AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 29, Number 7, 2013

DOI: 10.1089/aid.2013.0004

Liver Stiffness Is Associated With Monocyte Activation in HIV-Infected Ugandans Without Viral Hepatitis

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Abstract

A high prevalence of liver stiffness, as determined by elevated transient elastography liver stiffness measurement, was previously found in a cohort of HIV-infected Ugandans in the absence of chronic viral hepatitis. Given the role of immune activation and microbial translocation in models of liver disease, a shared immune mechanism was hypothesized in the same cohort without other overt causes of liver disease. This study examined whether HIV-related liver stiffness was associated with markers of immune activation or microbial translocation (MT). A retrospective case-control study of subjects with evidence of liver stiffness as defined by a transient elastography stiffness measurement ≥ 9.3 kPa (cases = 133) and normal controls ($n = 133$) from Rakai, Uganda was performed. Cases were matched to controls by age, gender, HIV, hepatitis B virus (HBV), and highly active antiretroviral therapy (HAART) status. Lipopolysaccharide (LPS), endotoxin IgM antibody, soluble CD14 (sCD14), C-reactive protein (CRP), and D-dimer levels were measured. Conditional logistic regression was used to estimate adjusted matched odds ratios (adjMOR) and 95% confidence intervals. Higher sCD14 levels were associated with a 19% increased odds of liver stiffness (adjMOR = 1.19, $p = 0.002$). In HIV-infected individuals, higher sCD14 levels were associated with a 54% increased odds of having liver stiffness (adjMOR = 1.54, $p < 0.001$); however, the opposite was observed in HIV-negative individuals (adjMOR = 0.57, $p = 0.001$). No other biomarker was significantly associated with liver stiffness, and only one subject was found to have detectable LPS. Liver stiffness in HIV-infected Ugandans is associated with increased sCD14 indicative of monocyte activation in the absence of viral hepatitis or microbial translocation, and suggests that HIV may be directly involved in liver disease.

Introduction

Liver disease has emerged as a significant complication of HIV disease and contributes to approximately 12% of AIDS-related deaths.[1] Moreover, liver disease staging using noninvasive transient elastography measurements (LSM) has been validated in HIV infection as representative of liver disease stage as determined by biopsy.[2] We recently found evidence of significant liver stiffness among HIV-infected persons in the Rakai District of Uganda: HIV infection was associated with a 50% increased odds of liver stiffness as defined by transient elastography measurement.[3] This population had a low seroprevalence of chronic viral hepatitis [5% $HBsAg^+$, 0% anti-hepatitis C virus (HCV^+)][3] (C. Mullis *et al.*, unpublished observations). Underlying causes of liver stiffness in this HIV-infected population without chronic viral hepatitis, therefore, are unknown and warrant study.

Lipopolysaccharide (LPS) is a marker for translocation of bacterial byproducts from the lumen of the gut into the circulation. This is commonly referred to as microbial translocation, which has been found to be associated with increased immune activation and advanced HIV disease state, although the nature of this association is unclear.[4–6] A proposed mechanism for microbial translocation's effects on HIV disease is through LPS-induced activation of monocytes and the subsequent increased production of soluble CD14 (sCD14).[4,7,8] Immune activation

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and intestinal microbial translocation (MT) have been implicated as causes of numerous types of liver disease in humans and in animal models.[4,9,10] Moreover, immune activation appears to drive HIV progression.[11] Based on these previous studies, we hypothesized that liver stiffness in HIV-infected Ugandans was associated with innate immune activation. Previously, we did not find evidence of MT contributing to HIV progression in this region.[12] However, others have found evidence of MT in HIV–HCV coinfection, but only in the presence of cirrhosis or advanced fibrosis.[9,10] We performed a retrospective case-control study of subjects with detectable liver stiffness and cirrhosis from Rakai, Uganda, to examine the contribution of immune activation and MT to liver disease within this cohort.[3]

Materials and Methods

Study population

Details of the Rakai liver study have been described previously.[3] Briefly, the Rakai Health Science Programs (RHSP) recruited 500 HIV-infected and 500-uninfected individuals at five different clinics. Liver stiffness was determined by transient elastography (FibroScan, Echosense, Paris, France), and individuals with a liver stiffness measurement (LSM) greater than or equal to 9.3 kPa were considered to have significant liver disease, as previously documented.[2,3,13] All subjects from the previous liver disease study with LSM ≥ 9.3 kPa were selected as cases for a retrospective 1:1 case-control study.[3] Cases ($n = 133$) were matched to controls ($n = 133$) by age groups (18–29, 30–38, and 39–65), gender, HIV status, highly active antiretroviral therapy (HAART) initiation, and hepatitis B virus (HBV) status. A sensitivity analysis was performed using matched pairs in which the case had evidence of cirrhosis (LSM ≥ 12.3 kPa) and their matched control had no indication of liver stiffness (LSM < 8.0 kPa).[14] All participants provided informed consent.

