

Unhealthy Alcohol Use is Associated with Monocyte Activation Prior to Starting Antiretroviral Therapy

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Background: Alcohol use may accelerate HIV disease progression, but the plausible biological mechanisms have not been clearly elucidated.

Methods: HIV-positive persons who were not on antiretroviral therapy (ART) completed the baseline assessment for a longitudinal study examining the association of alcohol use with HIV disease markers. Oversampling drinkers, baseline samples were tested for markers of monocyte activation (soluble CD14 [sCD14]), inflammation (interleukin-6 [IL-6]), and coagulation (D-dimer). We defined “unhealthy alcohol use” as testing positive using the Alcohol Use Disorders Identification Test—Consumption (≥ 3 for women and ≥ 4 for men) in the past 3 months or testing positive using a biomarker of heavy drinking, phosphatidylethanol (≥ 50 ng/ml). Multiple linear regression was used to examine the associations of unhealthy alcohol use with sCD14, \log_{10} IL-6, and D-dimer.

Results: Compared to those who were abstinent from alcohol, unhealthy drinkers had significantly higher sCD14 levels (mean = 1,676 vs. 1,387 ng/ml; mean difference [95% confidence interval (CI)] = 289 [83, 495], $p < 0.01$). In analyses adjusted for demographic factors, current cigarette smoking, and HIV disease markers, unhealthy drinkers continued to display significantly higher sCD14 levels compared to those who were abstinent from alcohol (adjusted mean = 1,670 vs. 1,406 ng/ml; adjusted mean difference [95% CI] = 264 [47, 480], $p = 0.02$). Unhealthy alcohol use was not significantly associated with IL-6 or D-dimer levels.

Conclusions: Unhealthy alcohol use was independently associated with a marker of monocyte activation (i.e., higher sCD14) that predicts mortality in treated HIV infection. Longitudinal research should examine whether unhealthy alcohol use predicts changes in sCD14 prior to and following ART initiation.

Key Words: Alcohol, HIV/AIDS, Immune Activation, Microbial Translocation.

ALCOHOL IS THE most commonly used recreational drug in the world, yet it remains unclear whether and how unhealthy drinking may be linked to faster HIV disease progression (Hahn and Samet, 2010). Unhealthy alcohol use is associated with frailty, hospitalization, and prevalent cardiovascular disease in HIV-positive persons (Akgun et al., 2013; Freiberg et al., 2010; Womack et al., 2013). Unhealthy drinkers on antiretroviral therapy (ART) are also more likely to have difficulties with adherence, experience treatment

interruptions, and may display elevated HIV viral load (Baum et al., 2010; Conen et al., 2013; Hendershot et al., 2009). Despite these difficulties with HIV disease management, cohort studies with HIV-positive persons receiving ART have provided inconclusive support for an association of unhealthy alcohol use with HIV disease progression via biological pathways (Hahn and Samet, 2010).

Studies with those not yet receiving ART could elucidate the biological pathways whereby unhealthy alcohol use may contribute to morbidity and mortality in HIV-positive persons, independent of ART nonadherence. Understanding these biological processes could support efforts to optimize HIV-related health outcomes in sub-Saharan Africa and other areas of the world where unhealthy drinking is prevalent. The potential deleterious effects of unhealthy drinking prior to initiating ART are particularly relevant in sub-Saharan Africa because many are unaware that they are infected with HIV or are not currently receiving ART (Hahn et al., 2011).

Unhealthy alcohol use may amplify the effects of HIV on translocation of microbial products such as lipopolysaccharide (LPS) across the gastrointestinal tract (Brenchley et al., 2006), which predicts greater immune activation and faster clinical progression among those not receiving ART (Marchetti et al., 2011). In fact, prior research has established that

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higher soluble CD14 (sCD14), a marker of LPS-induced monocyte activation, predicts faster mortality in treated HIV infection (Hunt et al., 2014; Justice et al., 2012). To the extent that unhealthy alcohol use contributes to innate immune activation, this could lead to increases in markers of inflammation such as interleukin-6 (IL-6) and coagulation (i.e., D-dimer) that also predict hastened mortality in HIV-positive persons receiving ART (Justice et al., 2012; Kuller et al., 2008; Tenorio et al., 2014). Understanding the extent to which unhealthy alcohol use potentiates these interacting pathophysiologic processes prior to starting ART could inform efforts to mitigate its potentially deleterious effects on HIV disease progression (Justice, 2011).

