Can HTLV-1 Infection Be a Potential Risk Factor for Atherosclerosis?

Parvaneh Layegh^a Ali Shoeibi^b Karim Nikkhah^b Ali Ghabeli Juibary^b
Samaheh Raftari^b Sahar Darbarpanah^c Reza Boroumand^d
Mahmoudreza Azarpazhooh^b

Departments of ^aRadiology and ^bNeurology, at ^cSchool of Medicine, Mashhad University of Medical Sciences, Mashhad, and ^dPrivate Practice of Neurology, Torbat Heydarieh, Iran

Key Words

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that this significant difference in IMT might support the hypothesis that HTLV-1 infection is an independent risk factor for atherogenesis.

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Abstract

Introduction: Chronic inflammations including infectious disorders such as HIV infection are now considered as risk factors for atherosclerosis. In this study, conducted for the first time on human subjects, human T-lymphotropic virus type 1 (HTLV-1) infection was examined as a potential risk factor for atherosclerosis. **Materials and Methods:** This is a matched-pair cross-sectional study on 58 HTLV-1-infected cases and 55 healthy control subjects. The subjects did not have any major cerebrovascular risk factors. Carotid intima-media thickness (IMT) was measured for each patient using the standard protocol of the Atherosclerosis Risk in Communities (ARIC) Study. **Results:** The mean age of the subjects was 42.9 ± 10.52 years, and males made up 33% of the population. The difference between the mean IMT of the infected case group and that of the healthy control group was significant ($p < 0.05$). **Discussion:** This study indicated that the HTLV-1-infected individuals showed a greater carotid IMT than the age- and sex-matched control subjects. Observing no other known risk factor for atherosclerosis, we concluded

Introduction

Chronic inflammation and activation of the immune system are presently considered as major risk factors for atherosclerosis [1]. Based on the conception that the host immune system remains chronically activated in untreated and to some extent in treated patients with a history of infectious diseases, it has recently been postulated that chronic T-cell activation is associated with atherosclerosis [1, 2]. Viral infections, as a known prototype of immune activators, have been investigated as a potential cause of atherosclerosis in previous studies [3]. In this sense, evidence has been accumulated for more than 25 years suggesting that herpesviruses may possibly play a role in the development of atherosclerosis [4]. It was found that Marek's disease virus, an avian herpesvirus, tended to cause typical atherosclerotic lesions in chickens' blood vessels. According to this study, in vitro infection of smooth muscle cells with this virus led to the ac-

cumulation of cholesterol in the cells' cytoplasm [5]. Likewise, HIV-infected patients are more prone to the development of atherosclerosis and cardiovascular disorders than age-matched HIV-seronegative adults. However, the precise mechanisms responsible for this incidence remain poorly understood [6].

Human T-lymphotropic virus type 1 (HTLV-1) is reportedly the earliest recognized human retrovirus, isolated from peripheral blood samples of patients with adult T-cell lymphoma in the early 1980s [7]. HTLV-1 infection is endemic in certain parts of the world including southwestern Japan, Central Africa, the Caribbean islands, regions of South America, Australo-Melanesia as well as Khorasan Razavi, a province in northeastern Iran [7]. Based on epidemiological studies, it is estimated that about 2.3–3% of the general population in this province are infected with the HTLV-1 virus [8].

HTLV-1 is believed to trigger several diseases such as adult T-cell leukemia, HTLV-1-associated myelopathy/tropical spastic paraparesis, sensorimotor polyneuropathy and optic neuritis [8]. There have been a few animal studies regarding HTLV-1 atherogenicity [9]. Koizumi et al. [10] studied mice with the pX transgene carrying an HTLV-1 env-pX region and found that the pX gene plays an important role in the induction of hypercholesterolemia in BALB/c mice, which are genetically less susceptible to hypercholesterolemia and the atherosclerosis process, and concluded that patients with rheumatoid arthritis who are carrying the HTLV-1 virus have a tendency toward developing hypercholesterolemia, a main risk factor for cardiovascular disorders. However, to the best of our knowledge, there has been no clinical research before this study involving human subjects focusing on HTLV-1 as a potential risk factor for atherosclerosis.

