3-26-2020

Gut Integrity, Microbial Translocation, Immune Activation, Inflammation and Vitamin D in Drug Users Living with HIV

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GUT INTEGRITY, MICROBIAL TRANSLOCATION, IMMUNE ACTIVATION, INFLAMMATION AND VITAMIN D IN DRUG USERS LIVING WITH HIV

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

DIETETICS AND NUTRITION

by

Jacqueline Hernandez

2020

To: Dean Tomas Guilarte
Robert Stempel College of Public Health and Social Work

This dissertation, written by Jacqueline Hernandez, and entitled Gut Integrity, Microbial Translocation, Immune Activation, Inflammation and Vitamin D in Drug Users Living with HIV, having been approved in respect to style and intellectual content, is referred to you for judgment.

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Date of Defense: March 26, 2020

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Florida International University 2020

DEDICATION

First and foremost, I thank my parents, Carlos and Jacqueline Hernandez for their love, encouragement and support throughout my life. They always believed in me and in my passion for learning and academic success. They are the best examples of hard work, honesty and humility that I have ever had.

A special feeling of gratitude to my loving wife, Juliet who gave me an incredible support and unconditional love every single day of my PhD journey. She was always there when I needed her the most. To my entire family and friends for showing their understanding, admiration and support.

ACKNOWLEDGMENTS

This dissertation work would not have been possible without the assistance, support, and guidance of my committee members. I am very grateful to Dr. Baum, my Major Professor, who provided an excellent guidance and support during the entire process. I will never forget all the time and effort you invested in making sure I succeeded. Thank you for giving me the precious opportunity to work in your research and for allowing me to have access to your research resources for my dissertation. Dr. Campa, thank you for your incredible help with my dissertation supplies, support and guidance during my PhD journey. I am grateful to both of you, Dr. Baum and Dr. Campa, for giving me the honor of learning from you and teaching me how to conduct outstanding research. You are wonderful examples of successful and hardworking researchers, who always thrive for excellence. Dr. Huffman, thank you for always having an open-door policy, for your kindness and willingness to always listen. You always made feel welcome and supported. Thank you, Dr. George for your help and expertise. You always were patience and kind during this journey.

I would also like to acknowledge Dr. Sabrina Martinez who has served as a special mentor and inspiration in my PhD journey and professional career, and to the MASH Cohort research team for their collaborative effort and support during this process.

ABSTRACT OF THE DISSERTATION

GUT INTEGRITY, MICROBIAL TRANSLOCATION, IMMUNE ACTIVATION, INFLAMMATION AND VITAMIN D IN DRUG USERS LIVING WITH HIV

by

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Florida International University, 2020

Miami, Florida

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The purpose of this study was to examine associations between MT, immune activation, inflammation, gut integrity and vitamin D status in the context of cocaine use among People Living with HIV (PLWH). One hundred cocaine and non-cocaine users living with HIV were selected from the Miami Adult Studies in HIV cohort. Blood samples were collected to assess MT (LPS), immune activation (sCD163 and sCD27), inflammation (IL-6, TNF-α, hs-CRP, IL-17 and IL-22), gut integrity damage (I-FABP) and vitamin D status. Data on socio-demographic characteristics, anthropometrics, diet and disease progression were collected. Descriptive statistics, independent t-test, multiple linear and logistic regressions were conducted to analyze the data.

The non-cocaine group had a greater proportion of participants (66%, n=66), than cocaine group (34%, n=34). For the two groups, mean age was 53.37±6.88 years old, most participants were male (51%), and African Americans (66%). All participants were on ART and had controlled viral load (<200 copies/mL). Half of the participants were considered vitamin D deficient. Cocaine users had higher levels of MT and I-FABP than non-users (P<0.001 and P=0.014). Cocaine use was a associated with MT (LPS)

(P=0.048), immune activation (sCD163, P<0.001 and sCD27, P=0.03) and gut integrity damage (I-FABP) (P=0.042). Cocaine users had 6.66 times more likely to exhibit high MT levels than non-users (OR: 6.65 95% CI: 2.42, 18.25; P<0.001). In vitamin D deficient participants, those who used cocaine had higher MT levels than non-users (P=0.043). In cocaine users, alcohol use and BMI were associated with inflammation (P=0.037 and P=0.045). Cocaine users with intestinal inflammation, had 3.42 times higher odds to exhibit high levels of gut integrity damage than cocaine users without intestinal inflammation (OR: 3.42 95% CI: 1.07, 10.90; P=0.037). Vitamin D status was not associated with immune activation, inflammation and gut integrity damage in our participants.

