Human T lymphotropic virus type 1 and risk of cardiovascular disease: High-density lipoprotein dysfunction versus serum HDL-C concentrations

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Abstract

High-density lipoprotein (HDL) is thought to be protective against cardiovascular disease (CVD), and HDL dysfunction is considered to be a risk factor for CVD. It is unclear whether there is an association between Human T lymphotropic virus type 1 (HTLV1) infection and CVD risk. We have assessed HDL lipid peroxidation (HDLox) as a marker of HDL dysfunction and CVD risk in a subgroup of the MASHAD cohort study. One hundred and sixty two individuals including 50 subjects positive for HTLV1 infection and 112 individuals negative for HTLV1 infection were recruited. Anthropometric and biochemical parameters including serum hs-CRP, fasted lipid profile (HDL-C, LDL, triglycerides, and cholesterol), and fasting blood glucose were determined. Serum HDLox was also measured in the study participants. Multivariate analyses were used to

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evaluate the association between serum HDLox and HTLV1 infection. None of the traditional CVD risk factors were associated with HTLV1 infection, including serum HDL-C. However, serum HDLox was independently associated with the presence of HTLV1 infection. Logistic regression analysis showed that subjects who were positive for HTLV1 infection were also significantly more likely than uninfected individuals to have higher HDLox (odds ratio 9.35, 95%CI: 3.5–24.7; $P < 0.001$). HDLox was increased approximately 20% ($P < 0.001$) in infected subjects compared to the uninfected group. Serum HDLox is a marker of CVD risk factor and increased in individuals affected by HTLV1 infection compared to healthy subjects.

1. Introduction

Human T lymphotropic virus type 1 (HTLV1) is a deltavirus and a member of the Retroviridae family [1]. It predominantly infects CD4+ T cells, but CD8+ may also be infected [2]. HTLV1 is an oncogenic retrovirus comprised a single-stranded RNA that can cause adult T-cell leukemia lymphoma [2,3]. It is also the causative agent for a neurological disease, namely HTLV1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [4]. Fortunately, both diseases only develop in a small percentage of infected individuals; most (95%) remain asymptomatic carriers throughout their lives [3]. In parts of the world in which the prevalence rate of HTLV infection is >1%, it is assumed to be endemic [5]; these include: Japan, Africa, Caribbean Islands, South America [6], and northeastern of Iran [7]. The prevalence varies between 1 and 7.2% in the general population of the mentioned areas [7,8]. It is estimated that 10−20 million individuals were infected with the virus globally [2]. The virus is transmitted via four principal routes: (1) Mother to child transmission which is mainly through breastfeeding, (2) Sexual transmission with a higher transmission rate from men to women, (3) through contaminated blood products, and (4) transmission between intravenous drug users [2,8]. Chronic infections induce a state of systemic inflammation and may increase the risk for comorbidities like cardiovascular disease (CVD) [9]. However, there are limited data about the contribution of chronic HTLV1 infection in CVD and the potential mechanisms. High-density lipoprotein (HDL) is involved in reverse cholesterol transport, and its levels have been shown to be inversely related to CVD risk [10,11]. The athero-protective effects of HDL, apart from its well described role in reverse cholesterol transport (RCT), include: antioxidant, antithrombotic, anti-inflammatory, regulating immune response, and endothelial protection [12]. The atheroprotective effects of HDL may be altered, and it may develop proatherogenic properties as a result of changes in lipid and protein content of HDL (abnormal HDL) in conditions such as acute inflammation. Niacin and the CETP inhibitors that have been used to increase HDL-C concentrations have no significant impact on CVD outcomes [13]. Although evaluating HDL-C and apoA1 (the principal HDL apolipoprotein) provide information about the number of circulating HDL particles, their composition and function remain unclear and the functionality of all HDL particles is not the same [14]. Therefore, the development of standard assays for HDL

