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Interleukin-6 Is Associated with Noninvasive Markers of Liver Fibrosis in HIV-Infected Patients with Alcohol Problems

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Abstract

Both HIV and hepatitis C virus (HCV) cause chronic inflammation and alterations in serum inflammatory cytokines. The impact of inflammatory cytokines on liver fibrosis is not well understood. We studied the association between interleukin (IL)-6, IL-10, and tumor necrosis factor (TNF)-α and liver fibrosis in HIV-infected patients with current or past alcohol problems (CAGE ≥2 or physician investigator diagnosis). Liver fibrosis was estimated with FIB-4 (FIB-4 <1.45 defined the absence of liver fibrosis and FIB-4 >3.25 defined advanced fibrosis). Logistic regression was used to assess the association between cytokines and fibrosis, adjusting for age, sex, CD4, HIV RNA, current antiretroviral therapy, body mass index, and HCV. Secondary analyses explored whether the association between HCV and liver fibrosis was mediated by these cytokines. Participants ($n=308$) were all HIV-infected; 73% were male with a mean age of 42 years; half had detectable HCV-RNA, 60.7% had an absence of liver fibrosis, and 10.1% had advanced fibrosis. In models that adjusted for each cytokine separately, higher levels of IL-6 were significantly associated with an absence of fibrosis [adjusted OR (95% CI): 0.43 (0.19, 0.98), $p=0.05$] and were borderline significant for advanced fibrosis [adjusted OR (95% CI): 8.16 (0.96, 69.54), $p=0.055$]. In the final model, only higher levels of IL-6 remained significantly associated with advanced liver fibrosis [adjusted OR (95% CI): 11.78 (1.17, 118.19), $p=0.036$]. Adjustment for inflammatory cytokines attenuated the adjusted OR for the association between HCV and fibrosis in the case of IL-6 [for the absence of fibrosis from 0.32 (0.17, 0.57) $p<0.01$ to 0.47 (0.23, 0.96) $p=0.04$; and for advanced fibrosis from 7.22 (2.01, 25.96) $p<0.01$ to 6.62 (1.20, 36.62) $p=0.03$], suggesting IL-6 may be a partial mediator of the association between HCV and liver fibrosis. IL-6 was strongly and significantly associated with liver fibrosis in a cohort of HIV-infected patients with alcohol problems. IL-6 may be a useful predictive marker for liver fibrosis for HIV-infected patients.

Introduction

Liver disease causes significant morbidity and mortality in HIV-infected patients.[1,2] Chronic hepatitis C virus (HCV) coinfection is common in this setting; especially among past injection drug users.[3] In addition, other forms of liver disease such as fatty liver disease, antiretroviral-related liver toxicity, and noncirrhotic portal hypertension have been described in HIV infection, even in the absence of HCV.[2]

Liver fibrosis is the main predictor of progression to end-stage liver disease in chronic HCV infection and other forms of liver disease.[4] Liver fibrosis is preceded by liver inflammation; it is characterized by the progressive deposition of collagen in the extracellular matrix and is mainly driven by the activation and proliferation of hepatic stellate cells.[5,6] Evaluation of liver fibrosis is a cornerstone in the clinical management of HIV/HCV-coinfected patients,[2] as it may predict evolution to end-stage liver disease and the likelihood of response to HCV antiviral therapy and of antiretroviral

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hepatotoxicity.[7] HIV infection is associated with reduced spontaneous resolution of acute HCV infection,[8,9] faster progression of liver fibrosis, and an earlier onset of end stage liver disease.[10,11] Even though immune suppression, male gender, alcohol abuse, and age at infection have been recognized as major contributors to accelerated liver fibrosis progression,[10,12] the underlying molecular mechanisms that accelerate liver injury in HIV-infected patients are not completely understood.[13,14]