Our data suggested that cocaine use was associated with MT, immune activation, inflammation and the damage to the gut integrity in PLWH despite ART use and viral suppression. Therefore, there is a need to develop effective interventions that target gut health and counseling on drug abuse, to improve MT and immune activation among PLWH.

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ABBREVIATIONS AND ACRONYMS

AIDS Acquired Immune Deficiency Syndrome

ART Antiretroviral Therapy

BMI Body mass index

CCR5 C-C chemokine receptor type five

CD4+ T cells Cluster Differentiation T helper cells

CVD Cardiovascular disease

ELISA Enzyme Linked Immunosorbent Assay

EndoCab Endogenous endotoxin-core antibodies

GALTs Gut-Associated Lymphoid Tissues

GI Gastrointestinal

HIV Human Immunodeficiency Virus

hs-CRP Highly sensitive C-reactive protein

I-FABP Intestinal Fatty Acid Protein

IL-6 Interleukin 6

IL-17 Interleukin 17

IL-22 Interleukin 22

INRs Immunological Non-Responders

LAL Limulus amebocyte lysate

LBP Lipopolysaccharide Binding Protein

LPS Lipopolysaccharide

MT Microbial Translocation

NOD Nucleotide-Binding Oligomerization Domain

PBMCs Peripheral Blood Mononuclear Cells

PLWH People Living with HIV

sCD14 Soluble CD14

sCD27 Soluble CD27

sCD163 Soluble CD163

TLR Toll-like receptor

TNF-α Tumor necrosis factor alpha

DBP Vitamin D-binding protein

VDR Vitamin D Receptor

ZO Zonula occluden

GUT INTEGRITY, MICROBIAL TRANSLOCATION, IMMUNE ACTIVATION, INFLAMMATION AND VITAMIN D IN DRUG USERS LIVING WITH HIV

CHAPTER I: INTRODUCTION

Statement of Problem

The Centers for Disease Control and Prevention ranks Florida as first among 50 states in the number of Human Immunodeficiency Virus (HIV) diagnosis in 2017.[1] Miami-Dade County alone accounts for 27,969 presumed living HIV cases as of June 2018, posing it as one of the geographical areas with the highest HIV rates in the nation.[2]

HIV infection is characterized for destruction of immune system cells, generating a broad range of physio pathological consequences that affects all organ systems, in particular the gastrointestinal tract.[3]

The gastrointestinal (GI) tract mucosa is considered one of the most active and important tissues of the immune system. The anatomical, histological and physiological characteristics of the GI mucosa provide a barrier against microorganisms. The enterocyte is the basic unit of the GI mucosa, serving as the single layer of cells for absorption of nutrients, water, and protection from external pathogens. Therefore, preserving the integrity of the GI mucosa is an essential task for the human body and the immune system.[4]

The integrity of the GI mucosa is affected by several diseases, including HIV.[4] Studies revealed that People Living with HIV (PLWH) develop dramatic changes in the gut, debilitating structures of the enterocyte and creating a dysbiotic microbiome. These changes promote the passage of GI microflora or its products from the GI tract into the systemic circulation, a process called Microbial Translocation (MT); immune activation,

inflammation and ultimately HIV disease progression.[5–17] Cumulative evidence demonstrated that products of MT, specifically Lipopolysaccharide (LPS) induce a massive immune activation as assessed by soluble CD14 (sCD14), and inflammation, as measured by pro-inflammatory cytokines Tumor Necrosis Factor- alpha (TNF-α) and C-reactive protein (CRP), which are considered hallmarks in predicting clinical outcomes and disease progression during HIV infection regardless of Antiretroviral Therapy (ART) use.[18–24]

Vitamin D plays an essential role in several physio pathological processes since Vitamin D Receptor (VDR) is expressed in many areas of human body.[25] VDR is highly expressed in the intestine[26] and it regulates transcellular calcium transport, protects mucosa barrier integrity and controls mucosal inflammation.[27] Vitamin D deficiency has been linked to reduced expression of VDR in the intestine, intestinal inflammation and permeability in patients with inflammatory bowel disease.[28] In PLWH, vitamin D deficiency prevalence ranges from 70 to 85% in U.S.[25,29] affecting not only compromised immunity but also gut integrity in this population.

