IL-1β Levels Are Associated With Chronic Multisite Pain in People Living With HIV

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Background: The pathophysiology of chronic pain experienced by people living with HIV (PLWH) in the current antiretroviral treatment era is poorly understood. We sought to investigate the relationship between inflammation and chronic pain in PLWH. We hypothesized that, among PLWH who have undetectable HIV viral loads, those with chronic multisite pain (CMP) would have higher levels of circulating pain-related inflammatory markers than those without chronic pain.

Setting: This study was conducted at the University of Alabama at Birmingham's Center for AIDS Research Network of Integrated Clinical System site.

Methods: We compared inflammatory markers in 70 PLWH with CMP and 70 PLWH without chronic pain. Custom multiplex human inflammatory assays were completed on banked plasma specimens to measure cytokines commonly associated with chronic inflammatory pain: interleukin 1β (IL-1β), eotaxin, IL-15, IL-6, tumor necrosis factor α, and leptin. Logistic regression models were built using group status (CMP vs no pain) as the outcome variable, with each cytokine as independent variables and age, sex, substance use, and prescribed opioid medications as covariates.

Results: Participants were mostly men (71%); 53% were 50 years or older. The most common sites of pain were low back (86%), hands/feet (81%), and knee (66%). Median CD4+ T-cell count was 676 cells per milliliter. IL-1β was significantly higher in the CMP group than in the individuals without chronic pain (odds ratio: 1.35, 95% confidence interval: 1.01 to 1.82, $P < 0.05$). Eotaxin, IL-15, IL-6, tumor necrosis factor α, and leptin were not significantly different between groups.

Conclusions: We found that PLWH who also have CMP have significantly higher levels of IL-1β than PLWH who do not have any pain. Future work on the role of IL-1β on chronic pain pathogenesis in this population may inform novel approaches to chronic pain management.

Key Words: HIV, chronic pain, inflammation, IL-1β, chronic multisite pain

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INTRODUCTION

Chronic pain is a common chronic illness associated with substantial functional impairment.[1] Defined as pain lasting >3 months, chronic pain affects approximately 15% of the US population.[2,3] It often occurs in patients with complex chronic illness, including medical, psychiatric, and substance use comorbidities.[4] However, chronic pain is not simply a symptom of these comorbidities. Its distinct neurobiologic basis and substantial impact on physical and emotional function make it a serious illness in itself.[1] The Institute of Medicine[5] and recent National Pain Strategy[1] have identified research on chronic pain, particularly in populations most affected, to be a priority.

The burden of chronic pain in people living with HIV (PLWH) is substantial, with prevalence estimates ranging from 39% to 85%.[6,7] Chronic pain in PLWH includes the classically described syndromes of HIV and avascular necrosis, as well as other regional musculoskeletal pain and multisite pain.[8,9] Mounting evidence suggests that chronic pain in PLWH may be associated with worse HIV outcomes, including suboptimal adherence to antiretroviral therapy[10] and retention in HIV primary care,[6] and has serious health consequences, including up to 10 times greater odds of functional impairment[11] and increased health care utilization.[9]

Despite the importance of chronic pain in PLWH, there has been little research in this area. Specifically, the reasons why PLWH have a high burden of chronic pain are unknown. The pathophysiology of HIV neuropathy has been well-described and includes activation of macrophages around peripheral neurons.[12,13] However, a large burden of chronic pain in PLWH in the current treatment era is musculoskeletal and not directly attributable to peripheral neuropathy.[9] Additionally, multisite pain is common.[9] To illustrate, in one study,[7] the median

number of pain locations was 5. Therefore, investigating the pathophysiology of chronic pain in PLWH is a critical next step in both pain prevention and treatment.

Systemic inflammation that is generalized to the central nervous system may be an important driver of chronic pain in PLWH. The HIV envelope protein GP120 has been shown to stimulate cellular and molecular changes in the glial cells that support the central nervous system, a process referred as gliosis.[14] Once these glial cells are activated as part of the gliosis process, they release large amounts of proinflammatory cytokines, including tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and IL-6.[15] Glial activation and concomitant inflammation have been implicated in the development and perpetuation of various chronic pain conditions.[16] Accordingly, elevated levels of the proinflammatory cytokines TNF-α and IL-1β have been found in the spinal dorsal horn of PLWH who have chronic pain.[13] Peripherally circulating levels of cytokines have not been investigated in PLWH, even though they may represent a more accessible proxy measurement of central inflammation. Peripheral markers of inflammation are higher in PLWH than in the general population, even when they are treated with antiretroviral therapy and achieve virologic suppression.[17]

To expand on the small amount of existing evidence, our aim was to investigate the relationship between inflammation and chronic pain in PLWH. We hypothesized that PLWH who reported chronic pain would have higher levels of circulating pain-related inflammatory markers than PLWH without chronic pain.