Adaptive immunity is crucial in HCV infection. In non-HIV-infected patients, acute HCV infection is followed by a Th1 response[15,16] that produces cytokines [interleukin (IL)-2, interferon (IFN)-γ, IL-6, and tumor necrosis factor (TNF)-α] and promotes inflammation and cell-mediated immunity, as well as macrophage and stellate cell activation, in an attempt to control infection.[15,17] It is followed by a Th2 response, which is antiinflammatory and is aimed at regulating the proinflammatory Th1 response.[15] An imbalance between Th1 and Th2 responses influences progression and persistence of HCV infection,[15,17,18] and intrahepatic expression of Th1-associated cytokines has been related to progressive liver injury.[19] IL-6, IL-10, and TNF-α have been associated with the immune response to both HIV and HCV infection,[14] and they are also associated with the immune effects of heavy alcohol use.[20] Most of the studies of immune response to hepatitis C virus infection have been conducted *in vitro* and in animal models and studies with serum levels in HCV-infected humans and its impact on liver damage are scarce. Whether HIV/HCV coinfection contributes to this imbalance and whether this is an important mechanism leading to liver fibrosis are unclear. Furthermore, HIV infection by itself leads to hepatocyte death and to hepatic stellate cell activation.[21,22] Detectable HIV RNA, even in the absence of HCV coinfection, has been associated with noninvasive markers of liver fibrosis in cross-sectional studies of HIV-infected cohorts.[23,24]

There is a need to better characterize the underpinnings of liver fibrosis in HIV-infected patients. This study was undertaken to determine whether levels of inflammatory cytokines IL-6, IL-10, and TNF-α are associated with liver fibrosis as measured by FIB-4 (a noninvasive index that includes platelets, age, and liver enzyme levels)[25] in HIV-infected patients with alcohol problems. Second, we explored whether higher levels of these cytokines in the setting of HCV may explain increased risk for fibrosis (i.e., whether these cytokines mediate the known association between HCV infection and liver fibrosis in HIV-infected patients).

Materials and Methods

Design

This is a cross-sectional analysis examining the association between inflammatory cytokines (IL-6, IL-10, and TNF-α) and liver fibrosis in HIV-infected patients with current or past alcohol problems. Data were obtained from a prospective, observational cohort study [HIV-Longitudinal Interrelationships of Viruses and Ethanol (HIV-LIVE)] in which assessments occurred at 6-month intervals over a maximum of 42 months.[26]

Subjects

Recruitment for the HIV-LIVE cohort occurred from different sources: a previous cohort study, an intake clinic for HIV-infected patients, HIV primary care and specialty clinics at two hospitals, homeless shelters, drug treatment programs, subject referrals, and flyers. Enrollment occurred between August 2001 and July 2003. Eligibility criteria were (1) documented HIV antibody test by ELISA and confirmed by Western blot; (2) two or more affirmative responses to the CAGE alcohol screening questionnaire,[27] or physician investigator diagnosis of alcoholism; and (3) an ability to speak English or Spanish. Exclusion criteria were (1) scoring <21 on the 30-item Mini-Mental State Examination (i.e., cognitive impairment)[28,29] and (2) an inability to provide informed consent.[30] In addition, the present analysis included only those HIV-LIVE subjects with laboratory parameters available to calculate the FIB-4. The Institutional Review Boards of Boston Medical Center and Beth Israel Deaconess Medical Center approved this study.

Measures

Dependent variable. The two primary outcomes of this study were the absence of liver fibrosis and the presence of advanced liver fibrosis based on FIB-4 (a noninvasive index) at study entry or at the earliest time point at which laboratory results were available during the study period. FIB-4 values were calculated as follows:

FIB-4 = [age × AST (IU/liter)/platelet count (10^9/liter) × ALT (IU/liter)$^{1/2}$]. As validated in a cohort of HIV/HCV-coinfected patients, FIB-4 values <1.45 are consistent with the absence of liver fibrosis with a negative predictive value of 90% and a sensitivity of 70%. Also, FIB-4 values >3.25 are consistent with significant liver fibrosis with a positive predictive value 67% and a specificity of 97%, with an area under the receiver operating curve (AUROC) of 0.765.[25]

Independent variables. The inflammatory cytokines IL-6, IL-10, and TNF-α were chosen based on research demonstrating that serum levels are significantly different in the setting of HCV infection,[31] and that they are related to immune response in HCV infection and with liver inflammation and liver fibrosis in HCV-infected patients.[13]

TNF-α and IL-6 were measured using Bio-Rad Luminex Flow Cytometry (Millipore) and IL-10 was measured using Chemiluminescent ELISA (R&D Systems). Laboratory testing was conducted on baseline samples at the University of Vermont's Laboratory for Clinical Biochemistry Research.