Studies on drug use have reported that 40% of PLWH used illicit drugs in the preceding 12 months and 12% had screened positive for drug dependence.[30–32] Cocaine is considered one of the most addictive and abused illicit drugs among PLWH.[33] Drug abuse, particularly crack-cocaine, seem to accelerate HIV disease progression by reducing CD4+ cell count and increase HIV viral load, promoting immunosuppression, liver fibrosis, and altering microbiome composition in PLWH.[34–38] The above evidence may suggest that PLWH who use illicit drugs and have an impaired vitamin D status have

higher risk for disruption of gut integrity, increased MT and immune activation. However, no substantial evidence exists to show association among gut integrity, MT, immune activation, inflammation and vitamin D status in the context of drug abuse among PLWH. This study examined these associations providing evidence for the development of adjunct therapies for the management of the co-occurring HIV and illicit drug abuse, gut health and nutrition. To the best of our knowledge, this is the first study assessing relationship among gut integrity, MT, immune activation, inflammation and vitamin D status in cocaine users living with HIV.

Significance of the study

Several lines of evidence showed that HIV poses unfavorable consequences for GI mucosa promoting MT and immune activation.[18–21] Chronic and persistent immune activation is documented to be a reliable predictor of HIV disease progression regardless of ART use.[39,40] Illicit drug abuse, particularly cocaine, worsen the immune system and deteriorates nutritional status during HIV infection.[41–43] Vitamin D deficiency is highly prevalent among PLWH[25,29] and due to its relationship with gut integrity, some studies have shown association among vitamin D deficiency, MT and immune activation in PLWH.[44–47]

Currently, understanding the interactions of multiple elements that promote immune activation and inflammation continue to be a major goal toward improving health and lifespan in PLWH. Therefore, this study is significant because it assessed the relationships among key elements such as gut integrity, MT, immune activation, inflammation and vitamin D status in drug users living with HIV, which eventually could

postulate new strategies to target cocaine abuse, gut health and nutrition as important factors in the management of immune activation and inflammation during HIV.

The evidence from the literature generates the following questions:

- Is cocaine use associated with MT, immune activation, and inflammation among PLWH despite ART use and virologic control?
- Is cocaine use associated with poor gut integrity among PLWH despite ART use and virologic control?
- Is cocaine use associated with an impaired vitamin D status among PLWH?

Innovation

There is enough scientific evidence that support the mechanisms behind MT and immune activation as key factors for HIV disease progression.[39,40] The role of vitamin D in gut integrity and immune system in HIV infection is less known[25] despite deficiency of this essential vitamin being highly prevalent in this population.[29] However, the interplay among MT, immune activation, inflammation and vitamin D status in the context of drug abuse have not been investigated. To the best of our knowledge, this is the first study incorporating cocaine abuse as a key player in the deterioration of gut integrity, MT, immune activation, inflammation and vitamin D status in PLWH, which ultimately could provide additional evidence to support the development of therapies to target gut health and substance abuse programs to ameliorate the persistent immune activation and inflammation among PLWH.

AIM FOR CHAPTER III: ASSOCIATIONS BETWEEN MICROBIAL TRANSLOCATION, IMMUNE ACTIVATION AND VITAMIN D STATUS IN DRUG USERS LIVING WITH HIV

Specific Aim 1: to assess markers of MT, immune activation and vitamin D status in cocaine users living with HIV.

Hypothesis 1a: Cocaine users living with HIV will have higher levels of MT, as measured by Lipopolysaccharide (LPS), and immune activation as measured by sCD14, sCD163 and sCD27, than cocaine non-users living with HIV.