Laboratory assays

Serum and plasma were collected from venous blood at the time of liver examination. Platelet (PLT), alanine aminotransferase (ALT) levels, $CD4^+$ T cell counts (FACScalibur, BD biosciences), and HAART status were assessed.[3] HBV infection status was determined by detection of HBV surface antigen (HBsAg) and antibodies to HBV core (anti-HBc) using enzyme-linked immunosorbent assay (ELISA) (ETI-EKB s Plus and ET-AB-COREK Plus, Diasorin, Vercelli, Italy).[3] HCV status was tested with the ORTHO HCV Version 3.0 ELISA Test System and validated using the Abbott RealTime HCV Assay (Abbott Molecular Inc., Des Plaines, IL) (C. Mullis *et al.*, unpublished observations). Schistosomiasis infection was determined with soluble egg antigen ELISA (Schisto-96; IVD Research Inc., Carlsbad, CA).[3]

Circulating LPS levels were measured with an adapted LAL assay (Lonza QCL-1000, Walkersville, MD) that was modified by using a higher dilution and an additional diazo-coupling step to avoid overlap between the background and the signal readout.[15] Each sample was tested in duplicate, and any positive samples were repeated.

Endotoxin core IgM antibody levels (EndoCAb; Hycult Biotechnology, The Netherlands), sCD14 (R&D Systems, Minneapolis, MN), CRP (Invitrogen Corporation; Camarillo, CA), and D-dimer (American Diagonistica Inc., Stamford, CT) were measured by ELISA on stored serum samples except for 21 samples, which were run on plasma.

Statistical analysis

Conditional logistic regression was used to analyze the association of immunologic biomarkers with liver stiffness. D-dimer and CRP levels were $\log_{10}$ transformed prior to analysis. EndoCAb and sCD14 levels were analyzed by 100 MMU/ml and 500 ng/ml increments, respectively. The associations were also assessed, and stratified by HIV and HAART initiation status. Suspected confounding factors associated with increased risk of liver disease (self-reported alcohol consumption and herbal medicine use, schistosomiasis antibody, and $CD4^+$ T cell count) were included in a multivariable analysis with each biomarker.[3] Stata version 11.2 (StataCorp, College Station, TX) was used for statistical analysis.

Results

Population analysis

In this study 58% ($n = 154/266$) of the individuals were HIV infected and 55% ($n = 148/266$) were female (Table 1). Three percent of the HIV-uninfected and 5% of the HIV-infected individuals were HBsAg positive. No confirmed HCV was found in the population (C. Mullis *et al.*, unpublished observations). In the HIV-infected individuals, 58% had initiated HAART ($n = 89/154$). The $CD4^+$ T cell counts (HIV^+ only; adjMOR = 1.22; 95% CI = 0.75–1.96; $p = 0.42$), ALT (adjMOR = 0.74; 95% CI = 0.47–1.12; $p = 0.15$), and PLT levels (adjMOR = 1.13; 95% CI = 0.64–1.99; $p = 0.68$) of the cases and controls

Table 1. Characteristics of Study Participants

	Cases (≥ 9.3 kPa)		*Controls (< 9.3 kPa)*	
Liver disease	*HIV^+ n = 77 (%)*	*HIV^- n = 56 (%)*	*HIV^+ n = 77 (%)*	*HIV^- n = 56 (%)*
Gender[a]				
Male	34 (56)	25 (45)	34 (56)	25 (45)
Female	43 (44)	31 (55)	43 (44)	31 (55)
Age range[a]				
18–29	13 (17)	6 (11)	13 (17)	6 (11)
30–38	34 (44)	25 (45)	34 (44)	25 (45)
39–65	30 (39)	25 (45)	30 (39)	25 (45)
HAART[a]				
Yes	44 (57)	—	44 (57)	—
No	33 (43)	—	33 (43)	—
Hepatitis B[a]				
Yes	4 (5)	2 (4)	4 (5)	2 (4)
No	73 (95)	54 (96)	73 (95)	54 (96)
CD4 count[b]	484 (±283)	—	462 (±296)	—
ALT level[b]	28 (±25)	25 (±16)	36 (±42)	22 (±8)
PLT level[b]	242 (±68)	236 (±101)	232 (±76)	238 (±99)

[a]Denote variables that were matched for the case/control experiment. These were reported as number of individuals and percentage in parentheses.