Covariates. Potential confounders controlled for in the analyses were age, sex, and CD4 count/mm^3 (categorized as <200, 200–500, and >500), HIV RNA (categorized as <500 copies/ml or ≥ 500 copies/ml], current antiretroviral therapy (yes/no), body mass index (BMI), and HCV infection. HCV infection was defined as a positive HCV antibody result confirmed with the presence of detectable HCV RNA on polymerase chain reaction (PCR).

Statistical analysis

Descriptive statistics were used to describe the study sample overall and were stratified by liver fibrosis status [FIB-4 <1.45 (absence of liver fibrosis), FIB-4 1.45–3.25 (intermediate values), and FIB-4 >3.25 (presence of advanced liver fibrosis)]. Baseline

characteristics were compared across groups using Chi-square/Fisher's exact test for categorical values and ANOVA for continuous variables. To avoid assumptions of linearity, IL-6, IL-10, and TNF-α were categorized in tertiles. The lowest tertile was the reference category.

Logistic regression models were fit to evaluate the association between each of the three inflammatory cytokines (IL-6, IL-10, and TNF-α) and the outcomes (i.e., the absence of liver fibrosis and the presence of advanced liver fibrosis), controlling for age, gender, CD4 count, HIV RNA, current antiretroviral therapy, BMI, and HCV.

Additional analyses were performed to assess whether serum levels of cytokines IL-6, IL-10, and TNF-α mediated the association between HCV infection and liver fibrosis. Based on the approach described by Baron and Kenny,[32] first, a multivariate adjusted analysis was performed to assess the association between HCV infection and the absence of liver fibrosis and the presence of advanced liver fibrosis in this cohort. Then a series of additional regression models were fit that included each inflammatory cytokine (in tertiles) one at a time as well as all three simultaneously in order to assess whether HCV coefficient estimates were attenuated by inclusion of the potential mediators (IL-6, IL-10, and TNF-α).

Adjusted odds ratios (AORs) and 95% confidence intervals are reported for each model. Spearman correlations were used to identify strong correlations between independent variables and covariates that could result in potential collinearity. No pair of variables included in the same regression model was highly correlated ($r > 0.40$). All analyses were conducted using two-sided tests and a significance level of 0.05. Due to the exploratory nature of the analyses, adjustments were not made for multiple comparisons. However, pairwise comparisons were not made unless the global *p*-value for the

Table 1. Baseline Characteristics of HIV-LIVE Study Participants—HIV-Infected Persons with a History of Alcohol Problems