Hypothesis 1b: Cocaine users living with HIV, who have vitamin D deficiency or insufficiency, will have higher levels of MT, as measured by Lipopolysaccharide (LPS), and immune activation as measured by sCD14, sCD163 and sCD27, than cocaine non-users living with HIV

AIM FOR CHAPTER IV: MICROBIAL TRANSLOCATION, COCAINE USE AND OBESITY ARE ASSOCIATED WITH INFLAMMATION AMONG DRUG USERS LIVING WITH HIV

Specific Aim 2: To assess markers of inflammation and vitamin D status in cocaine users living with HIV.

Hypothesis 2a: Cocaine users living with HIV will have higher levels of inflammation, as measured by IL-6, TNF-α and high sensitive C-reactive protein (hs-CRP) than cocaine non-users living with HIV.

Hypothesis 2b: Cocaine users living with HIV, who have vitamin D deficiency or insufficiency, will have higher levels of inflammation, as measured by IL-6, TNF-α and high sensitive C-reactive protein (hs-CRP) than cocaine non-users living with HIV

AIM FOR CHAPTER V: GUT INTEGRITY, INTESTINAL INFLAMMATION AND VITAMIN D STATUS IN DRUG USERS LIVING WITH HIV

Specific Aim 3: To assess marker of gut integrity and vitamin D status in cocaine users living with HIV.

Hypothesis 3a: Cocaine users living with HIV will have reduced gut integrity, as measured by Intestinal Fatty Acid Binding Protein (I-FABP), Interleukin 17 (IL-17), and Interleukin 22 (IL-22) than cocaine non-users living with HIV.

Hypothesis 3b: Cocaine users living with HIV, who have vitamin D deficiency or insufficiency, will have reduced gut integrity, as measured by Intestinal Fatty Acid Binding Protein (I-FABP), Interleukin 17 (IL-17), and Interleukin 22 (IL-22) than cocaine non-users living with HIV.

Sample Size

The study recruited 100 participants from the Miami Adults Studies in HIV (MASH) Cohort Study in the FIU-Borinquen Research clinic who are currently participating in an observational study, which investigates the impact of cocaine use on HIV infection, HIV/HCV co-infection and long-term morbidity with a focus on liver disease, directed by P.I. Dr. Marianna K. Baum.

Statistical Analyses

Table 1 describes the dependent and independent variables and statistical analyses conducted in each chapter for all hypotheses.

Table 1. Statistical Analyses of Hypotheses

Hypothesis	Dependent Variable	Independent variables	Statistical Analyses
Chapter III Hypothesis 1a	MT levels as measured by LPS Immune activation as measured by sCD14, SCD27 and sCD163	Cocaine use, gender, age, CD4 T cell, HIV viral load, BMI	T-test for independent samples, multiple linear regression, Pearson correlation
Chapter III Hypothesis 1b	MT levels as measured by LPS above median Immune activation as measured by sCD14, SCD27 and sCD163 above median	Cocaine use, gender, age, vitamin D status, obesity, seasonality	T-test for independent samples. Logistic regression
Chapter IV Hypothesis 2a	Inflammation as measured by IL-6, TNF-α and hs-CRP	Cocaine use, gender, age, CD4 T cell, HIV viral load, BMI	T-test for independent samples, multiple linear regression, generalized linear model. Pearson correlation
Chapter IV Hypothesis 2b	Inflammation as measured by hs-CRP >3mg/dl	Cocaine use, gender, age, vitamin D status, obesity, seasonality	T-test for independent samples. Logistic regression
Chapter V Hypothesis 3a	Gut integrity as measured by I-FABP levels, IL-17 and IL-22	Cocaine use, gender, age, CD4+ T cell count, BMI	T-test for independent samples, multiple linear regression, Pearson correlation
Chapter V Hypothesis 3b	Gut integrity as measured by I-FABP above median	Cocaine use, gender, age, CD4+ T cell count, BMI, vitamin D status	T-test for independent samples, logistic regression, generalized linear model

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CHAPTER II: LITERATURE REVIEW