[b]CD4 Count, ALT level, and PLT level are reported as median with standard deviation in parentheses.

HAART, highly active antiretroviral therapy; ALT, alanine aminotransferase; PLT, platelet.

Table 2. Matched Case-Control Status for Known Causes of Liver Disease

	Case/control (N = 133 pairs)				
	Neg/neg	*Pos/pos*	*Pos/neg*	*Neg/pos*	*MOR (95% CI)*
Schistosomiasis	93	4	15	21	0.8 (0.415–1.54)
Herbal medication	111	10	11	1	1.1 (0.47–2.59)
Alcohol use	80	19	18	16	1.05 (0.55–2.01)

MOR, matched odds ratio.

were similar and had no significant association with liver stiffness (Table 1). There were no significant differences between cases and controls in exposure to alcohol, herbal medicine, or schistosomiasis (Table 2). The subjects for this study were previously screened for a variety of other risks factors for liver disease, which are described in detail in the previous study.[3]

Immune activation markers

EndoCAb, CRP, and D-dimer measurements did not differ between cases and controls (Tables 3 and 4). However, the odds of liver stiffness were increased with each 500 ng/ml increment in sCD14 (adjMOR = 1.19; 95% CI = 1.16–1.32; $p = 0.002$) (Table 4). To further examine this association, individuals were stratified by HIV status. Among HIV-uninfected individuals, there was a decreased odds of liver stiffness with each increase of 500 ng/ml in sCD14 (adjMOR = 0.57; 95% CI = 0.41–0.8; $p = 0.001$) (Fig. 1A). Conversely, HIV-infected individuals had increased odds of liver stiffness with each 500 ng/ml increment in sCD14 (adjMOR = 1.54; 95% CI = 1.25–1.89; $p < 0.001$) (Fig. 1A). Individuals who were HIV infected were further divided according to HAART status. There was an increased odds of liver stiffness in HIV-infected, HAART-naive individuals with increased sCD14 (adjMOR = 1.93; 95% CI = 1.21–3.07; $p = 0.005$) as compared to a modest increased odds among those subjects who had initiated HAART therapy (adjMOR = 1.36; 95% CI = 1.08–1.70; $p = 0.008$) (Fig. 1B).

LPS testing

Only one sample had a positive LPS signal using a highly accurate adapted Lonza assay.[15] The individual with a positive LPS signal was HIV infected, currently on HAART, HBV negative, and liver stiffness negative (LSM = 7.6 kPa). Therefore, LPS levels were excluded from further analyses.

Sensitivity analysis

We performed a sensitivity analysis of 61 matched pairs in which the case had a liver stiffness measurement indicative of cirrhosis (LSM ≥ 12.3 kPa) and the controls had no discernible liver stiffness (LSM < 8.0 kPa).[14] In this subanalysis, 56% ($n = 68/122$) of the individuals were HIV infected and 64% ($n = 78/122$) were female. Within the HIV-infected individuals, 58% had initiated HAART therapy ($n = 40/68$). This analysis demonstrated a similar association with an increased odds of cirrhosis with increased sCD14 (adjMOR = 1.17, 95% CI = 1.02–1.35; $p = 0.029$). Both HIV-infected (adjMOR = 1.48, 95% CI = 1.31–1.93; $p = 0.004$) and HIV-uninfected individuals (adjMOR = 0.57, 95% CI = 035–0.9; $p = 0.017$) displayed trends similar to those seen in the larger population. Within this smaller group of HIV-infected matched pairs, the relationship between cirrhosis and increased sCD14 seemed to be modulated by HAART status [(HAART-initiated adjMOR = 1.29, 95% CI = 0.99–1.7; $p = 0.059$) and (HAART-naive adjMOR = 2.02, 95% CI = 0.95–4.18; $p = 0.055$), although this was of borderline statistical significance.

