Study group (*n*=168)	SD	Control group (*n*=150)	SD	Subgroup A (*n*=124)	SD	Subgroup B (*n*=44)	SD
Age (years)	33.04	6.47	32.64	6.84	32.96	6.72	33.25	5.77
Duration of HIV (years)	3.45	2.56			4.08	2.58	1.63	1.36
Pulse (beats/min)	81.46	9.59	82.49	9.76	81.72	9.54	80.75	9.82
Systolic blood pressure (mmHg)	119.13	10.38	118.47	9.55	119.87	10.19	117.05	10.73
Diastolic blood pressure (mmHg)	75.57	7.67	76.61	7.37	76.08	7.58	74.14	7.82
Height (cm)	161.37	8.21	160.44	8.13	161.92	7.84	159.82	9.09
Weight (Kg)	53.87	6.63	58.05	6.51	54.23	6.56	52.86	6.79
BMI (Kg/m^2)	20.66	2.43	22.60	2.26	20.66	2.53	20.66	2.15
Fasting blood sugar (mg/dL)	94.19	6.13	94.92	6.52	94.37	6.39	93.68	5.33
Urea (mg/dL)	27.04	9.50	27.32	10.62	27.40	9.93	26.02	8.18
Creatinine (mg/dL)	0.74	0.20	0.76	0.20	0.742	0.20	0.75	0.21
Uric acid (mg/dL)	4.24	1.96	4.33	2.08	4.33	1.94	4.00	2.00
Total cholesterol (mg/dL)	151.43	23.96	146.00	23.81	156.03	24.71	138.48	15.86
LDL (mg/dL)	70.20	15.75	68.86	15.55	72.50	15.49	63.70	14.77
HDL (mg/dL)	49.06	7.77	49.56	8.51	49.97	7.33	46.50	8.47
VLDL (mg/dL)	32.27	16.67	27.58	14.03	33.69	17.86	28.27	12.05
Triglycerides (mg/dL)	161.37	83.37	137.88	70.14	168.47	89.30	141.36	60.27
Total protein (g/dL)	6.76	1.14	6.82	1.22	6.66	1.14	7.05	1.09
Albumin (g/dL)	3.47	0.74	3.49	0.70	3.41	0.75	3.67	0.68
Globulin (g/dL)	3.29	0.82	3.32	0.94	3.26	0.82	3.37	0.85
Calcium (mg/dL)	8.19	0.51	8.09	0.46	8.18	0.49	8.21	0.55
Phosphate (mg/dL)	3.71	0.72	3.66	0.68	3.67	0.71	3.79	0.73
Bilirubin (mg/dL)	0.65	0.49	0.59	0.199	0.61	0.19	0.75	0.90
Direct bilirubin (mg/dL)	0.32	0.19	0.31	0.15	0.31	0.16	0.34	0.27
Indirect bilirubin (mg/dL)	0.35	0.46	0.28	0.14	0.30	0.14	0.49	0.87
Haemoglobin (g/dL)	10.12	1.70	10.29	1.88	10.07	1.73	10.25	1.64
Total leukocyte count (/mm^3)	9180	2846	8005	2347	8978	2835	9752	2830
Platelet count (cells/mm^3)	271,000	73,000	275,000	73,000	275,000	71,000	263,000	78,000
CD4 cell count (cells/mm^3)	332.41	172	–	–	330	153	339	213
cIMT left (mm)	0.71	0.04	0.61	0.027	0.72	0.034	0.68	0.043
cIMT right (mm)	0.71	0.041	0.62	0.02	0.72	0.037	0.68	0.04
Mean cIMT (mm)	0.71	0.038	0.61	0.02	0.72	0.033	0.68	0.04

- A history of myocardial infarction, unstable angina, stroke, coronary revascularisation procedure, congestive heart failure or an affirmative response to the Rose Questionnaire [5]; ECG suggestive of coronary artery disease as per Minnesota code were excluded [6].

After an overnight fast, a 10-mL venous blood sample was drawn for routine blood investigations such as fasting/post prandial blood glucose level, kidney and liver function tests, lipid profile (indirect measurement) and complete haemogram. A Becton-Dickinson FACS flow cytometer was used to obtain CD4 cell counts.

cIMT is a validated measure and a surrogate marker of cardiovascular disease and atherosclerosis approved by the American Diabetes Association (ADA) and was measured by the technique of carotid colour doppler ultrasound performed using the HD 11 machine (Philips, USA) equipped with colour flow imaging and pulse doppler, with an electrical linear transducer (mid-frequency of 7.5 MHz). The cIMT was calculated as the mean of four measurements taken for both the arteries.