METHODS

This study was conducted at the University of Alabama at Birmingham's Center for AIDS Research Network of Integrated Clinical System (CNICS) site. All procedures were approved by the Institutional Review Board of the University of Alabama at Birmingham. Pain Patient Reported Outcome (PRO) questionnaires were part of CNICS between July 2015 and June 2016. The pain PROs consisted of a Brief Chronic Pain Questionnaire that asks about pain intensity and duration, which has undergone validation work in PLWH.[18,19] Individuals with moderate or greater pain for at least 3 months went on to additional questions. These included the 3-item PEG,[20] a well-validated questionnaire about pain severity and its impact on enjoyment of life and general activities on a scale of 0–10, where 0 is "no pain" and 10 is "pain as bad as you can imagine," and a pain location questionnaire that included a drop-down menu of the following pain locations: numbness or tingling in hands and/or feet; headache; abdominal pain; low back pain; hip pain; shoulder pain; knee pain; pain everywhere in your body, other please specify. Participant responses to other PROs, including those that measure depression [Patient Health Questionnaire 9 (PHQ-9)[21]] and the use of anti-inflammatory medications (statins, steroids, and nonsteroidal anti-inflammatory drugs) were collected.

Two groups were selected for this study: a PLWH group with chronic pain (n = 70) and a PLWH group without chronic pain (n = 70). Individuals were selected from a CNICS database query between July 2015 and June 2016. Individuals

were identified as having chronic multisite pain (CMP) if they reported pain in 3 or more body sites. Individuals were eligible for the no pain group if they responded "none" to the pain intensity question of the Brief Chronic Pain Questionnaire. If participants had more than 1 PRO during that time, we required them to have the same pain status (CMP or no pain) at every measurement.

Because of the relationship between inflammation and viral load, participants were limited to those with undetectable viral loads (<400 copies/mL). Additionally, the 2 groups were frequency matched based on 2 factors strongly associated with both inflammation and chronic pain: age (≥50 vs <50 years) and sex.

Custom Meso Scale Discovery (Meso Scale Diagnostics, Rockland, MD) multiplex human inflammatory assays were completed on banked cryopreserved plasma specimens to measure cytokines most commonly associated with both HIV and chronic pain: IL-1β, eotaxin, IL-15, IL-6, TNF-α, and leptin.[16,22] Analytes were assayed using an electrochemiluminescence immunoassay technique per the manufacturer's protocol and analyzed on a Meso Scale Discovery Sector Imager 2400 (MSD, Rockville, MD). IL-1β, IL-6, and TNF-α were analyzed simultaneously in a 3-plex analysis. Eotaxin and IL-15 were measured in separate single-plex analyses. All were measured according to the manufacturer's instructions in duplicate and results are reported in picograms per milliliter. Leptin was analyzed by radioimmunoassay using EMD Millipore RIA kits (EMD Millipore, Billerica, MA) in duplicate. Results are reported in nanograms per milliliter.

Logistic regression models were built using group status (CMP vs no pain) as the outcome variable, with each cytokine as independent variables and age and sex as covariates. A statistical threshold level of $P < 0.05$ was set for all analyses.

We also considered other potential confounders of the relationship between cytokine levels and pain group. Opioids can modulate immune system activity[23] and can substantially impact the experience of pain.[24] Additionally, activation of mesolimbic reward structures by current substance use (eg, cocaine, amphetamine, nonmedical use of opioids) is known to engage descending pain modulatory systems that decrease pain.[25] Although the relationship between being prescribed opioid pain medications, current substance use, and circulating levels of inflammatory markers is less clear, we constructed models that include these variables as potential confounders.