Discussion

Our study found that increased liver stiffness indicative of fibrosis and cirrhosis, as determined by transient elastography, was associated with increased monocyte activation in HIV-infected individuals. This was in the absence of detectable microbial translocation and controlling for other risk factors associated with liver disease. Transient elastography has been shown to provide an accurate noninvasive appraisal of liver disease, and in some cases outperforms histologic diagnosis of cirrhosis.[13]

sCD14 is an indicator of monocyte activation, and its secretion can be induced by LPS and other stimulants.[7,16] These results suggest that elevated sCD14 levels seen in individuals with liver stiffness and cirrhosis are independently associated with HIV and not a secondary effect caused by viral hepatitis. However, it is possible that the subjects with liver stiffness in this study were exposed or infected with a different viral hepatitis not tested in this study such as hepatitis E or a previously uncharacterized virus, and future studies are planned to explore this question.

There was a significant amount of variability in the levels of sCD14 in the cases and controls, which suggests that monocyte activation does not fully explain the increase in liver stiffness seen in this population. There was only one LPS-positive sample, showing almost no evidence of biologically relevant microbial translocation in this population. Similarly, antibodies to the endotoxin core have been previously shown

Table 3. Biomarkers According to Case/Control Group and HIV Status

Biomarkers	*sCD14 (ng/ml)*	*EndoCAb (MMU/ml)*	*CRP (mg/ml)*	*D-dimer (ng/ml)*
Overall population	1,770 (±1163)	126 (±106)	2.28 (±2.71)	19.1 (±41.2)
Cases (≥9.3 kPa)	2,029 (±1320)	138 (±128)	2.05 (±2.17)	23.5 (±50.9)
Controls (<9.3 kPa)	1,509 (±914)	113 (±77.8)	2.50 (±3.18)	14.5 (±27.8)
HIV^+	1,840 (±1369)	102 (±77.7)	2.32 (±2.81)	19.5 (±45.2)
HIV^-	1,672 (±793)	159 (±129)	2.23 (±2.62)	18.5 (±35.1)

Median values are shown with standard deviation in parentheses.
EndoCAb, endotoxin core antibody; CRP, C-reactive protein.

Table 4. Matched Odds Ratios and Confidence Intervals for Liver Stiffness in the Total Population

Biomarkers	*MOR (95% CI)*	p-*value*
sCD14 (500 ng/ml)	1.19 (1.06–1.32)	0.002
Endotoxin (100 MMU/ml)	1.24 (0.99–1.69)	0.057
CRP (log mg/ml)	0.89 (0.74–1.08)	0.25
D-Dimer (log ng/ml)	1.17 (0.99–1.38)	0.057

Adjusted for self-reported alcohol consumption and herbal medicine use, schistosomiasis, and $CD4^+$ T cell count.

to be associated with microbial translocation in HIV-infected individuals, but were not associated with liver stiffness in the present study.[4] These findings differ from studies examining liver disease in HIV/HCV-coinfected individuals.[9,10] It should be noted that elevated transient elastography measurements may not predict morbidity and mortality in the same way they do in the presence of HCV coinfection. In addition, two markers of overall immune activation, CRP and D-dimer, were not found to be associated with liver stiffness in this population. This was somewhat surprising given the strong association of monocyte activation and liver disease, and the association of increased CRP levels with HIV disease progression observed in this area.[11]

