Human T-cell leukemia virus-1 infection is associated with atherosclerosis as measured by carotid intima-media thickness in Japanese community-dwelling older people

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ABSTRACT

The association between human T-cell leukemia virus-1 (HTLV-1) and atherosclerosis remains to be determined. This case–control study aimed to investigate this association as measured by carotid intima–media thickness (CIMT). HTLV-1 infection was positively associated with CIMT, independent of atherosclerotic risks.

Introduction

Human T-cell leukemia virus-1 (HTLV-1) infects approximately 5 to 10 million people worldwide, including endemic areas, such as Southwest Japan, North Iran, Central Africa, the Caribbean, and South America [1]. Most HTLV-1 infections are latent, however, HTLV-1 is known to associate with a number of diseases, such as adult T-cell leukemia/lymphoma, HTLV-1-associated myelopathy/tropical spastic paraparesis, and autoimmune/inflammatory diseases, such as the arthropathies, Sjögren's syndrome, and uveitis [2]. Therefore, asymptomatic carriers need to be examined with further onset of varying diseases.

Recent studies have shown that human immunodeficiency virus (HIV) infection is a predictor of increased carotid intima–media thickness (CIMT) [3,4]. CIMT is measured by high-resolution B-mode ultrasound, which is a widely accepted method of assessing atherosclerosis. An increased CIMT is a potent predictor of future cardiovascular and cerebrovascular events [5].

Similar to HIV-1, an Iranian case–control study reported an association between HTLV-1 and atherosclerosis as measured by CIMT [6]. Limitations in this prior study with small sample size prompted the current study in a large, older community-dwelling population in Japan.

Methods

Study settings and participants

We conducted this nested case–control study using data from the Nagasaki Islands Study, which was a prospective cohort study performed in Goto City in the western islands of Japan [7]. The participants were recruited at medical check-ups, and

members of the general population aged > 40 years living in Goto City were targeted for enrollment. The recruitment process has been described elsewhere [7]. The protocol for the Nagasaki Islands Study was approved by the Ethics Committee for the Use of Humans of Nagasaki University (project registration number: 14051404, 20141002-3; Nagasaki, Japan). Written informed consent was obtained from all participants. A total of 4428 participants were enrolled from 2014–2017. A total of 3070 subjects were included after exclusion of the following: age<60 years, history of stroke or ischemic heart disease, and missing data regarding blood pressure, CIMT, or HTLV-1 serostatus. Finally, a total of 2184 participants (810 men and 1374 women) with a mean age of 72.5 years (standard deviation, 7.2 years; range, 60–97 years) were evaluated.

Data collection and laboratory measurements

Body weight and height were measured with an automatic body composition analyzer (BF-220; Tanita, Tokyo, Japan) at the time of phlebotomy, and the body mass index (BMI) was then calculated. Abdominal circumference was measured with an inelastic measuring tape that was placed over the skin at the level of the umbilical point. Trained medical technologists measured systolic blood pressure and diastolic blood pressure at rest using cuff oscillometry with a PASESA AVE-1500 (Shisei Datum, Tokyo, Japan). After coagulation, each blood samples were centrifuged and the serum was separated. Serum levels of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, creatinine, and glycated hemoglobin were measured using standard laboratory procedures. Trained interviewers obtained information on the participants' smoking status, alcohol intake, medical history, and use of antihypertensive agents, hypoglycemic agents, and lipid-lowering drugs. The

smoking status and drinking status were categorized as “never”, “ex”, or “current”. The risk of atherosclerotic disease was estimated using the risk equation (Framingham–D’Agostino score) based on the Framingham study [8]. The Framingham–D’Agostino score includes age, total cholesterol, high-density lipoprotein cholesterol, and systolic blood pressure as quantitative variables, as well as sex, drug treatment for hypertension, smoking, and a history of diabetes mellitus as dichotomous variables.

Measurements of HTLV-1

A chemiluminescent enzyme immunoassay (CLEIA) kit (Fujirebio Inc., Tokyo, Japan) was used for HTLV-1 detection. As a confirmatory test, we used real-time RT-PCR using the Hydrolysis probe method with the LightCycler 480 (Roshe, Basel, Schweiz). Sample DNA was purified from whole blood using GENE PREP STAR NA-480 (KURABO, Osaka, Japan). We additionally used western blotting (Problot HTLV-1, Fujirebio Inc., Tokyo, Japan) for the real-time RT-PCR-negative cases.