This study examined whether unhealthy alcohol use was associated with greater sCD14, IL-6, and D-dimer after adjusting for demographics, cigarette smoking, and HIV disease markers among HIV-positive persons who were not yet receiving ART in rural Uganda. It is also recognized that cirrhosis of the liver, from alcohol or other causes, contributes to impaired filtration of microbial products, which could explain these pathophysiologic processes. Consequently, a secondary aim was to assess possible mediation by the extent of liver disease as assessed by fibrosis-4 (FIB-4) score.

MATERIALS AND METHODS

Study Design and Procedures

HIV-positive persons not yet receiving ART were recruited from the Immune Suppression Syndrome Clinic of the Mbarara University Teaching Hospital in Mbarara, Uganda, for a cohort study (i.e., the Uganda cohort of the Uganda Russia Boston Alcohol Network for Alcohol Research Collaboration on HIV/AIDS [URBAN ARCH]) examining the pathways whereby unhealthy drinking may contribute to HIV disease progression. Adults diagnosed with World Health Organization Stage I or II clinical HIV disease with last T helper (CD4+) cell count >350 cells/mm³ that resided within 60 km of the Immune Suppression Syndrome Clinic and who spoke English or Runyankole were eligible. After completing an informed consent at the enrollment visit, self-report measures were administered and participants provided a peripheral venous blood sample to measure CD4+ T-cell count, HIV viral load, platelet count, and phosphatidylethanol (PEth). All study procedures were approved by the Institutional Review Boards of the University of California—San Francisco, Boston University Medical Center, the Mbarara University of Science and Technology, and the Uganda National Committee on Science and Technology.

This study tested baseline plasma samples from 180 participants to examine whether unhealthy drinking is independently associated with biomarkers of monocyte activation, inflammation, and coagulation. Plasma samples from all 60 participants who reported unhealthy alcohol use were selected. We also selected a random subset of samples (fre-

quency matched for gender) from participants who reported lower risk drinking ($n = 60$) and participants who reported abstaining from alcohol ($n = 60$). All samples with an HIV viral load of 1,000 copies/ml or greater were included. Of the 38 samples with an HIV viral load $<1,000$ copies/ml, 11 that tested negative for HIV antibodies or positive for ART medications (i.e., efavirenz and nevirapine) were excluded. Thus, we conducted this analysis on the 169 confirmed HIV-positive participants who were not yet receiving ART.

Monocyte Activation, Inflammation, Coagulation, and Liver Function

Plasma levels of sCD14 and IL-6 were measured in duplicate using quantitative sandwich enzyme immunoassay methods (R&D Systems, Minneapolis, MN). D-dimer was measured once using an immuno-turbidimetric assay (Liatest D-DI; Diagnostica Stago, Parsippany, NJ). To measure risk factors for cirrhosis, these samples were also tested for aspartate transaminase, alanine transaminase, and hepatitis B virus surface antigen using standard methods.

Alcohol Use

Alcohol use was characterized using the Alcohol Use Disorders Identification Test—Consumption (AUDIT-C) in the past 3 months and PEth levels (Hahn et al., 2012a). PEth was assayed at a commercial laboratory using liquid chromatography paired with tandem mass spectrometry as previously described (Jones et al., 2011). Alcohol use was modeled as a 3-level variable (unhealthy alcohol use, lower risk alcohol use, and abstinent) based on AUDIT-C score and PEth. Unhealthy alcohol use was defined as screening positive using the AUDIT-C (i.e., ≥ 3 for women and ≥ 4 for men) or a PEth of 50 ng/ml or greater, a conservative cutoff for unhealthy drinking to maximize specificity and thereby minimize the likelihood of false-positive results, as noted previously (Stewart et al., 2010). Lower risk alcohol use was operationalized as self-reported drinking or quantifiable PEth levels (PEth ≥ 8 ng/ml), but no evidence of unhealthy alcohol use (i.e., AUDIT-C less than cutoffs and PEth <50 ng/ml). These alcohol-using groups were compared to a reference group that reported being abstinent from alcohol (i.e., AUDIT-C = 0), which was biologically confirmed (i.e., PEth less than the limit of quantitation, 8 ng/ml).