	Total (n = *308*)	*FIB-4 < 1.45* (n = *187*)	*FIB-4 1.45–3.25* (n = *90*)	*FIB-4 > 3.25* (n = *31*)	p-*value*
Male [*n* (%)]	225 (73.1)	136 (72.7)	68 (75.6)	21 (67.7)	0.69
Age (mean ± SD)	42 ± 7.3	40 ± 6.9	46 ± 6.6	46 ± 6.6	<0.01
HCV infection (HCV RNA) (*n* = 307) [*n* (%)]	150 (48.9)	67 (35.8)	57 (64.0)	26 (83.9)	<0.01
CD4 count [cells/mm^3] (*n* = 289)					<0.01
< 200 [*n* (%)]	54 (18.7)	27 (15.3)	18 (21.4)	9 (31.0)	
200–500 [*n* (%)]	121 (41.9)	63 (35.8)	40 (47.6)	18 (62.1)	
> 500 [*n* (%)]	114 (39.4)	86 (48.9)	26 (31.0)	2 (6.9)	
HIV RNA viral load [copies/ml] (*n* = 274)					0.72
< 500 [*n* (%)]	121 (44.2)	76 (46.1)	34 (42.0)	11 (39.3)	
≥ 500 [*n* (%)]	153 (55.8)	89 (53.9)	47 (58.0)	17 (60.7)	
Current antiretroviral therapy	186 (60.4)	113 (60.4)	52 (57.8)	21 (61.7)	0.62
Body mass index [kg/m^2] (*n* = 293) (mean ± SD)	27 ± 5.5	27 ± 6.0	26 ± 5.0	26 ± 3.2	0.08
Diabetes mellitus[a]	18 (5.8)	5 (2.7)	9 (10)	4 (12.8)	0.01
Injection drug use, ever [*n* (%)] (*n* = 307)	165 (53.7)	74 (39.6)	65 (73.0)	26 (83.9)	<0.01
Current marijuana use	119 (38.6)	75 (40.1)	32 (35.6)	12 (38.7)	0.77
Heavy alcohol use (NIAAA definition) [*n* (%)] (*n* = 307)	101 (32.9)	61 (32.8)	30 (33.3)	10 (32.3)	0.99
IL-6 (*n* = 237)					
Median (IQR)	2.80 (1.52–4.78)	2.34 (1.26–3.67)	3.21 (1.77–5.36)	6.17 (3.26–10.05)	<0.01
Range	0.15–45.17	0.15–18.56	0.15–45.17	1.28–14.06	
Tertiles [*n* (%)]					
1st tertile (< 1.96)	79 (33.3)	57 (38.8)	20 (27.8)	2 (11.1)	<0.01
2nd tertile (1.96–3.70)	90 (33.8)	55 (37.4)	22 (30.6)	3 (16.7)	
3rd tertile (≥ 3.71)	89 (32.9)	35 (23.8)	30 (41.7)	13 (72.2)	
IL-10 (*n* = 263)					
Median (IQR)	5.15 (3.08–8.39)	4.52 (2.76–7.43)	6.3 (4.25–9.16)	5.68 (3.3–14.94)	0.02
Range	0.12–61.08	0.12–59.95	0.12–28.89	1.12–61.08	
Tertiles [*n* (%)]					
1st tertile (< 3.64)	83 (31.6)	60 (37.0)	16 (20.8)	7 (29.2)	0.08
2nd tertile (3.64–6.90)	93 (35.4)	57 (35.2)	28 (36.4)	8 (33.3)	
3rd tertile (≥ 6.91)	87 (33.1)	45 (27.8)	33 (42.9)	9 (37.5)	
TNF-α (*n* = 263)					
Median (IQR)	7.25 (4.87–10.01)	6.44 (4.82–9.22)	7.68 (5.41–10.67)	8.31 (5.67–13.19)	0.06
Range	0.63–149.68	1.66–49.04	0.63–149.68	1.30–30.27	
Tertiles [*n* (%)]					
1st tertile (< 5.41)	85 (32.3)	60 (37.0)	19 (24.7)	6 (25.0)	0.06
2nd tertile (5.41–9.06)	91 (34.6)	59 (36.4)	25 (32.5)	7 (29.2)	
3rd tertile (≥ 9.07)	87 (33.1)	45 (26.5)	33 (42.9)	11 (45.8)	

[a]Self-reported.

SD, standard deviation; IQR, interquartile range; HCV, hepatitis C virus; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha.

inflammatory cytokine was statistically significant. All statistical analyses were conducted using SAS version 9.3 (SAS Institute, Inc., Cary, NC).

Results

Out of 400 HIV-LIVE study participants, 308 had sufficient data to calculate the FIB-4 score and comprised the sample for the current analyses. There was no significant difference according to gender, age, and HCV coinfection for the 308 participants included in the study compared to the 92 subjects from the cohort without available FIB-4 data.

Table 1 shows the main characteristics in the total study group stratified by liver fibrosis status [FIB-4 <1.45 (absence of liver fibrosis), FIB-4 1.45–3.25, and FIB-4 >3.25 (presence of advanced liver fibrosis)]. Participants were 73% male with a mean age of 42 years and a mean BMI of 27; 49% had a detectable HCV-RNA, 59% were on antiretroviral therapy, 55.8% had an HIV RNA load >500 copies/ml, and 19% had a CD4 count <200 cells/mm^3. One hundred and sixty-five participants (53.7%) had prior or current injection drug use and the mean duration of injection drug use was 11.7 years. Based on FIB-4, 60.7% had an absence of liver fibrosis and 10.1% had advanced liver fibrosis. The median (IQR) levels in pg/ml of IL-6, IL-10, and TNF-α were 2.80 (1.52–4.78), 5.15 (3.08–8.39, and 7.25 (4.87–10.01), respectively.