function appears to be necessary along with HDL-C levels to be clinically useful. Several studies have used novel techniques to determine HDL function and have shown that HDL function represents a more reliable and accurate predictor of CVD outcomes as compared to circulating HDL-C [14–16]. In the chronic inflammatory situations, the antioxidant function of HDL is impaired and individuals may then be exposed to enhanced risk of CVD as a consequence of the presence of abnormal HDL [17]. We have also shown that oxidized dysfunctional HDL (HDLox) has a major role in systemic inflammation and immune activation in chronic viral infections like chronic HIV-1 infection [18]. The impact of HTLV1 infection on the properties of HDL has not been studied previously. Therefore, the aim of the current study was to assess HDL lipid peroxidation as a CVD risk factor in HTLV1-infected individuals in a population recruited from the Mashhad-Stroke and Heart-Atherosclerotic-Disorders (MASHAD) cohort.

2. Experimental procedures

2.1. Study design

The MASHAD study is a cohort study of a representative sample population from north-eastern Iran; recruitment started in 2010, and participants will be followed up until 2020. Eligible participants of 35−65 years, without prevalent CVD, were recruited [19]. The study was approved by Ethics Committee of Mashhad University of Medical Sciences and informed written consent was obtained from all participants.

Study subjects underwent physical examinations and medical interviews. 112 individuals without clinical HTLV1 infection and 50 infected subjects with HTLV1 (matched by sex and age) were assessed from the MASHAD cohort study. Demographic and anthropometric information of 162 participants included as age, sex, smoking status, history of diabetes, educational level, hypertension, and diabetes were applied to our study.

2.2. Reagents

The assay was conducted using reagents from Amplex Red Cholesterol Assay Kit (Invitrogen, Life Technologies, Grand Island, NY), phosphate buffered saline PBS (BioShop, Burlington, ON, Canada), polyethylene glycol (PEG) molecular weight (MW) 6000 Sigma-Aldrich (St. Louis, MO), deionized water, catalase enzyme Sigma-Aldrich (St. Louis, MO), and Black 96-well plates (SPL life science, Pocheon, South Korea). Serum HDL-C (mg/dL) was quantified by routine methods on an auto analyzer (Eppendorf, Germany).

2.3. HTLV1 infection assessment

All participants of MASHAD study were screened for HTLV1-specific antibodies using ELISA (Dia.Pro Diagnostic, Italy). A confirmatory test for HTLV1 antibody positive cases was undertaken using specific PCR primers as follows: TAX (5′ – AGGGTTTGG ACAGAGTCTT – 3′ and 5'-AAGGACCTTGAGGGTCTTA-3′) and LTR (5'-CATAAGCTCAGACCTCCGGG-3′ and 5′ -GGATGGCGGCCTCA GGTAGG-3′). If either of the two genes was present, the patient was confirmed to be infected with HTLV1 [20].

2.4. Determination of HDL lipid peroxidation

PEG precipitation was used to obtain ApoB depleted serum as follows: 40 μl PEG was added to 100 μl serum of each sample with proportion of 1:2.5 and incubated by 30 min at room temperature and centrifuged at 1000 rpm (4°C). A validated fluorometric assessment based on cell-free method was used to measure HDL lipid peroxidation (HDLox) [14]. In brief, 50 μl of ApoB depleted serum was added to the wells of a 96-well plates in duplicate. The negative control was 1X reaction buffer and positive control included 20 mM hydrogen peroxide (H_2O_2) working solution. The readout of conversion of Amplex Red to fluorescence resorufin was quantified for each sample every 5 min for an hour, at wavelengths of 530/590 nm using a plate reader (Biotek, Vermont). Pooled ApoB depleted serum of the healthy group was used as a control for each plate aiming at minimizing experimental variability. Using mean fluorescent readout of the pooled control and HDL-C level, mean fluorescence of each sample was normalized by the following calculation: "normalized" oxidized HDL (nHDLox) = [HDLox_sample × 40 (mg/dL)]/ [HDLox_control × HDLC sample (mg/dL)], where 40 mg/dL represents HDL-C of the pooled control [21].