Statistical Analyses

Multiple linear regression analyses were employed to examine whether unhealthy alcohol use was independently associated with higher sCD14, IL-6 (\log_{10}), and D-dimer levels.

For statistical analyses, IL-6 was \log_{10} -transformed due to skewness in its distribution; final results were back-transformed for interpretation. Because D-dimer levels were not

normally distributed after \log_{10} transformation, we fit median regression models (results not shown) in secondary analyses to confirm the results of the multiple linear regression analysis (Hao and Naiman, 2007). Model covariates included the following: demographic factors (i.e., age, gender), socioeconomic status (i.e., asset index), current cigarette smoking, time since HIV diagnosis, HIV disease markers (i.e., CD4+ T-cell count, HIV viral load), and hepatitis B virus coinfection (surface antigen positive). Because liver cirrhosis could partially explain the association of unhealthy alcohol use on these outcomes, we examined whether FIB-4 score differed by alcohol use category to assess whether it should be tested as a mediator.

RESULTS

The majority of participants in this analysis were women (52%) and the median age was 32 years. Most participants (90%) had a CD4+ T-cell count of 350 cells/mm³ or greater and 83% had an HIV viral load of 1,000 copies/ml or greater. As shown in Table 1, unhealthy drinkers were more likely to be current cigarette smokers compared to those who were abstinent from alcohol, $\chi^2(2, N = 169) = 11.10$, $p = 0.004$, and lower risk drinkers were more recently diagnosed with HIV compared to those who were abstinent from alcohol (Kruskal–Wallis test $H(2) = 8.43$, $p = 0.01$).

In unadjusted analyses, unhealthy drinkers displayed significantly higher sCD14 levels compared to those who were abstinent from alcohol (mean = 1,676 vs. 1,387 ng/ml;

mean difference [95% confidence interval (CI)] = 289 [83, 495], $p < 0.01$). However, no statistically significant differences in back-transformed IL-6 (1.62 vs. 1.27 pg/ml; ratio of means [95% CI] = 1.28 [0.91, 1.79], $p = 0.16$), and D-dimer (mean = 0.86 vs. 0.80 ng/ml; mean difference [95% CI] = 0.06 [−0.36, 0.47], $p = 0.79$) levels were observed. In analyses adjusted for participant characteristics (i.e., demographic factors, current cigarette smoking, and HIV disease markers; see Table 2), unhealthy drinkers displayed significantly higher sCD14 levels compared to those who were abstinent from alcohol (adjusted mean = 1,670 vs. 1,406 ng/ml; adjusted mean difference [95% CI] = 264 [47, 480], $p = 0.02$). However, no statistically significant differences in IL-6 (adjusted mean = 1.54 vs. 1.38 pg/ml; adjusted ratio of means [95% CI] = 1.12 [0.78, 1.61], $p = 0.55$) and D-dimer (adjusted mean = 0.51 vs. 0.50 ng/ml; adjusted mean difference [95% CI] = 0.01 [−0.43, 0.46], $p = 0.95$) levels were observed.