There have been certain problems with regard to the identification of a noninvasive method for the diagnosis of atherosclerosis in both subclinical and symptomatic stages, which recent imaging technologies might be able to solve in the near future [11, 12]. New research, for example, has focused on imaging techniques with the capacity to identify not only atherosclerotic plaques but also those which are most prone to rupture and thrombosis [12]. However, carotid ultrasonography as a conventional surrogate for measuring the risk for atherosclerosis in patients with HIV infection remains the best option [11, 12].

The purpose of this study is to examine the role of HTLV-1 infection in the pathogenesis of atherosclerosis, that is, we aimed to decide whether HTLV-1 per se exerts

atherogenic effects as well as to observe the frequency and degree of carotid atherosclerosis measured by carotid ultrasonography in HTLV-1-infected adults.

Materials and Methods

Population

In this matched-pair cross-sectional study, 58 HTLV-1-infected subjects and 55 healthy control subjects were recruited. An enzyme-linked immunosorbent assay (kit from Dia.Pro, Milan, Italy) was used as the screening test for HTLV-1 detection in sera, and all positive samples were confirmed by Western blot (kit from Genelabs Diagnostics, Pte. Ltd., Redwood City, Calif., USA). The subjects in both groups did not have any known risk factors for vascular disease such as smoking, high blood pressure, diabetes mellitus, elevated serum total (and LDL) cholesterol, low serum HDL cholesterol, ischemic heart disease, peripheral vascular disorders or other major health problems. In other words, we excluded subjects with any of the mentioned vascular risk factors. Age and sex, as confounding factors, were matched between the two groups. The study's case subjects were recruited from HTLV-1-positive donors from the Khorasan Razavi Blood Bank who had been referred to the HTLV-1 clinic of the Ghaem Hospital (Mashhad, Iran), and the healthy control cases were selected from the general population.

Carotid Intima-Media Thickness Measurements

Carotid B-mode ultrasound recordings were obtained from each patient using the standard protocol of the Atherosclerosis Risk in Communities (ARIC) Study [13]. Briefly, patients were asked to rest supine in a dimmed quiet room, and the blood pressure was recorded at the beginning and end of the study. Both carotid arteries were studied with the head in the midline position and tilted slightly upward. Carotid intima-media thickness (IMT) was measured in 12 predefined segments [6 per side, 3 for the common carotid artery (CCA) at the proximal, mid and distal portions, and 3 for the internal carotid artery (ICA) at the proximal, mid and distal segments]. Imaging was performed by an experienced vascular technician (P.L.) blinded to the patients' clinical features, including HTLV infection status. Atherosclerotic plaques were studied by ultrasound examination of both carotid arteries, employing a high-resolution ultrasound scanner (Medison, Accuvix V20) equipped with a 5- to 12-MHz linear-array transducer. The maximum IMT in CCAs and ICAs was measured on frozen B-mode images. An integrated distance of 5 mm was used in multigate Doppler examinations. The mean value of the proximal, mid and distal IMTs from each artery on each side (common carotid and internal carotid) was recorded and registered as the IMT of that specific artery in data analysis (four values in total for each individual).

Statistical Analyses

Intergroup differences were assessed using the t and Mann-Whitney tests for continuous variables and the χ^2 and Fisher's exact tests for categorical variables. SPSS (Statistical Package for the Social Sciences) version 21 was used for data analysis. The Kolmogorov-Smirnov test was used to detect whether data were normally distributed. $p < 0.05$ was defined as significant.

Ethics

The study was approved by the Regional Ethics Committee of the Mashhad University of Medical Sciences. All cases and controls signed informed consent forms.

Results

The mean age of the subjects was 42.9 ± 10.52 years (42.44 ± 11.77 years in the case group and 43.40 ± 9.13 years in the control subjects) and 68% of the population were females (71% of the cases and 67% of the control subjects). The mean right CCA (RCCA) IMT was 0.57 ± 0.16 mm in the HTLV-1-positive patients and 0.48 ± 0.12 mm in the healthy control cases. This difference was statistically significant ($p = 0.005$). The mean right ICA (RICA) IMT measured was 0.55 ± 0.18 mm in the cases, which was significantly greater than what was observed in the control group (0.42 ± 0.10 mm, $p < 0.001$). The mean left CCA (LLCA) IMT in the cases was 0.55 ± 0.17 mm and 0.46 ± 0.11 mm in the control group. Again, the difference was found to be significant ($p = 0.001$). The mean left ICA (LICA) IMT recorded was 0.52 ± 0.17 mm in the cases and 0.44 ± 0.14 mm in the control subjects, which, similar to previous patterns, showed a significant difference between the two groups ($p = 0.002$) (table 1). As indicated by the above values, the mean IMT was greater in the cases than in the controls in all measured arteries.