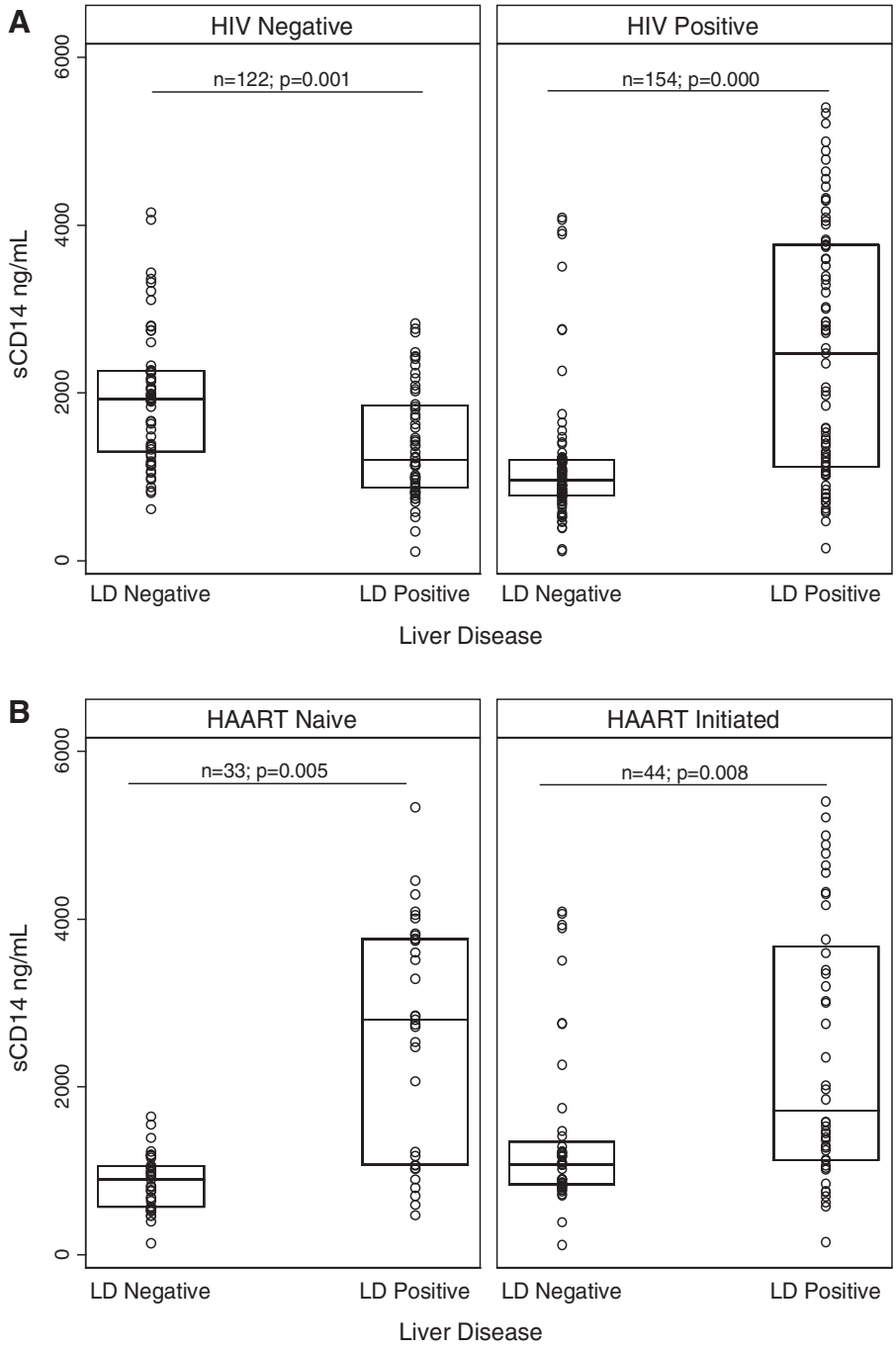

FIG. 1. sCD14 levels of cases and controls segregated by HIV **(A)** and highly active antiretroviral therapy (HAART) status **(B)** are shown. Conditional logistic regression was used to determine significance ($p < 0.05$). All points are included with the median and interquartile ranges denoted by the box plot.

The contrasting relationship between sCD14 and liver stiffness by HIV status could have many possible causes, and this may indicate that HIV is playing a more direct role in liver disease than previously observed. One possible explanation for this association is that HIV infection may be causing an increase in monocyte activation in the liver due to increased bystander cell death or loss of Kupfer cells, which in turn could promote fibrosis.[17] However, due to the cross-sectional nature of this study, no causal inference can be made in the absence of longitudinal follow-up to determine if sCD14 is a predictor of HIV-associated liver disease progression.

Acknowledgments

Institutional Review Board approvals were obtained from the Uganda Virus Research Institute's Scientific and Ethics Committee, the Uganda National Council for Science and Technology, and the Institutional Review Boards of collaborating U.S. institutions (National Institutes of Health and Johns Hopkins University). This study was supported by the Division of Intramural Research, NIAID, the Office of AIDS research, NIH, NICHD (grant F31HD063345), NIAID (grants 5P30HD06826, U01AI075115), Henry M. Jackson Foundation, Fogarty Foundation (5D43TW00010), and the Bill and Melinda Gates Foundation (grant 22006.03). The authors with to thank the Office of Cyberinfrastructure and Computational Biology (OCICB), NIAID for support.

Author Disclosure Statement

No competing financial interests exist.

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