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Herpes simplex virus type 2 (HSV-2) as a coronary atherosclerosis risk factor in HIV-infected men: Multicenter AIDS Cohort Study

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Abstract

We assessed associations of herpes simplex virus types 1 and 2 (HSV-1 and -2), cytomegalovirus (CMV), and human herpesvirus 8 (HHV-8) infection with subclinical coronary atherosclerosis in 291 HIV-infected men in the Multicenter AIDS Cohort Study. Coronary artery calcium (CAC) was measured by non-contrast coronary CT imaging. Markers for herpesviruses infection were measured in frozen specimens collected 10-12 years prior to case identification. Multivariable

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logistic regression models and ordinal logistic regression models were performed. HSV-2 seropositivity was associated with coronary atherosclerosis (adjusted odds ratio [AOR] =4.12, 95% confidence interval [CI] =1.58-10.85) after adjustment for age, race/ethnicity, cardiovascular risk factors, and HIV infection related factors. Infection with a greater number of herpesviruses was associated with elevated CAC levels (AOR=1.58, 95% CI=1.06-2.36). Our findings suggest HSV-2 may be a risk factor for subclinical coronary atherosclerosis in HIV-infected men. Infection with multiple herpesviruses may contribute to the increased burden of atherosclerosis.

Keywords

herpesvirus; HSV-2; atherosclerosis; HIV-1/AIDS; risk factors

1. Introduction

Cytomegalovirus (CMV), herpes simplex virus (HSV) and human herpesvirus 8 (HHV-8) are common infectious agents among men who have sex with men (MSM), particularly those infected with HIV-1 [1, 2] It has been postulated that because there is an impaired immune response to viral antigens, herpesviruses could have more severe cardiovascular consequences in those infected with HIV-1. Previous studies have analyzed subclinical carotid artery atherosclerosis [3,4] in relation to concurrent measures of HIV infection, HIV disease parameters and antiretroviral medication use in the Multicenter AIDS Cohort Study (MACS), an ongoing prospective study of the natural and treated histories of HIV-1 infection in homosexual and bisexual men [5]. The present analysis is a nested case-control study to evaluate the association of subclinical coronary atherosclerosis with markers of infections with CMV, HSV types 1 and 2, and HHV-8 in HIV-infected MSM in MACS.

2. Materials and methods

Two hundred and ninety one HIV-infected men enrolled through 1984-1985 and 1987-1991 recruitment, who had completed non-contrast coronary CT imaging between April 2004 and March 2006 and had adequate frozen blood specimen collected at a visit before March 1995 were included in this analysis. All participants were 39 years or older and had no history of self-reported or confirmed coronary disease (heart attack, heart surgery, or other major heart illness) or cerebrovascular disease before the study entry, and provided informed consent. The study protocol was approved by local Institutional Review Boards.

2.1 Subclinical atherosclerosis measurement

Non-contrast coronary CT imaging was performed as previously described [4] to assess coronary artery calcium (CAC, Agatston method). Two consecutive cardiac CT scans were done for each subject within 5-7 minutes and read centrally with repeated analysis of 5% of scans with intra-observer reliability of 0.97. The mean CAC score of the two consecutive scans was used to determine the presence and amount of calcification in coronary arteries. CAC scores of 0-10, 11-100, 101-400, >400 were used to categorize individuals into groups having minimal, mild, moderate, or extensive amounts of calcification, respectively, according to the Rumberger Scale [6]. Men with a mean CAC score > 10 were considered as subclinical coronary atherosclerosis cases in this analysis. The degree of agreement between the two CAC score readings was low among those with a CAC score under 10.

2.2 Detection of herpesvirus infections

HSV-1 and HSV-2 IgG type-specific antibody ELISA tests were performed (Focus Diagnostics, Inc. Cypress, CA). A randomly selected 5% of the serum samples were tested in duplicate, yielding 100% concordant results. Presence of antibodies to HHV-8 lytic

antigens was examined in duplicate as described [7] by an indirect immunofluorescence assay (IFA) on induced body cavity B-cell lymphoma (BCBL)-1 cells (NIH AIDS Research Reagent Program, Rockville, MD) that contained the HHV-8 genome [8]. As the prevalence of CMV seropositivity approached 100% in this study population, we measured plasma CMV DNA instead of CMV antibody. CMV DNA was extracted using a NucliSENS easyMAG nucleic acid extractor (BioMérieux Inc., Durham, NC) with a known amount of phocine herpesvirus 1 (PhHV-1) ($1 \times 10^{4.5}$ $TCID_{50}$/ml) added to each sample as an internal control. Real-time PCR assays were performed on plasma samples in duplicate to detect CMV US17 and UL54. An optimal cutoff of 500 normalized copies was calculated based on high sensitivity, specificity, and positive and negative predictive values [9].