As shown in Table 1, one-third of the study participants had heavy alcohol use [defined as meeting the NIAAA (National Institute on Alcohol Abuse and Alcoholism) definition for risky drinking: >14 drinks/week or ≥ 5 drinks on one occasion for men ≤ 65 years of age, and >7 drinks/week or ≥ 4 drinks on one occasion for all women and for men >65 years of age] in the past 30 days, and they were evenly distributed among the three groups according to FIB-4 thresholds.

Compared to patients with FIB-4 values consistent with the absence of liver fibrosis, participants with values consistent with advanced liver fibrosis were significantly older, had a significantly lower CD4 count, and were more likely to be coinfected with HCV. Also, they had significantly higher levels of both IL-6 and IL-10, and had higher levels of TNF-α that were borderline significant ($p=0.06$).

The results from multivariate logistic regression models showing the relative odds for the absence of fibrosis are demonstrated in Table 2, with serial adjustments for each inflammatory cytokine and a final fully adjusted model with all cytokines. Table 3 shows similar models for the outcome of advanced liver fibrosis. In models that included each cytokine separately higher levels of IL-6 were significantly associated with the absence of fibrosis [adjusted OR (95% CI): 0.43 (0.19, 0.98), $p=0.05$] and were borderline significant for advanced fibrosis [adjusted OR (95% CI): 8.16 (0.96, 69.54), $p=0.055$]. In models that included all three cytokines, the highest tertile of IL-6 was significantly associated with the presence of advanced liver fibrosis [adjusted OR (95% CI): 11.78 (1.17, 118.19), $p=0.04$] (Table 3). Levels of IL-10 and TNF-α were not significantly associated with either the absence or presence of fibrosis.

Table 2. Associations Between Hepatitis C Virus, Inflammatory Cytokines, and Absence of Liver Fibrosis Among HIV-Infected Persons with a History of Alcohol Problems

	Model I[a] *OR (95% CI)*	*Model II*[a] *OR (95% CI)*	*Model III*[a] *OR (95% CI)*	*Model IV*[a] *OR (95% CI)*	*Model V*[a] *OR (95% CI)*
HCV	0.32 (0.17, 0.57)	0.37 (0.19, 0.71)	0.33 (0.17, 0.63)	0.43 (0.22, 0.86)	0.47 (0.23, 0.96)
Age	0.89 (0.85, 0.93)	0.88 (0.84, 0.93)	0.88 (0.84, 0.93)	0.90 (0.85, 0.95)	0.89 (0.84, 0.94)
Gender					
Female	1.13 (0.56, 2.30)	1.11 (0.49, 2.54)	1.10 (0.48, 2.55)	1.22 (0.52, 2.87)	1.25 (0.52, 2.99)
Male	1	1	1	1	1
CD4 count					
<200	0.45 (0.19, 1.07)	0.63 (0.24, 1.63)	0.63 (0.24, 1.66)	0.63 (0.22, 1.81)	0.69 (0.24, 2.04)
200–500	0.50 (0.25, 0.98)	0.64 (0.31, 1.33)	0.62 (0.30, 1.28)	0.71 (0.33, 1.51)	0.76 (0.35, 1.64)
>500	1	1	1	1	1
HIV RNA					
≤500	1.36 (0.67, 2.78)	1.32 (0.60, 2.87)	1.29 (0.59, 2.81)	1.44 (0.64, 3.25)	1.34 (0.58, 3.09)
>500	1	1	1	1	1
Antiretroviral therapy	1.49 (0.75, 2.99)	1.45 (0.67, 3.14)	1.43 (0.67, 3.04)	1.51 (0.68, 3.32)	1.48 (0.65, 3.35)
BMI	1.05 (0.98, 1.13)	1.09 (1.01, 1.18)	1.09 (1.01, 1.18)	1.07 (0.99, 1,.16)	1.08 (0.99, 1.17)
IL-10					
1st tertile		1			1
2nd tertile		0.65 (0.29, 1.49)			0.66 (0.27, 1.63)
3rd tertile		0.47 (0.20, 1.08)			0.58 (0.22, 1.53)
TNF-α					
1st tertile			1		1
2nd tertile			0.80 (0.36, 1.75)		0.81 (0.34, 1.90)
3rd tertile			0.46 (0.21, 1.02)		0.69 (0.27, 1.76)
IL-6				*b*	
1st tertile				1	1
2nd tertile				1.09 (0.47, 2.56)	1.24 (0.51, 3.00)
3rd tertile				0.43 (0.19, 0.98)	0.54 (0.23, 1.29)