2.5. Statistical analysis

All statistical analyses were performed using SPSS software, version 22. Results for normal and non-normally distributed data were reported as mean ± SD and median (interquartile range), respectively. Baseline characteristics of participants with and without HTLV1 were compared by Student's *t* test for normal distributed parameters, chi-square for categorical ones, and Mann–Whitney test for variables with skewed distribution. The association between HDLox and HTLV1 infection was assessed by logistic regression model after adjustment for potential confounders including age, sex, BMI, smoking status, total cholesterol, diabetes, and hypertension.

3. Results

3.1. General characteristics of studied population across HTLV1 infection

Twenty nine percent of HTLV1 carriers were males and 71% were females. Our findings revealed that the mean age of the total population was 50 ± 8.4 years. As expected, HTLV1 infection was significantly higher in women than in men. We assessed demographic, anthropometric, and biochemical variables in HTLV1 and non-HTLV1 studied population (n = 161) (Table 1). There were no significant differences in weight, waist circumference, physical activity, diastolic blood pressure, fasting blood glucose among the two groups of individuals with and without HTLV1 infection ($P > 0.1$). The uninfected group had-higher systolic blood pressure (126.2 ± 19.9 mm Hg vs. 120.5 ± 19.8 mm Hg) than HTLV1 carriers.

3.2. Lipid alterations

Based on our data, triglyceride, LDL, total cholesterol, and HDL-C level were not associated with HTLV1 status. There was no difference in mean serum HDL-C in subjects without HTLV1 infection (51.31 ± 18.03 mg/dl) and HTLV1 carriers (47.9 ± 10.5 mg/dl) ($P = 0.6$). Our results showed that although the fasted lipid profile was not affected by HTLV1 status, HDLox was significantly different between the groups ($P < 0.001$). Serum HDLox was 0.90

± 0.27 for the total population (n = 162) and was significantly higher in the HTLV1 group (1.05 ± 0.3) compared to the healthy subjects (0.83 ± 0.24) (P < 0.001) (Figure 1 and Table 1).

We assessed the association between HDLox and HTLV1 infection in studied population and found a significant difference between infected subjects and healthy individuals. Univariate logistic regression analysis confirmed the association between HDLox and HTLV1 infection (odds ratio 6.38, CI: 3–13.6; P < 0.001). In a multivariate model, adjusted for age, sex, BMI, smoking, cholesterol, diabetes and hypertension, and HTLV1 infection were associated with increased HDLox (odds ratio 9.35, CI: 3.5–24.7; P < 0.001) (Table 2).

4. Discussion

There are limited data evaluating cardiovascular risk in individuals with HTLV1 infection [22]. Herein, we assessed HDL lipid peroxidation as a surrogate measure of HDL dysfunction and a predictor of CVD in the MASHAD cohort study. Subjects who were positive for HTLV1 infection had an increased level of HDL lipid peroxidation, compared to those who were negative. Serum HDLox was approximately 20% higher in HTLV1-infected subjects than the uninfected group. In univariate logistic regression, HDLox but not serum HDL-C was associated with HTLV1 infection. After adjustment for potential confounders, the association remained significant. To our knowledge, this is the first demonstration that patients with HTLV have impaired HDL function compared to uninfected subjects. HDL dysfunction may be a contributor to CVD in chronic HTLV infection.