Data analysis

Data were analysed using IBM SPSS software for Windows version 20. Quantitative variables are reported as mean ± standard deviation. Correlation and chi-squared tests were used for statistical correlation of continuous variables and other appropriate statistical tests were used for analysing categorical variables, a *P*-value of <0.05 was considered significant.

Results

The group of HIV-positive individuals (hereafter called the study group) (*n*=168) included 105 men (62.5%) and 63 women (37.5%) and the control group (*n*=150) consisted of 100 men (66.7%) and 50 women (33.3%). The study group was further subdivided into two subgroups:

- Subgroup A: individuals on ART, 124 subjects (73.8%).

- Subgroup B: ART-naive HIV-positive individuals, 44 subjects (26.2%).

The mean cIMT in HIV-positive subjects (study group) and HIV-negative subjects (control group) was 0.712±0.04 mm and 0.616±0.02 mm, respectively and the difference was found to be statistically significant (*P*<0.001) (Table 2).

Amongst the 124 individuals in subgroup A the mean cIMT was 0.723±0.03 mm as compared to 0.682±0.04 mm in subgroup B, and the difference was statistically significant (*P*<0.01). Similarly the difference in cIMT between subgroup B and control group was statistically significant (*P*<0.001) implying the direct effect of virus per se on the atherosclerotic process.

A significant correlation was found between mean cIMT and the duration of HIV infection (*P*<0.01, Pearson's correlation coefficient 0.289). Similarly cIMT was higher in patient with longer duration of ART (*P*<0.01, Pearson's correlation coefficient 0.216). HIV-positive individuals taking ART in our study were taking combination therapies as per NACO guidelines and four different combinations of treatments, including two nucleoside reverse transcriptase inhibitors [NRTI] and one NNRTI, were given to these individuals.

The mean cIMT was found to be higher in individuals who were on nevirapine-based regimens as compared to efavirenz (Table 3), and the difference was found to be statistically significant (*P*<0.05), with a *t* test value of 2.172 with a degree of freedom of 122.

On applying Jocksheere–Terpstra test for ordered alternatives and using the ART regimen as grouping variables it was seen that the distribution of mean cIMT was the same across different ART regimens. Also, on applying the Kruskall–Wallis test it was seen that there was no statistically significant difference in mean cIMT amongst HIV-positive individuals taking different ART regimens.

The mean total cholesterol of individuals in subgroup A, was 156.03±24.7 mg/dL as compared to 138.48±15.85 mg/dL in individuals in subgroup B and the difference was statistically significant (*P*<0.05).

On applying multivariate regression analysis in the study group (keeping mean cIMT as the dependent variable) low CD4 cell counts (odds ratio [OR] 0.68, 95% CI 0.58–0.83), long duration of HIV infection (OR 2.15, 95% CI 1.70–3.33), exposure to ART (OR 4.71, 95% CI 2.66–7.82) and duration of taking ART (OR 1.70, 95% CI 1.57–2.13) were found to be independent strong predictors of a higher carotid intimal medial thickness. On applying multivariate regression analysis in the control group, age (OR 2.2, 95% CI 0.4–9.1), high BMI (OR 1.5, 95% CI 1.1–1.9), smoking (OR 1.4, 95% CI 1.1–1.8), DBP (OR 1.3, 95% CI 1.1–1.7) and low HDL (OR 0.72, 95% CI 0.62–0.84) were found to be the predictors of higher mean carotid intimal medial thickness.

Discussion

In our study, HIV-positive individuals had a higher cIMT as compared to HIV-negative healthy people (Table 1 and 2). In the control group, cIMT was positively correlated with age, BMI, diastolic blood pressure, low HDL and history of smoking, which is consistent with current medical knowledge (Table 1). However, these known risk factors for atherosclerosis had no association or correlation with higher cIMT in our HIV-positive individuals. Mean cIMT in HIV-positive individuals was found to be 0.712 mm, which was significantly higher than 0.616 in the control group (*P*<0.001). HIV-positive ART-naive individuals also had a significantly higher cIMT compared to HIV-negative healthy people (*P*<0.001) implying the direct effect of the virus itself on atherosclerosis. HIV-positive individuals on ART had significantly higher cIMT than HIV/AIDS patients not on ART signifying the metabolic and atherogenic effect of ART (even non-protease inhibitors) on cardiovascular disease. This emphasises that HIV infection by itself is a prominent and major cardiovascular risk factor in these individuals that is independent of other traditional cardiac risk factors. Superimposed upon that, ART itself has an additive effect on this cardiovascular risk. This result is similar to a study carried out by Papita *et al.* to assess arterial stiffness and cIMT in HIV-positive individuals [2], comparing 65 HIV-positive and 36 healthy subjects where they found that mean cIMT in HIV-positive subjects was 0.60±0.15 mm and 0.51±0.08 mm in HIV-negative subjects. However, a higher mean cIMT in both our study and control groups may be related to higher baseline prevalence of metabolic syndrome and CVD risk markers in people of Indian origin.