Measurements of CIMT

Research doctors or laboratory technicians measured CIMT by ultrasonographic examination of the left and right carotid arteries using a LOGIQ Book XP with a 10-MHz transducer (GE Healthcare, Milwaukee, WI, USA) that was programed with the CIMT measurement software Intimascope (Cross Media Ltd., Tokyo, Japan). The mean CIMT was calculated as the mean of the right and left CIMT measurements with carotid plaques excluded.

Statistical analyses

Differences in mean values or proportions of variables by HTLV-1 positivity were analyzed by using the paired Student's t-test for continuous variables, or the McNemar chi-square test or the Wilcoxon signed-rank test for categorical variables. The mean CIMT met the assumptions of linear regression analysis, which justified using this analysis. In linear regression analysis, the association between HTLV-1 and CIMT was analyzed, and confounding of age and sex strongly attenuated this association. Therefore, further analysis was performed using HTLV-1-infected cases vs. age- and sex-matched controls. Simple and multivariable linear regression analyses were performed to investigate the association between HTLV-1 and CIMT. We used a stepwise regression algorithm to build the multivariable model. Age, BMI, systolic blood pressure, glycated hemoglobin, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol (continuous variables), sex (dichotomous variable), and smoking status (categorical variable; never, ex, current; 1, 2, 3, respectively) were included in the adjustment Model 1. The Framingham–D'Agostino score (continuous variable) and BMI were included in Model 2.

All P values for statistical tests were two-tailed and $P < 0.05$ was considered significant. All statistical analyses were performed using STATA v14 (StataCorp, College Station, TX, USA).

Results

Among 2184 subjects, 406 (18.6%) HTLV-1-infected participants were detected using CLEIA. Of these 406 participants, 393 were positive for HTLV-1 by real-time RT-PCR. For the 13 real-time RT-PCR negative cases, western blotting showed that eight cases were positive, two were negative, two were indeterminant, and one had a

shortage of specimen. Finally, we detected 401 HTLV-1 infected participants who remained for analysis. The characteristics of the 401 cases and 401 matched controls are shown in **Table 1**. The atherosclerotic risks, including sex, smoking status, systolic blood pressure, glycated hemoglobin, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and the Framingham–D'Agostino score were not different between cases and controls. However, mean (standard deviation) CIMT was higher in cases (0.74 ± 0.14 mm) than in controls (0.72 ± 0.13 mm, P = 0.020). Mean age was older in cases than in controls (P = 0.030).

In linear regression analysis, the standardized ß coefficient was positively significant for HTLV-1 infection, age, male sex, smoking status, systolic blood pressure, and the Framingham–D'Agostino score (ß = 0.08, P = 0.031; ß = 0.39, P < 0.001; ß = 0.09, P = 0.010; ß = 0.10, P = 0.005; ß = 0.11, P = 0.002; and ß = 0.24, P < 0.001, respectively) (**Supplementary table 1**). This coefficient was negatively significant for diastolic blood pressure and high-density lipoprotein cholesterol (ß = −0.08, P = 0.032 and ß = −0.08, P = 0.029, respectively).

In multivariable linear regression analysis, HTLV-1 infection was positively associated with CIMT after adjusting for age, sex, BMI, systolic blood pressure, glycated hemoglobin, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and smoking status (Model 1, ß = 0.07, P = 0.028). This association was also positive after adjusting for the Framingham–D'Agostino score and BMI (Model 2, ß = 0.08, P = 0.015).

Discussion

This population-based study demonstrated an association between HTLV-1 infection and atherosclerotic risks. We found that HTLV-1 infection was positively associated with preclinical atherosclerosis as measured by CIMT, independent of atherosclerotic risk factors. Although an Iranian case–control study showed a positive association between HTLV-1 infection and CIMT [6], the mean age of the participants was 42.9 years and mean CIMT was 0.52–0.57 mm. Therefore, this result is difficult to extrapolate to older populations and/or those with a higher mean CIMT.

A plausible mechanism of atherosclerosis induced by HTLV-1 infection might be related to inflammation. HTLV-1 preferentially infects into CD4+ T cells. However, HTLV-1 also infects synovial fibroblasts [9], salivary epithelial cells [10], and CD68+ macrophages [11]. Additionally, HTLV-1 enhances production of cytokines through activation of transcription factors or activators, such as nuclear factor-κB, cyclic AMP response element-binding protein (CREB) and CREB/activating transcription factor [12]. Increased expression of these molecules may be induced via activation of transcription factors by cytokines and chemokines in a bystander fashion [10].