When lower risk drinkers were compared to those who were abstinent from alcohol, in adjusted analyses, no significant differences were observed in sCD14 (adjusted mean = 1,500 vs. 1,406 ng/ml; adjusted mean difference [95% CI] = 93.66 [−155.46, 342.79], $p = 0.46$), IL-6 (adjusted mean = 1.09 vs. 1.14 pg/ml; adjusted ratio of means [95% CI] = 0.82 [0.54, 1.25], $p = 0.36$), and D-dimer (adjusted mean = 0.39 vs. 0.50 ng/ml; adjusted mean difference [95% CI] = −0.10 [−0.61, 0.40], $p = 0.69$) levels. Because there were no differences in FIB-4 score by alcohol use group (Fisher's exact test, $p = 0.33$), it was not examined as

Table 1. Demographics, Cigarette Smoking, and Health Status Indicators by Alcohol Use Group ($N = 169$)

	Abstinent ($n = 48$) n (%)	Lower risk drinking ($n = 37$) n (%)	Unhealthy drinking ($n = 84$) n (%)	p -Value
Female	27 (56.3)	22 (59.5)	39 (46.4)	0.33
Asset index				
Lowest	15 (31.3)	14 (37.8)	40 (47.6)	0.10
Middle	26 (54.2)	15 (40.5)	25 (29.8)	
Highest	7 (14.6)	8 (21.6)	19 (22.6)	
Current cigarette smoker	1 (2.1)	3 (8.1)	18 (21.4)	0.004
CD4+ T-cell count (cells/mm ³)				
<350	5 (10.4)	3 (8.1)	9 (10.7)	0.95
350 to 499	15 (31.3)	12 (32.4)	31 (36.9)	
≥500	28 (58.3)	22 (59.5)	44 (52.4)	
HIV viral load (copies/ml)				
≥1,000	38 (80.9)	31 (83.8)	69 (84.2)	0.33
40 to 999	5 (10.6)	6 (16.2)	11 (13.4)	
<40	4 (8.5)	0 (0)	2 (2.4)	
FIB-4 score				
>3.25	3 (6.3)	3 (8.33)	9 (11.1)	0.35
1.45 to 3.25	12 (25.0)	8 (22.2)	29 (35.8)	
<1.45	33 (68.8)	25 (69.4)	43 (53.1)	
Hepatitis B surface antigen positive	4 (8.3)	1 (2.7)	11 (13.1)	0.19
	M (SD)	M (SD)	M (SD)	
Age	32 (28, 39.5)	29 (25, 37)	35 (29, 42)	.09
Time since HIV diagnosis (years)	2.7 (0.6, 6.6)	0.4 (0.1, 4.9)	1.1 (0.1, 6.4)	0.01
Soluble CD14 (ng/ml)	1,387.0 (435.2)	1,456.0 (554.2)	1,675.9 (652.9)	0.01
IL-6 (pg/ml)	1.68 (1.26)	2.85 (7.82)	3.01 (5.33)	0.36
D-dimer (ng/ml)	0.80 (0.73)	0.69 (0.60)	0.86 (1.48)	0.76

Table 2. Multivariate Analyses Examining Associations of Unhealthy Drinking with Soluble CD14 (sCD14), IL-6, and D-Dimer Levels (*N* = 169)

	sCD14 (ng/ml) adjusted mean difference (95% CI)	<i>p</i> -Value	IL-6 (pg/ml) adjusted ratio of means (95% CI)*	<i>p</i> -Value	D-Dimer (ng/ml) adjusted mean difference (95% CI)	<i>p</i> -Value
Alcohol						
Abstinent (Ref)	–	0.05	–	0.29	–	0.88
Lower risk drinking	93.66 (–155.46, 342.78)		0.82 (0.54, 1.25)		–0.10 (–0.61, 0.41)	
Unhealthy drinking	263.80 (47.22, 480.37)		1.12 (0.78, 1.61)		0.01 (–0.43, 0.46)	
Age	3.89 (–7.07, 14.85)	0.48	1.01 (0.99, 1.03)	0.33	0.01 (–0.01, 0.03)	0.34
Female	105.61 (–96.48, 307.69)	0.30	1.06 (0.75, 1.48)	0.75	–0.08 (–0.49, 0.33)	0.70
Asset index						
Highest (Ref)	–	0.88	–	0.80	–	0.28
Middle	63.45 (–188.52, 315.43)		0.87 (0.57, 1.33)		0.31 (–0.21, 0.83)	
Lowest	52.96 (–193.52, 299.45)		0.88 (0.58, 1.34)		0.41 (–0.10, 0.91)	
Current cigarette smoker	–101.74 (–376.89, 173.41)	0.45	0.98 (0.62, 1.55)	0.93	–0.35 (–0.91, 0.21)	0.22
Time since HIV diagnosis (years)	–7.70 (–33.90, 18.50)	0.56	0.97 (0.93, 1.01)	0.14	–0.01 (–0.06, 0.05)	0.78
CD4+ count						
<350 (Ref)	–	0.11	–	0.78	–	0.64
350 to 499	–306.98 (–625.24, 11.29)		0.83 (0.48, 1.41)		–0.30 (–0.95, 0.35)	
≥500	–308.83 (–605.94, –11.71)		0.86 (0.52, 1.42)		–0.17 (–0.78, 0.43)	
HIV viral load (log ₁₀)	129.32 (31.16, 227.48)	0.01	1.24 (1.05, 1.47)	0.01	0.29 (0.08, 0.49)	0.006
Hepatitis B surface antigen positive	15.38 (–303.58, 334.34)	0.92	0.95 (0.56, 1.63)	0.86	–0.46 (–1.11, 0.19)	0.16