A growing body of genetic and clinical evidence suggests that chronic viral infections such as HIV and hepatitis virus infections are related to heightened inflammation and CVD prevalence [23,24]. It has also been demonstrated that subjects with HTLV1 infection have increased inflammation in various tissues [25]. HIV-1 is a retrovirus that contributes to increased inflammation, endothelial dysfunction, and accelerated atherosclerosis [26], and HTLV1 has similar replication enzymes including retroviral proteases [27] and may also contribute to CVD. Lipoproteins like HDL and LDL are important for pathogenesis of atherosclerotic CVD [28]. In states of systemic inflammation like atherosclerosis and chronic infections, lipoproteins can be modified and get oxidized. Although many studies described the relation of LDLox and atherosclerosis, the impact of LDLox on atherogenesis remains unclear [29]. Unlike LDL, HDL is part of the innate immunity and also has the ability to influence cholesterol availability in lipid rafts in immune cells resulting in the modulation of toll-like receptors, MHC-II complex, as well as B-and T-cell receptors, while specific molecules shuttled by HDL such as sphingosine-1-phosphate (S1P) contribute to immune cells trafficking. Thus, as a platform integrating innate and adaptive immunity, HDL may have a major role in pathogenesis of chronic viral infections [12]. We confirmed this hypothesis in chronic HIV infection, where we found that HDLox rather than LDLox was consistently and independently associated with several biomarkers of systemic inflammation and immune dysfunction that predict morbidity, CVD, and mortality in chronictreated HIV infection [18]. In addition, emerging evidence suggests that HDLox may also be important for pathogenesis of atherosclerotic CVD [14]. Thus, HDLox rather than LDLox may be important for pathogenesis of chronic viral infections (like HIV and HTLV) and

atherosclerosis. Our data in this study provide further evidence for this hypothesis that need to be validated in further models of chronic viral infections (e.g., chronic hepatitis and other chronic viral infections).

HTLV1 carriers showed a higher carotid intima-media thickness compared to the control group, and HTLV1 infection may consider to be an independent CVD risk factor [30]. Consistent with these findings, Shabestari et al. showed that the rate of HTLV1 sero-positivity in individuals who suffered from CVD is about three times higher than the general population [31]. However, the mechanism of HTLV-related CVD remains unclear.

Chronic inflammation present in chronic viral infections may contribute to CVD. In a cohort study in the HTLV1 endemic region of Japan, it has been shown that the interaction between HTLV1 infection and TNFα 1031 T/C as a polymorphism of inflammation may be a risk factor for CVD. Of note, several studies have reported changes of the immune-inflammatory status in subjects infected by HTLV1. TNFα plays a key role in the metabolism of lipid, insulin resistance, endothelial function, and coagulation, and particularly TNFα 1031 allele C was correlated with CVD risk and dyslipidemia [32,33]. However, consistent to our findings in other studies, serum hs-CRP and IL-6 were not significantly associated with the risk of CVD in the HAM/TSP patients [22]. Thus, other factors such as lipid alter ations may contribute to pathogenesis of CVD in chronic HTLV infection. We found no association between lipid parameters, including HDL-C, LDL, triglyceride, and total cholesterol, nor serum hsCRP with HTLV1 infection, as compared to the healthy subjects.

These data are in consistent with prior cross-sectional studies that did not demonstrate consistent associations between traditional cardiovascular risk factors [34], lipid levels, and HTLV serostatus [22]. In a study that evaluated lipid alterations in women infected with HTLV1, increased levels of very low-density lipoproteins and triglyceride were found in the women with HTLV1 and HAM/TSP groups as compared with healthy subjects [35]. Thus, changes in function of HDL rather than alterations in the levels of serum lipids and HDL-C may be a better predictor of CVD in chronic HTLV infection.

Alterations in HDL function may contribute to both increased CVD risk and impaired host immune responses to pathogens such as viruses [36]. Multiple cohort studies using new techniques to evaluate HDL functionality rather than HDL-C level have consistently demonstrated HDL function represents a more accurate biomarker for predicting CVD compared to serum HDL-C. The inverse association between serum HDL and CVD risk is likely to be related to the multiple characteristics of HDL that include reverse cholesterol transport and its antioxidant properties [37,38]. Serum HDLox levels may reflect HDL dysfunction and is an independent predictor of CVD risk. Lipid hydroperoxides are produced during the oxidative modification of LDL, and this process in turn is involved in the formation of fatty streak. The role of lipid oxidation is well documented in the inflammation of arterial wall. The increased amount of lipid peroxide and oxidation of low-density lipoprotein have been demonstrated in systemic inflammation as well as atherosclerosis conditions [39]. Clinical studies have previously demonstrated the association between high-serum HDLox with cardiovascular events and obesity [40]. High-serum HDLox is found in oxidative stress such as Human Immunodeficiency Virus infection

(HIV) [14]. Thus, similar to chronic HIV-1 infection, chronic HTLV infection is also associated with impaired HDL function that may contribute to increased CVD risk.