Discussion

This study showed that HTLV-1-infected patients tend to have significantly greater carotid IMT than age- and sex-matched control subjects. A lack of relevant data in the literature on HTLV-1 infection, besides the fact that the HTLV-1 virus belongs to the viral species of the Retroviridae family, left the researchers no choice but to compare the results of this study with those of the studies investigating the impact of HIV infection on atherosclerosis. The first reports of severe premature atherosclerosis among HIV-infected patients appeared in the literature in 1998 [3]. Generally, it is accepted that uncontrolled viremia in HIV infection raises the chances of cardiovascular disorders [14]. In a meta-analysis of 13 cross-sectional studies, HIV-infected patients demonstrated thicker carotid IMTs than matched controls [6]. Another study revealed that an increased level of lymphocyte activity is also associated with higher carotid IMT in

Table 1. Mean IMT of patients

Artery	Study group	Mean $\pm$ SD, mm	Range, mm	p value
RCCA	Case	0.572 ± 0.0217	0.3–1.0	0.005
	Control	0.480 ± 0.0173	0.1–0.8	
	Total	0.527 ± 0.0146	0.1–1.0	
LCCA	Case	0.553 ± 0.1743	0.1–1.0	0.001
	Control	0.469 ± 0.1136	0.3–0.9	
	Total	0.512 ± 0.1531	0.1–1.0	
RICA	Case	0.557 ± 0.1846	0.3–1.1	0.000
	Control	0.422 ± 0.1031	0.3–0.7	
	Total	0.491 ± 0.1645	0.3–1.1	
LICA	Case	0.522 ± 0.1785	0.1–0.9	0.002
	Control	0.444 ± 0.1424	0.3–1.0	
	Total	0.484 ± 0.1645	0.1–1.0	

HIV-infected patients [15]. Even though HIV infection appears to be associated with a higher risk for progression of IMT over time [14], this has not been confirmed in all studies [12]. Since traditional risk factors such as smoking and dyslipidemia are commonly observed in the HIV-infected populations studied to date, in our study the entire population (both the infected and the control subjects) was devoid of any known vascular risk factors such as hypertension, diabetes mellitus, hyperlipidemia and smoking. Therefore, based on the achieved results, it is fair to assume that HTLV-1 infection might be an independent risk factor for atherosclerosis. Likewise, in light of the facts that subclinical carotid atherosclerosis is an independent predictor of adverse cardiovascular events [13–15] and that increasing carotid IMT is an independent predictor of stroke and myocardial infarction in other populations [11–13], the findings of this study might suggest that the rate of vascular events could possibly increase in HTLV-1-infected patients.

As for diagnosis, high-resolution B-mode ultrasound imaging of the carotid arteries, particularly the measurement of carotid IMT, has been well validated in the non-infected population as a surrogate marker of vascular risk [2]. The carotid IMT measurement, which correlates accurately with pathologic specimens [1], is highly reproducible over time, is relatively inexpensive and does not expose the patient to radiation. Thus, carotid ultrasonography has been widely adopted as a research tool to compare HIV-infected populations with matched controls and to identify atherosclerosis in HIV [3, 6, 13]. Accord-

ingly, we applied the same method for the diagnosis of atherosclerosis in our subjects.

For the establishment of HTLV-1 infection as an independent risk factor for atherosclerosis, understanding the underlying pathogenesis seems to be a prerequisite and calls for extensive research. However, some theories can be derived from the registered literature. Both immunodeficiency and immune reconstitution might be atherogenic [16]. T lymphocytes, of which CD4-positive cells constitute the major population, play a key role in atherogenesis [16, 17]. The activation of CD4-positive cells appears to stimulate atherosclerosis through the induction

of proinflammatory cytokines, including interleukins as well as tumor necrosis factor [6, 18]. Despite all, it should be emphasized that sufficient data to definitely prove a causal role for infection in atherosclerosis pathogenesis are still lacking, and even though there is great temptation to initiate clinical trials testing whether interventions targeting HTLV-1 infection will decrease the evolution of atherosclerosis, we cannot ignore the importance of performing additional animal and human studies that can further address the validity of the concept and provide more information as to how the HTLV-1 virus may be related to atherogenesis.

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