Finally, chronic pain and depression commonly occur together; pain may lead to depression and vice versa.[26] Given the known association between depression and inflammation, we planned exploratory analyses incorporating depressive symptoms into models with significant results. To do this, we added the PHQ-9 total score to the model as a continuous variable, given that both clinical and subclinical depressive symptoms have been associated with elevated inflammatory markers.[27,28]

RESULTS

Plasma samples from 70 PLWH with CMP and 70 PLWH without chronic pain were analyzed. Ninety-four percent of samples were obtained on the same day as the

TABLE 1. Sample Characteristics

Characteristic	Chronic Multisite Pain (N = 70)	No Pain (N = 70)	P*
Age ≥50 yrs, N (%)	37 (52.9)	37 (52.9)	1
Female sex, N (%)	22 (31.4)	18 (25.7)	0.57
Race, N (%)			0.93
Black	40 (57.1)	38 (54.3)	
White	29 (41.4)	31 (44.3)	
Other/unknown	1 (1.4)	1 (1.4)	
CD4+ T-lymphocyte count, median (IQR), cells/mL	624 (435–889)	688 (408–879)	0.98
PHQ-9 ≥10 (moderate or greater depressive symptoms), N (%)	33 (48.5)	0 (0)	<0.0001
PHQ-9 total score, median (IQR)	9 (5–14)	0 (0–2)	<0.0001
PHQ-9 somatic score, median (IQR)	5 (2.5–8)	0 (0–1)	<0.0001
PHQ-9 nonsomatic score, median (IQR)	4 (1–6.5)	0 (0–0)	<0.0001
Substance use (cocaine, methamphetamine, nonmedical use of opioids)			0.02
Current	9 (14.3)	5 (7.6)	
Prior	27 (42.9)	17 (25.8)	
Never	27 (42.9)	44 (66.7)	
Prescribed anti-inflammatory medications, N (%)			
Statins	30 (42.9)	24 (34.3)	0.39
Corticosteroids	3 (4.3)	0 (0)	0.24
Nonsteroidal anti-inflammatory drugs	19 (27.1)	9 (12.9)	0.06
Prescribed opioid medications	21 (30.0)	1 (1.4)	<0.0001
Pain locations (not mutually exclusive, does not include "pain everywhere"), median (IQR)	4 (3–5)		
Numbness/tingling in hands and/or feet, N (%)	57 (81.4)		
Headache, N (%)	34 (48.6)		
Abdominal pain, N (%)	25 (35.7)		
Low back pain, N (%)	60 (85.7)		
Hip pain, N (%)	26 (37.1)		
Shoulder pain, N (%)	38 (54.3)		
Knee pain, N (%)	46 (65.7)		
Pain "everywhere in your body"	23 (32.9)		
Cytokines			
IL-1β	0.63 (0.05–1.77)	0.15 (0.05–0.64)	0.02
IL-6	0.72 (0.44–1.35)	0.65 (0.44–0.98)	0.35
TNF-α	2.90 (2.12–3.74)	2.66 (2.13–3.49)	0.45
Eotaxin	134 (103–209)	126 (91–188)	0.09
IL-15	2.47 (1.92–3.25)	2.39 (1.92–2.92)	0.35
Leptin, ng/mL	20.0 (11.4–39.2)	18.2 (9.7–30.0)	0.46

Missing data: CD4+ T-cell count 12, PHQ-9 3, PHQ-Anxiety 1, substance use 11.

*Wilcoxon rank sum test for continuous variables and Fisher exact test for categorical variables. All assay results are reported in nanograms per milliliter.

IQR, interquartile range.

pain questionnaires; the remainder occurred no more than 91 days before the pain questionnaires. See Table 1 for sample characteristics. Participants were mostly men (71%); 53% were 50 years or older. The most common sites of pain were low back (86%), numbness/tingling in hands/feet (81%), and knee (66%). Median CD4+ T-cell count was 676 cells per milliliter. Among individuals with CMP, the median score on the 3-question PEG was 7.2 (interquartile range: 6.0–8.7) on a 0–10 scale. More individuals with CMP had depressed mood than individuals without pain; there were no differences in race, CD4+ T-cell count, or prescription of anti-inflammatory medications.

The logistic regression models investigating the association between cytokine levels and CMP vs no pain reached statistical significance for IL-1β only. No significant differences were found for IL-6, TNF-α, eotaxin, IL-15, or leptin. Therefore, Table 2 focuses on presenting the univariate and multivariable models for IL-1β only.