2.3 Statistical analyses

Characteristics at the atherosclerosis assessment visit were compared between the cases and controls using t-test or Pearson χ^2 test. Wilcoxon test was performed for continuous variables that were not normally distributed. We used logistic regression bivariate and multivariable models to examine the associations of herpesvirus infections and subclinical coronary atherosclerosis. Covariates were collected at MACS visits and were included in the multivariable models based on significance of association ($P<0.05$) in bivariate analysis and prior hypotheses. Three separate multivariable logistic regression models were used to model the adjusted associations: In model 1, we adjusted age, race/ethnicity and cardiac risk factors: cumulative cigarette pack-years (never-smokers coded as 0), alcohol consumption, cholesterol-lowering medication use, median BMI between herpesvirus test visit and CT visit, history of hypertension and diabetes, and family history of premature heart attack. In model 2, we further adjusted co-infection of herpesvirus. In the full model, further adjustment was made for HIV-1 infection duration, AIDS status, nadir CD4+ T-cell count, peak HIV viral load, use of protease inhibitor (PI)- based HAART, time since initiation of HAART, and adherence to HAART. An ordinal logistic regression proportional odds model was used to examine the associations of number of herpesviruses tested positive with the extent of subclinical atherosclerosis assessed by the 4 groups of the CAC score.

Analyses were carried out using the SAS program (SAS version 9.2, SAS Institute Inc., Cary, NC).

3. Results

There were 109 subclinical coronary atherosclerosis cases and 182 controls included in the analysis. Cases were older, more likely to be current smokers and had a binge drinking history (Table 1). There was a higher proportion of white and lower proportion of Black men among cases than among controls.

Seropositivity to HSV-2 was significantly associated with subclinical coronary atherosclerosis (adjusted odds ratio [AOR] =3.04, 95% confidence interval [CI] =1.38-6.71) after adjustment for traditional cardiovascular risk factors (Table2, model 1), and remained significant after further adjustment for herpesviruses co-infection (model 2, AOR= 3.04, 95% CI=1.22-6.92) and HIV infection related covariates (full model: AOR=4.12, 95% CI=1.58-10.85). Plasma CMV DNA and seropositivity to HSV-1 and HHV-8 were not significantly associated with subclinical coronary atherosclerosis (Table 2). Age was significantly associated with subclinical coronary atherosclerosis in the full model (every 5 years, AOR=2.25, 95% CI=1.57-3.25).

Eighty-two cases (75%) and 105 (58%) controls tested positive for two or more herpesviruses. In the ordinal logistic regression, the number of herpesvirus tested positive

was associated with elevated level of CAC score (AOR=1.58, 95% CI=1.06-2.36) after controlling for cardiovascular risk factors and HIV infection related covariates.

4. Discussion

HSV-2 was independently associated with subclinical coronary atherosclerosis, and infection with multiple herpesviruses was associated with a higher level of CAC in HIV-infected MSM. HSV-2 has been shown to cause thrombogenic and atherogenic changes to host cells [10]. Previous studies reported a positive association between HSV-2 infection and hypertension[11] and increased CIMT [12, 13]. Growing evidence showed that calcification of lesions may be specifically associated with inflammatory pathway(s) of atherosclerosis [14]. Our findings support the hypothesis that long-term HSV-2 infection may contribute to development of atherosclerosis.

The results from this study should be interpreted with acknowledgement of several limitations. The sample size was limited by availability of frozen specimens, which might have introduced selection bias and reduced the power to detect possible moderate effects of CMV, HSV-1 and HHV-8. There was a long interval between the collection of specimens and the measurement of subclinical atherosclerosis, during which some individuals might have become infected with herpesviruses. We were unable to identify those who acquired herpesviruses later as no repeated testing was performed. Finally, even though we have adjusted for several lifestyle variables (smoking, alcohol use, etc.) that could be associated with HSV-2 acquisition and cardiac risk factors, HSV-2 seropositivity could be a surrogate for other factors not considered in the analysis.

To our knowledge, this is the first study reporting an independent association of long-term HSV-2 infection with subclinical coronary atherosclerosis in HIV-1-infected MSM. Further investigations with a larger sample size are needed to confirm our findings.

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- We assessed associations between herpesviruses and subclinical coronary atherosclerosis among HIV-infected men.
- Coronary artery calcium (CAC) was measured by non-contrast coronary CT imaging to evaluate coronary atherosclerosis.
- This is the first report suggesting herpes simplex virus type 2 (HSV-2) may be a risk factor for coronary atherosclerosis in HIV-infected men.
- Infection with multiple herpesviruses may contribute to the increased burden of atherosclerosis.

Table 1

Characteristics of HIV-infected men who have sex with men at the subclinical coronary atherosclerosis assessment visit.