[a]Adjusted for age, gender, CD4 count, HIV RNA, current antiretroviral therapy, and BMI.
[b]Global p-value <0.05.
OR (95% CI), odds ratio (95% confidence interval); BMI, body mass index.

Table 3. Associations Between Hepatitis C Virus, Inflammatory Cytokines, and Presence of Advanced Liver Fibrosis Among HIV-Infected Persons with a History of Alcohol Problems

	Model I[a] *OR (95% CI)*	*Model II*[a] *OR (95% CI)*	*Model III*[a] *OR (95% CI)*	*Model IV*[a] *OR (95% CI)*	*Model V*[a] *OR (95% CI)*
HCV	7.22 (2.01, 25.96)	7.30 (1.90, 28.03)	6.47 (1.72, 24.31)	4.92 (0.96, 25.07)	6.62 (1.20, 36.62)
Age	1.06 (0.99, 1.13)	1.04 (0.97, 1.12)	1.05 (0.98, 1.12)	1.05 (0.96, 1.14)	1.05 (0.96, 1.14)
Gender					
Female	1.28 (0.45, 3.66)	1.47 (0.43, 4.98)	1.23 (0.36, 4.23)	1.56 (0.35, 6.98)	1. 90 (0.37, 9.86)
Male	1	1	1	1	1
CD4 count	*b*	*b*	*b*	*b*	*b*
<200	15.66 (1.72, 142.57)	11.48 (1.10, 119.85)	9.75 (0.96, 99.34)	8.65 (0.65, 115.35)	7.98 (0.52, 122.93)
200–500	12.56 (1.56, 101.14)	13.03 (1.53, 111.07)	11.56 (1.02, 95.66)	9.25 (0.97, 88.59)	11.32 (1.10, 116.58)
>500	1	1	1	1	1
HIV RNA					
≤500	0.65 (0.22, 1.94)	0.70 (0.21, 2.32)	0.68 (0.21, 2.22)	0.77 (0.18, 3.26)	0.83 (0.17, 4.01)
>500	1	1	1	1	1
Antiretroviral therapy	0.98 (0.32, 2.98)	0.87 (0.25, 2.98)	1.00 (0.31, 3.29)	0.96 (0.24, 3.95)	1.10 (0.24, 5.13)
BMI	0.98 (0.87, 1.10)	1.00 (0.88, 1.13)	0.99 (0.87, 1.13)	0.99 (0.85, 1.15)	0.99 (0.85, 1.14)
IL-10					
1st tertile		1			1
2nd tertile		0.41 (0.10, 1.62)			0.28 (0.04, 1.76)
3rd tertile		0.50 (0.14, 1.80)			0.19 (0.02, 1.40)
TNF-α					
1st tertile			1		1
2nd tertile			0.97 (0.25, 3.79)		1.42 (0.20, 10.20)
3rd tertile			1.70 (0.48, 6.01)		2.43 (0.32, 18.15)
IL-6				*b*	*b*
1st tertile				1	1
2nd tertile				1.64 (0.13, 20.28)	2.71 (0.20, 36.87)
3rd tertile				8.16 (0.96, 69.54)	11.78 (1.17, 118.19)

[a]Adjusted for age, gender, CD4 count, HIV RNA, current antiretroviral therapy, and BMI.
[b]Global *p*-value <0.05.
OR (95% CI), odds ratio (95% confidence interval).