IL-6, interleukin 6.

*Represents ratio of means after back transformation from log scale (base 10).

a mediator of the association of unhealthy drinking with greater sCD14.

DISCUSSION

This cross-sectional study examined whether unhealthy alcohol use was associated with surrogate markers of innate immune activation, inflammation, and coagulation among HIV-positive persons not yet receiving ART. Unhealthy drinking was independently associated with greater sCD14 (a marker of LPS-induced monocyte activation) after adjusting for demographics, cigarette smoking, and HIV disease markers. The association of unhealthy drinking with higher sCD14 did not appear to be mediated by the extent of liver disease indexed using FIB-4 score.

Permeability of the gastrointestinal tract in HIV leads to increased microbial translocation and innate immune activation, pathophysiologic processes that are only partially reversed with ART (Hunt et al., 2014). The clinical relevance of surrogate markers of innate immune activation is supported by prior research in which greater sCD14 predicted mortality in treated HIV infection (Hunt et al., 2014; Justice et al., 2012). In sub-Saharan Africa where delayed HIV testing and ART initiation are enduring challenges (Hahn et al., 2011), unhealthy alcohol consumption occurring prior to ART initiation may contribute to more rapid HIV disease progression.

Although unhealthy alcohol use was independently associated with higher sCD14 levels, findings from the present cross-sectional study should be interpreted in the context of some important limitations. Further longitudinal research is clearly needed to examine whether unhealthy drinking predicts changes in these pathophysiologic pro-

cesses and faster HIV disease progression. In addition, a minority of participants displayed relatively low and undetectable HIV viral loads which suggested that they were long-term nonprogressors or elite controllers. This may be due to the fact that this study enrolled only those with World Health Organization Stage I or II clinical HIV disease. The study design may have selected for a greater number of slower progressors. Finally, although unhealthy drinking was not associated with higher plasma IL-6 levels in the present study, further research is needed to examine plasma levels of other pro-inflammatory cytokines and stimulated cytokine production using peripheral blood mononuclear cells.

Despite these limitations, the present cross-sectional investigation is among the first to indicate that unhealthy alcohol use may be independently associated with greater innate immune activation among HIV-positive persons. An important strength of this study was also the use of PEth to mitigate misclassification of unhealthy drinking due to biases inherent in the measurement of self-reported alcohol use (Hahn et al., 2012a). This was needed given our previous studies finding under reporting in this setting (Bajunirwe et al., 2014; Hahn et al., 2012b). Longitudinal studies with objective measurements of alcohol use, such as biomarkers like PEth, are needed to prospectively examine whether distinct patterns of alcohol use predict surrogate markers of monocyte activation, inflammation, and coagulation prior to starting ART as well as following ART initiation. Understanding the drivers of HIV disease progression prior to starting ART is especially relevant in resource constrained settings where unhealthy alcohol use is prevalent and delayed HIV diagnosis and ART initiation are common.

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