	Cases (*N*=109)	Controls (*N*=182)	*P* value[a]
Age, median (Q1,Q3)	53.8(49.1, 58.3)	49.5(45.2, 54.1)	**<0.001**
Age Group			
<50 years	32 (29.4%)	94 (51.6%)	**<0.001**
>=50 years	77 (70.6%)	88 (48.3%)	
Race/Ethnicity			
White, non-Hispanic	96 (88.1%)	145 (79.7%)	**0.006**
Black, non-Hispanic	4 (3.7%)	29 (15.9%)	
Hispanic	6 (5.5%)	7 (3.8%)	
Asian, Pacific Islander, or Other	3 (2.7%)	1 (0.6%)	
Study Site			
Baltimore	26 (23.9%)	45 (24.7%)	0.66
Chicago	26 (23.9%)	39 (21.4%)	
Pittsburgh	19 (17.4%)	42 (23.1%)	
Los Angeles	38 (34.8%)	56 (30.8%)	
Cohort			
1984-85 cohort	96 (88.1%)	151 (83.0%)	0.17
1987-90 cohort	13 (11.9%)	31 (17.0%)	
Highest Education Level			
Up to 12th grade	18 (16.5%)	18 (9.9%)	0.21
At least one year college, no degree	24 (22.0%)	46 (25.3%)	
Four years college/degree	21 (19.3%)	52 (28.5%)	
Some graduate work	17 (15.6%)	22 (12.1%)	
Post-graduate degree	29 (26.6%)	44 (24.2%)	
Annual Household Income			
Less than $20,000	14 (12.8%)	36 (19.8%)	0.15
$20,000-39,999	27 (24.8%)	35 (19.2%)	
$40,000-59,999	21 (19.3%)	48 (26.4%)	
$60,000 or more	35 (32.1%)	52 (28.6%)	
Missing	12 (11.0%)	11 (6.0%)	
Family History of CV Disease			
Any CV disease among relatives	87 (82.9%)	152 (84.0%)	0.81
Premature heart attack among relatives	44 (42.7%)	67 (37.6%)	0.40
Alcohol Consumption			
None	15 (14.0%)	32 (17.7%)	**0.001**
Low to modest	62 (57.9%)	104 (57.4%)	
Modest to heavy	17 (15.9%)	42 (23.2%)	
Binge	13 (12.2%)	3 (1.7%)	
Smoking			

	Cases (*N*=109)	Controls (*N*=182)	*P* value[a]
Never smoked	28 (25.7%)	57 (31.3%)	0.30
Former smoker	55 (50.5%)	94 (51.7%)	
Current smoker	26 (23.8%)	31 (17.0%)	
Cumulative pack-years among smokers, median (Q1,Q3)[b]	16.3 (3.5, 23.6)	10.6 (0.5, 20.0)	0.07
History of Injection Drug Use (IDU)	22 (20.2%)	27 (14.8%)	0.24

[a] P-values were derived from Student t-tests or non-parametric Wilcoxon two-sample test for continuous, and χ^2 tests or Fisher's exact test for categorical variables.

[b] Among 81 former/current smoker cases and 125 former/current smoker controls.

Table 2

Association of subclinical atherosclerosis with presence of plasma CMV DNA, and antibodies to HSV-1, HSV-2 and HHV-8 in HIV-infected men who have sex with men.

Herpesvirus[a]	Unadjusted odds ratio (95% confidence interval)	Adjusted odds ratio (95% confidence interval)		
		Model 1[b]	Model 2[c]	Full model [d]
HSV-1	1.36 (0.81,2.26)	1.17 (0.60, 2.26)	1.65 (0.80,3.39)	1.99 (0.85,4.68)
HSV-2	**2.84 (1.53,5.26)**	**3.04 (1.38,6.71)**	**3.04 (1.22, 6.92)**	**4.12 (1.58, 10.85)**
HHV-8	1.30 (0.81, 2.11)	1.04 (0.57, 1.91)	0.86 (0.45, 1.64)	0.99 (0.45, 2.18)
CMV	0.91 (0.81, 2.11)	0.84 (0.29, 2.43)	0.80 (0.27, 2.40)	0.87 (0.24, 3.12)

[a]Equivocal HSV-1 and HSV-2 results for 9 subjects (3%) were recorded as missing. Equivocal HHV-8 results for 6 subjects (1%) were recorded as missing. 5% (14) of samples that failed internal controls twice for CMV DNA test were coded as missing.

[b]Model 1 controlled for age, race/ethnicity, cumulative cigarette pack-years (0 for non-smokers), alcohol consumption, cholesterol-lowering medication use, median BMI between herpesvirus test visit and CT visit, history of hypertension, history of diabetes, and family history of premature heart attack.

[c]Model 2 controlled for all variables in Model 1 and further controlled for the other three herpesviruses.

[d]Full model controlled for all variables in Model 2 and further controlled for HIV-1 infection duration, AIDS status, nadir CD4 T-cell, peak viral load, and history of HAART use (type, duration, and adherence).