In exploratory analyses assessing whether inflammatory cytokines are mediators of the relationship between HCV and liver fibrosis, adjustment for the three inflammatory cytokines simultaneously resulted in some, but not complete, attenuation of the HCV effect [the AOR for the absence of fibrosis attenuated from 0.32 (0.17, 0.57) to 0.47 (0.23, 0.96) and the AOR for advanced fibrosis attenuated from 7.22 (2.01, 25.96) to 6.62 (1.20, 36.62)]. Inclusion of IL-6 appeared to attenuate the HCV effect to a larger degree than IL-10 and TNF-α.

Discussion

In this cohort of HIV-infected patients with alcohol problems, we found that higher levels of IL-6 were associated with the presence of liver fibrosis. IL-6 also appeared to be a partial mediator of the association between HCV infection and liver fibrosis, suggesting an important role promoting fibrosis in the setting of HCV. We did not observe significant associations between TNF-α and IL-10 and liver fibrosis, but results should be cautiously interpreted due to the modest sample size.

Our results are consistent with the hypothesis that in the setting of HIV, inflammation from HCV leads to altered cytokine profiles that are linked to fibrosis. Both HIV and HCV infection are chronic infections that lead to immune stimulation and inflammation.[14] HIV/HCV coinfection could also contribute to an impaired cell-mediated immune response by altering the cytokine environment.[33] IL-6 is a downstream proinflammatory cytokine produced by Th1 lymphocytes that is elevated in HCV infection.[13] Furthermore, higher levels of inflammatory cytokines might be due to increased gut permeability, bacterial translocation, CD4 cell depletion, and loss of Kupffer cells in the liver, factors that have been recently related to fibrogenesis in HIV/HCV coinfection.[34–36] Other researchers have used noninvasive indices to estimate liver fibrosis in unselected HIV-infected patients,[23,37] but, to our knowledge, none has published results concerning the association between serum levels of IL-6, IL-10, and TNF-α and liver fibrosis measured with FIB-4 in HIV-infected patients.

Our findings might partially explain the high rate of liver fibrosis in patients with HIV/HCV coinfection[11,12,38] as well as in HIV-infected patients without viral hepatitis.[23,24] Of note, both age and immune suppression have been widely associated with greater liver injury in HIV/HCV coinfection,[12,39] and in our study those patients with values of FIB-4 consistent with advanced liver fibrosis had significantly lower CD4 counts and were significantly older.

The findings of this study are an important addition to the limited data on inflammatory biomarkers in HIV and HCV infection, as well as in alcoholic liver disease. In a prior study of this cohort, current heavy alcohol use was not significantly associated with Il-6, IL-10, or TNF-α levels.[40] Also, in another study we have shown that different measures of alcohol use (lifetime alcohol consumptions, years of heavy episodic drinking, and current heavy alcohol use) were not associated with noninvasive markers of liver fibrosis.[41]

However, the present study has several limitations. First, we measured levels of IL-6, IL-10, and TNF-α at baseline, and it is possible that fluctuations in cytokine levels might have a greater impact on liver injury. Moreover, serum cytokine levels might not reflect the levels of cytokine levels in other body compartments. Second, we do not have information from liver biopsy, so our results are based on a noninvasive index that has not been validated in HCV-negative/HIV-infected patients. However, it is impractical to justify the systematic use of an invasive procedure such as liver biopsy for research purposes only. Other investigators have used the noninvasive estimation of liver fibrosis in cohorts of HIV-infected patients without hepatitis coinfection so as to estimate the burden of liver disease.[23,42–44] Third, we did not have information regarding the CD4 percentage, so we could not include it as a covariate in the analysis, and the impact of CD4 discordance (low absolute CD4 count with preserved CD4 percentage) could not be evaluated.

In summary, in this cohort of HIV-infected patients with current or past alcohol problems, we found that higher levels of IL-6 were associated with liver fibrosis as measured by FIB-4. Furthermore, it appears that IL-6 may partially mediate the association between HCV infection and liver fibrosis. This study reinforces the link between altered cytokines and fibrosis in HIV-infected patients, and supports the hypothesis that inflammatory cytokines such as IL-6 may play an important causal role in the association between HCV and fibrosis. Further research is needed to elucidate the complex relationships between chronic inflammation and liver fibrosis, and to assess whether inflammatory cytokines such as IL-6 may serve as biomarkers for advanced liver disease.

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Author Disclosure Statement

No competing financial interests exist.

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