5. Conclusion

HDL lipid peroxidation is related to HDL dysfunction, and we have found higher serum levels of HDLox among subjects with HTLV1 infection than uninfected individuals, because HDLox is a marker of CVD risk, HDL lipid peroxidation may be associated with CVD outcomes in subjects with HTLV1 infection.

HDL dysfunction rather than traditional CVD risk factors and changes in lipid levels may be a predictor of CVD in subjects with HTLV1 infection. To our knowledge, these data are among the first to demonstrate that HDL dysfunction is present in chronic HTLV infection.

ACKNOWLEDGMENTS

Abbreviations:

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apoA1	Apolipoprotein A1
CETP	Cholesteryl ester transfer protein
CVD	cardiovascular disease
FBG	Fasting blood glucose
HAM/TSP	myelopathy/tropical spastic paraparesis
HDL	High-density lipoprotein
HDLox	HDL lipid peroxidation
hs-CRP	High sensitive C-reactive protein
HTLV1	Human T lymphotropic virus type
LDL	Low density lipoprotein
MASHAD	Mashhad-Stroke and Heart-Atherosclerotic-Disorders
PEG	polyethylene glycol
S1P	sphingosine-1-phosphate

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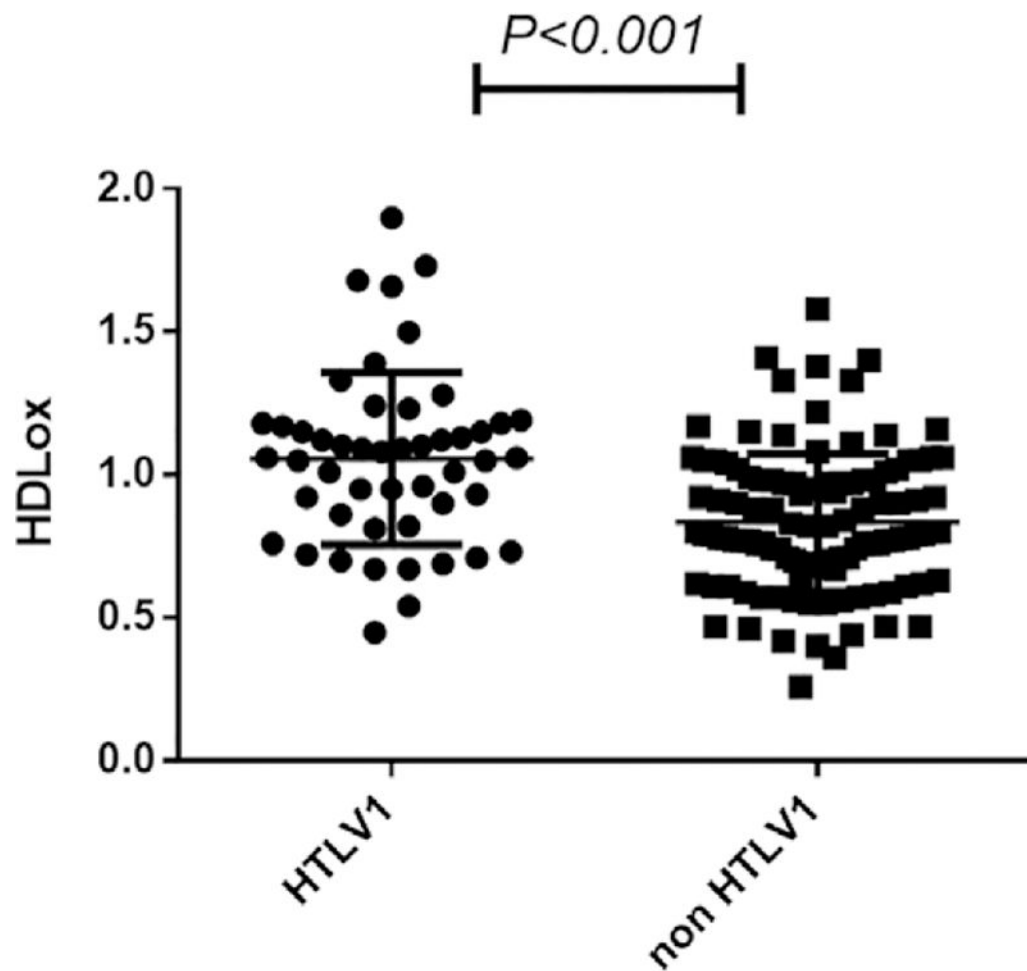
FIG 1.
The Amplex red assay was used to assess serum HDLox in subjects with HTLV1 infection. ApoB depleted sera were prepared by PEG precipitation from 50 HTLV1 infected individuals and 112 uninfected subjects. The HDL lipid peroxidation (HDLox) was quantified as a marker of cardiovascular events in study participants, so that subjects with higher serum HDLox are exposed to an increased risk of cardiovascular outcomes. The HTLV1 infected individuals have significantly higher HDLox (1.05 ± 0.3) compared to the healthy subjects (0.83 ± 0.24) ($P < 0.001$).