The mean duration of HIV infection in 168 HIV-positive individuals was 3.45±2.56 years, which showed a significant, positive correlation with the individuals' mean cIMT. Longer duration of HIV infection was positively correlated with a higher CD4 cell count. The higher CD4 cell counts in individuals with longer duration of infection can be explained as 124 out of 168 subjects (73.8%) in this study were taking ART, whereas the mean duration of HIV infection in HIV-positive ART-naive individuals was only 1.64±1.36 years. In a study by Hsue *et al.* on cIMT and the development of plaques in the carotid artery system in 300 HIV-infected individuals [7], it was found that the rate of progression of cIMT was greater in HIV-positive individuals (0.055 mm/year) as compared to healthy controls (0.024 mm/year) and the difference was statistically significant.

In the current study, we were able to establish a significant association between cIMT and ART, as cIMT was found to be significantly higher in HIV/AIDS patients taking ART

Table 2. cIMT in different groups of individuals

Group	Mean cIMT, left (mm)	Mean cIMT right (mm)	Mean cIMT (mm)
Study group (*n*=168)	0.709 ± 0.04	0.714 ± 0.04	0.712 ± 0.03
Subgroup A (*n*=124)	0.719 ± 0.03	0.724 ± 0.03	0.723 ± 0.03
Subgroup B (*n*=44)	0.680 ± 0.04	0.684 ± 0.03	0.682 ± 0.03
Control group (*n*=150)	0.615 ± 0.02	0.617 ± 0.02	0.616 ± 0.02

Table 3. cIMT and ART combinations

	Mean cIMT left (mm)	Mean cIMT right (mm)	Mean cIMT (mm)	Individuals	
				(*n*)	Frequency
Regimen I (zidovudine + lamivudine + nevirapine)	0.72	0.73	0.72	79	63.7
Regimen II (zidovudine + lamivudine + efavirenz)	0.70	0.71	0.70	16	12.9
Regimen Ia (stavudine + lamivudine + nevirapine)	0.72	0.73	0.72	28	22.6
Regimen IIa (stavudine + lamivudine + efavirenz)	0.72	0.70	0.71	1	0.8

(0.723±0.033 mm) than in those who were ART-naive (0.682±0.038 mm). cIMT in HIV-positive ART-naive individuals was also found to have a significant, positive correlation with low CD4 cell counts and so, indirectly with high viral load in these individuals. There was a positive correlation between exposure to ART and increasing duration of ART (two NRTI+NNRTI) with higher cIMT, further emphasising the dysmetabolic effect of ART. This means that the majority of Indian HIV/AIDS patients who are still on first-line ART (as per NACO guidelines) containing two NRTIs and one NNRTI are at equal risk to CVD as compared to individuals on protease inhibitors, which are the drugs given as first-line ART in many Western countries.

In a study by Lorenz *et al.* including 292 HIV-positive individuals and 1168 HIV-negative controls [8], cIMT was significantly higher in HIV-positive individuals receiving ART as compared to ART-naive HIV-positive individuals. In a study carried out on Indian an population by Bajaj *et al.* the duration of taking ART was the only significant predictor of cIMT in HIV-positive individuals [9]. However, contradictory results were found in a study done by Hsue *et al.* who studied 495 subjects, including 93 HIV-negative controls and 402 HIV-positive individuals in different stages of infection [1]. This study showed that cIMT was higher in HIV-positive individuals as compared to controls and ART exposure was also associated with a higher cIMT. However, there was no association or correlation between CD4 cell counts or viral load and cIMT. In this study, subjects were recruited independently of their cardiovascular risks and cIMT was measured using high resolution ultrasound instead of B-mode ultrasound or doppler and all measurements were carried out using manual callipers. Hence the values were not very reliable.