The odds ratio (OR) for IL-1β was significant in univariate model [OR: 1.33, 95% confidence interval (CI): 1.44 to 1.72], and the multivariable models incorporating age and sex (OR: 1.34, 95% CI: 1.04 to 1.72), and age, sex, current opioid prescription, and current substance use (OR: 1.35, 95% CI: 1.01 to 1.80). Incorporating the PHQ-9 total

TABLE 2. IL-1β Logistic Regression Models for Pain Group (Event = Chronic Multisite Pain)

	UV (OR, 95% CI)	MV Model 1 (OR, 95% CI)	MV Model 2 (OR, 95% CI)	MV Model 3 (OR, 95% CI)
IL-1β, ng/mL	1.33 (1.04 to 1.71)*	1.34 (1.04 to 1.72)*	1.35 (1.01 to 1.80)*	1.09 (0.74 to 1.62)
Age ≥50 vs <50, yrs		1.03 (0.52 to 2.06)	0.81 (0.35 to 1.88)	0.98 (0.28 to 3.38)
Sex male vs female		0.73 (0.34 to 1.56)		1.47 (0.31 to 7.13)
Substance use†				
Current vs never		—	3.09 (0.84 to 11.43)	5.39 (0.76 to 38.55)
Prior vs never		—	2.61 (1.06 to 6.44)*	1.29 (0.31 to 5.27)
Prescribed opioid medications			31.60 (3.93 to 253.88)*	35.25 (3.10 to 400.30)*
Depression (PHQ-9)			—	1.85 (1.44 to 2.38)*

*P < 0.05.
†Cocaine, methamphetamine, nonmedical use of opioid by any route (eg, oral, intravenous). Does not include marijuana.
MV, multivariate; UV, univariate.

score reduced the OR for IL-1β (OR: 1.09, 95% CI: 0.74 to 1.62). PHQ-9 total score was significant in the model (OR: 1.85, 95% CI: 1.44 to 2.38).

To examine if the primary analyses missed any important nonlinear relationships, we fit additional models with cytokines divided into quartiles. The resulting odds ratios were graphically examined and did not indicate any nonlinear relationships.

DISCUSSION

We investigated the relationship between chronic pain and circulating levels of inflammatory cytokines among PLWH. We found that individuals with CMP had significantly higher levels of IL-1β than PLWH who did not report pain, even after adjusting for current opioid prescription and current substance use. Additional analyses raise important questions about the roles of depression in this relationship. These findings are an important starting place for future work on the role of IL-1β on chronic pain pathogenesis in this population.

There is strong evidence for the relationship between IL-1β and various chronic pain conditions. IL-1β is strongly implicated in a range of autoinflammatory diseases (eg, familial Mediterranean fever) and autoimmune-mediated diseases (eg, rheumatoid arthritis, type II diabetes).[29] IL-1β has been shown to potentiate pain in the periphery[30] and maintain chronic pain including peripheral neuropathy and low back pain, both types of pain common among PLWH.[31,32] It also has several actions in the central nervous system, including potentiating a proinflammatory state in microglia, causing increased pain severity.[33] In animals, administration of IL-1β results in a hypersensitive pain state.[34] This evidence suggests that IL-1β operates mainly via central channels to cause increased pain sensitivity (hyperalgesia) and pain in the presence of nonpainful stimuli such as light touch (allodynia) and to maintain chronic pain states over long periods.

HIV infection has been associated with elevated IL-1β levels.[35] Furthermore, IL-1β levels have been associated with HIV-associated neurologic disorder symptom severity, and elevated IL-1β levels have been found in the cerebrospinal fluid of PLWH who have developed dementia.[36] Therefore, we hypothesize that the robust IL-1β response among PLWH may lead to passage of IL-1β through the blood–brain barrier, upregulation of microglia/astrocyte pre-inflammatory activity, and upregulation of pain signals. This could cause pain development, exacerbation, and/or maintenance. Our finding that this association is diminished when depression is added to the model is hypothesis generating—for example, depression may be on the causal pathway between inflammation and chronic pain.[37] Notably, the literature on the association between IL-1β levels and depression has been mixed.[27,38] These findings require further investigation.

Despite the promise of our findings, we note that only 1 of the 7 studied analytes reached significance in our tests. There is therefore a chance that the IL-1β result is a false-positive. The results of this study should be cautiously interpreted until repeated in an independent data set.

Our study has limitations. First, we only measured a small set of analytes intended to broadly query inflammatory pathways. We note that the measured immune components modulate each other in complex ways; this study did not investigate these inflammatory pathways or how they might interact. Hepatitis C can also cause inflammation; however, hepatitis C infection status was not readily available. Additionally, peripheral levels of inflammatory markers are limited by their short half-life, which may fluctuate based on the patient's current pain state, and are a surrogate for tissue levels.

We hope this study is the first in a new body of research on the role of inflammation, particularly IL-1β, in the pathogenesis, treatment, and prevention of chronic pain in PLWH. If inflammation leads to the development of CMP among PLWH, agents that block IL-1β could play an important role, as others have also proposed for chronic pain in general.[16]

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