TABLE 1

General characteristics of studied population by HTLV1 serostatus (independent of CVD risk) (n = 162)

Variable	Without HTLVI	With HTLVI	P-value
Anthropometrics			
Age (year)	50.8 ± 8.6	50.9 ± 8.2	0.9
Gender (female)	70.8% (N = 80)	71.4% (N = 35)	0.9
Weight (kg)	68.52 ± 13.9	68.4 ± 11.75	0.9
BMI (kg/m^2)	27.07 ± 4.84	27.37 ± 4.96	0.7
WC (cm)	94.49 ± 12.07	94.42 ± 11.5	0.9
PAL	1.63 ± 0.28	1.66 ± 0.3	0.5
Blood pressure			
SBP (mm Hg)	126.2 ± 19.9	120.5 ± 19.8	0.1
DBP (mm Hg)	81 ± 11.89	80.25 ± 14	0.7
Lipid profile			
Serum total cholesterol (mg/dl)	198.3 ± 34	194.4 ± 38	0.5
TG (mg/dl)	141.5 ± 107.7	146.43 ± 140.7	0.8
HDL–C (mg/dl)	51.31 ± 18.03	47.9 ± 10.5	0.06
LDL (mg/dl)	116.15 ± 33.22	115 ± 34.23	0.8
Blood glucose			
FBG (mg/dl)	93.26 ± 36.78	90.2 ± 24.7	0.6
Inflammation			
SerumUric Acid (mg/dl)	4.43 ± 1.36	4.85 ± 1.36	0.1
Serum Hs-CRP (mg/dl)	1.6(1.14–3.37)	1.96(1.07–4.9)	0.6
Serum HDLox	0.83 ± 0.24	1.05 ± 0.3	<0.001

Abbreviations: BMI, body mass index; WC, waist circumference; PAL, physical activity level; BSP, blood systolic pressure; BDP, blood diastolic pressure; TG, triglyceride; HDL–C, high–density lipoprotein cholesterol; LDL, low–density lipoprotein; FBG, fasting blood glucose; Hs-CRP, high sensitive C-reactive protein; HDLox, HDL lipid peroxidation.

aData reported as mean ± STD except for Hs-CRP.

TABLE 2

The association of serum HDL-C and HDLox with HTLV-1

Serum variable	Univariate		Multivariate	
	OR (95% CI)	P-value	OR (95% CI)	P-value
HDL-C	0.97 (0.4–2)	0.9	1.10 (0.5–2.45)	0.8
HDLox	6.38 (2.97–13.67)	<0.001	9.35 (3.5–24.7)	<0.001

Abbreviations: HDL-C, high-density lipoprotein cholesterol; HDLox, HDL lipid peroxidation.

The odds ratio was adjusted for age, sex, BMI, smoking, HDL-C, LDL, triglyceride, and total cholesterol level.