We did not complete a viral load assessment but a higher cIMT was found in subgroup A with higher CD4 cell counts and similarly a higher cIMT was found to be associated with low CD4 cell counts in subgroup B. This is because of a probable additive effect of ART on cIMT along with HIV infection in subgroup A whereas in subgroup B this correlation of cIMT with low CD4 cell counts may be directly because of high viral load and so viraemia per se, as these were relatively newly diagnosed individuals not on ART.

cIMT was not significantly higher in individuals taking any specific combination of antiretroviral drugs. Subjects in the present study were taking ART combinations comprising two NRTI drugs (lamivudine + zidovudine or stavudine) and one NNRTI drug (nevirapine or efavirenz). None of the individuals was taking a protease inhibitor-based regimen, which is a part of second-line ART in India. This could be a possible reason for the absence of any significant difference in cIMT values between individuals taking different combinations of antiretroviral drugs. Historically, high cardiovascular risk is seen with efavirenz, stavudine, abacavir and protease inhibitors [10–12]. However, we found that the individuals who had taken nevirapine as part of ART had a higher mean cIMT (*P*<0.05). This is contrary to the common view of efavirenz being more atherosclerotic. Our findings of nevirapine being more atherosclerotic may be a chance finding keeping in view the vast difference in number of individuals on nevirapine versus efavirenz in our study (107 *vs* 17). Nonetheless, this shows that nevirapine may not be entirely harmless as far as atherosclerotic cardiovascular risk is concerned and further studies with a greater number of individuals may be required to elaborate on this finding. In our study it was seen that the mean serum cholesterol was significantly higher in HIV-positive individuals taking ART as compared to HIV-positive ART-naive individuals. No significant difference was found between HIV-positive and negative subjects in terms of total cholesterol, LDL, VLDL, HDL or serum triglyceride levels. Various previous studies have shown varying results regarding association between total cholesterol, LDL, VLDL, HDL and serum triglycerides with ART and HIV infection [13,14]. This is expected in view of the fact that some subjects in our study were on stavudine, which is known to cause dyslipidaemia and lipodystrophy. Another reason for this lower value of cholesterol in ART-naive individuals may be the effect of viraemia and active disease, which causes loss of appetite and negative calorie balance.

In our study, no correlation was found between cIMT and traditional risk factors for cardiovascular diseases such as age, total serum cholesterol, LDL, HDL and BMI in HIV-positive individuals. In a study by Grunfeld *et al.* it was seen that after multivariable adjustment for cIMT and demographic factors (age, gender and race), the association of HIV infection with greater internal IMT was strengthened (+0.188 mm, *P*<0.0001) [15]. Grunfeld *et al.* discovered that the HIV association was somewhat attenuated after adjusting for the remaining traditional CVD risk factors (smoking, blood pressure, total cholesterol and HDL-cholesterol), but the HIV-infected participants still had significantly greater cIMT than the HIV-negative controls. On the other hand Bongiovanni *et al.* showed that traditional risk factors for cardiovascular diseases overshadow the role of ART in determining premature vascular lesions [16]. They also found that traditional risk factors for cIMT such as male gender, older age, higher BMI and hypertriglyceridaemia were confirmed to be predictive of cIMT >1 mm in HIV-positive individuals. However, in Bongiovanni *et al.* a cIMT of 1 mm was considered as a normal cut-off, which was considerably higher than the mean cIMT of healthy controls within the same study, which was 0.58 mm in both left and right common carotid arteries. We did not find the same correlation because metabolic syndrome in Indians is widespread and presents from adolescence and it may have exerted its effect equally in our cases as well as controls; thus, an additive effect of HIV and ART might have caused a significantly higher cIMT in our cases. Unfortunately not many Indian studies are available on cIMT in normal healthy subjects. Kasliwal *et al.* in their first ever Indian study on gender- and age-specific distribution of cIMT in 1229 subjects (SCORE India) revealed that cIMT increased progressively with age and men have higher cIMT than women (0.608±0.12 mm *vs* 0.579±0.11 mm) [17]. An average cIMT of 0.59±0.12 mm was seen in healthy Indian subjects in comparison to 0.635±0.10 mm and 0.624±0.10 mm in individuals with hypertension.

Although cIMT in our controls was only modestly higher than the above study but cIMT in our cases was much higher than that reported by Kasliwal (in individuals with diabetes or individuals with hypertension), it again reinforces the fact that HIV infection itself is a strong atherosclerotic risk marker like other chronic metabolic diseases.

Conclusion

We found that the effect of HIV itself, duration of HIV infection, exposure to and duration of ART, and drugs (nevirapine) are predominant factors causing accelerated atherosclerosis and so increased CVD in HIV-positive individuals. The drawbacks in our study were the nature of the study and a lack of viral load testing. In this era of availability of second- and third-line ART, individuals with HIV infection are living longer and causes of death are more likely to be comorbidities such as CVD and metabolic disease rather than HIV or opportunistic infections. It is now time to focus on these comorbidities and plan primary prevention for CVD in the same way we do for individuals with diabetes, hypertension or rheumatoid arthritis/connective tissue disorders.

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