The major responsible cell types of atherosclerotic lesions are macrophages and endothelial cells in vivo. Therefore, HTLV-1 might be involved in functional alteration of macrophages and endothelial cells, resulting in augmentation of atherosclerosis. Further studies, including *in situ* examination of HTLV-1 or HTLV-1-related molecules, are required to investigate this possibility.

Another possible pathway of atherosclerosis induced by HTLV-1 is an overabundance of reactive oxygen species (ROS). HTLV-1 encodes viral structural genes of the pX region encoding tax, which increases production of ROS [13]. ROS are chemically

unstable reactive free radicals and lead to endothelial dysfunction, which in turn progresses atherosclerosis [14].

HIV-associated atherogenesis can be explained by the complicated interaction of HIV with host immune cells and endothelial cells, triggered by ROS, endoplasmic reticulum stress, inflammasome formation, and dysregulation of autophagy [15]. Further experimental studies focusing on these molecular mechanisms may be useful for understanding our results.

Limitations of our study include using cross-sectional data for exposure and outcome. Because effects of HTLV-1 infection on atherosclerosis are thought to be cumulative, a cohort study would be ideal to investigate this association.

In conclusion, HTLV-1 was associated with atherosclerosis as measured by CIMT in an older population in an area with high HTLV-1 seroprevalence. Screening for HTLV-1 infection might be beneficial for reducing viral transmission and for controlling cardiovascular risks for those with HTLV-1 infection.

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Conflict of Interest:

All authors declare no conflict.

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Table 1. Demographic and clinical characteristics of HTLV-1-infected cases and controls.

	Control	Case	p
N	401	401	
Age (year)	74.0 ± 6.9	74.4 ± 7.2	0.030
Sex (male)	122 (30.4)	122 (30.4)	1.000
Body mass index	22.9 ±3.2	23.0 ± 3.4	0.555
Smoking status			0.722
Current	26 (6.5)	25 (6.2)	
Ex	75 (18.7)	84 (21.0)	
Never	300 (74.8)	292 (72.8)	
Antihypertensive drug use	178 (44.4)	176 (43.9)	0.881
Hypoglycemic drug use	23 (5.7)	31 (7.7)	0.258
Lipid-lowering drug use	89 (22.2)	77 (19.2)	0.296
Systolic blood pressure (mmHg)	140.2 ± 19.2	138.8 ± 21.0	0.306
Diastolic blood pressure (mmHg)	75.4 ± 11.5	74.5 ± 10.7	0.229
Glycated hemoglobin (%)	5.82 ± 0.68	5.77 ± 0.52	0.312
Low-density lipoprotein cholesterol (mg/dl)	121.7 ± 29.9	119.9 ± 29.0	0.351
High-density lipoprotein cholesterol (mg/dl)	61.6 ± 14.0	60.3 ± 14.5	0.184
Triglycerides (mg/dl)	102.8 ± 49.9	104.8 ± 60.3	0.595
Framingham–D'Agostino score	15.5 ± 3.8	15.4 ± 3.9	0.618
Mean CIMT (mm)	0.72 ± 0.13	0.74 ± 0.14	0.020

Data are shown as number (%) or mean ± standard deviation.

Supplementary table 1. Univariable linear regression analysis of the effect of each exposure on mean CIMT.

Variable	Standardized beta coefficient (ß)	95% confidence interval	*p*
HTLV-1 infection	0.08	(0.01, 0.15)	0.031
Age in years	0.39	(0.33, 0.46)	<0.001
Male sex	0.09	(0.02, 0.16)	0.010
Body mass index	-0.06	(-0.13, 0.01)	0.084
Smoking status	0.10	(0.03, 0.17)	0.005
Antihypertensive agent use	0.04	(-0.03, 0.11)	0.244
Hypoglycemic agent use	0.00	(-0.07, 0.07)	0.914
Lipid-lowing drugs use	-0.03	(-0.10, 0.04)	0.403
Systolic blood pressure (mmHg)	0.11	(0.04, 0.18)	0.002
Diastolic blood pressure (mmHg)	-0.08	(-0.15, -0.01)	0.032
Glycated hemoglobin (%)	0.06	(-0.01, 0.13)	0.105
Low-density lipoprotein cholesterol (mg/dl)	-0.02	(-0.09, 0.05)	0.637
High-density lipoprotein cholesterol (mg/dl)	-0.08	(-0.15, -0.01)	0.029
Triglycerides (mg/dl)	-0.02	(-0.09, 0.05)	0.630
Framingham–D'Agostino score	0.24	(0.17, 0.30)	<0.001