Gut integrity

The mucosal barrier is considered a complex anatomical structure that separates the internal milieu from the luminal intestine.[1,2] This anatomical structure represents a physical barrier with a cellular component that is essential for protection and function of the intestine. Intestinal barrier and intestinal permeability address different aspects of the mucosal barrier.[2] It constitutes a structure that divides intestinal lumen from the internal host in order to provide a shield against microorganism invasion.[3] The barrier contains mechanical elements (mucus and epithelial cell layer) and humoral and immunological factors (immunoglobulin A, lymphocytes, innate immune cells).[3]

Intestinal permeability, on the other hand, is known to be a measurable feature of the intestinal barrier,[2] that in a sense, represents the level of diffusion through the intestinal wall of large molecules. It has been shown that when the intestinal barrier is damaged, intestinal permeability increases, allowing translocation of bacterial products into the portal vein, causing endotoxemia and inflammation.[2] Intestinal epithelium is a key element of the intestinal barrier. It consists of a single-cell layer structure with an estimated surface area of 30 m^2 that maintains gut homeostasis, provides protection against pathogens, and plays a central role in intestinal immunity.[4–6]

The intestinal epithelium structure has a high cell turnover activity, renewing its cells every week. This cell renewal and differentiation is controlled by intestinal Cluster Differentiation T helper Cells (CD4+ T).[4,7] Cells composing the intestinal epithelium may be differentiated into 4 cell lineage including absorptive cells or enterocytes, Goblet

cells that produce mucus, chemokines and cytokines, entero-endocrine cells that produce hormones, and Tuft and Paneth cells that manage mucosal immunity response.[8,9]

The intestinal immune system is considered a specialized and multifaceted structure that has a substantial amount of organized lymphoid tissue. It contains more lymphocytes than any other anatomical area in the body, particularly CD4+ T cells.[4,10] As mentioned previously, CD4+ T cells play a critical role in intestinal cell renewal and pathogen elimination.[11] Cells in the lamina propia synthetize immunoglobulin A which helps in recognition of bacteria, and innate lymphoid cells that provide a defense line against microorganisms.[4,10] These elements of the intestinal immune system are called gut-associated lymphoid tissues (GALTs).

Preserving gut integrity is a vital task for the intestinal epithelium. Tight junctions are considered crucial elements that maintain gut integrity and intestinal permeability. Tight junctions are proteins located in the paracellular spaces in the gut, allowing the passage of water, nutrients and small molecules but blocking access for bacteria and toxins.[2]

The tight junction complex is comprised of many transmembrane and cytosolic proteins, including occludin, claudins, zonula occludens (ZOs), tricellulin, and junctional adhesion molecules, which work together to preserve gut integrity.[12,13] Tight junction complexes are programmed to rapidly expose and seal the intestinal epithelium in the occurrence of injury.[14]

Regulation of gut integrity

The tight junction proteins are controlled by various signaling proteins and molecules. Several molecules including small GTP-binding proteins, tyrosine kinases, and protein kinase C, play an important role in the protection of tight junction integrity.[14] Cytokines seem to also regulate tight junction protein during chronic diseases. Tumor necrosis factor-α (TNFα) is a key regulator that negatively affects the expression of occudins, promoting gut permeability.[14] In addition, modulation of the intestinal permeability is controlled by diet, short-chain fatty acids, prebiotics, probiotics and the microbiome.[2] The influence of diet depends upon host's genetic susceptibility and microbiome composition. However, studies have revealed that high fat[15] and high sugar diets, particularly high in fructose,[16] have the ability to create a dysbiosis in the microbiome, increase intestinal permeability and subsequent microbial translocation. These processes are now being accepted as key factors for the development of chronic diseases such as type II diabetes, non-alcoholic fatty liver, and cardiovascular diseases.[16–21] Among micronutrients, vitamin A and vitamin D play a critical role in the protection of gut integrity.[2,22]

Marker of Gut Integrity

Intestinal Fatty Acid Binding Proteins (I-FABP) are small water-soluble proteins found in mature enterocytes in both small and large intestines.[2] These proteins transport fatty acids from the apical membrane of the enterocyte to the endoplasmac reticulum where lipid synthesis takes place.[2] I-FABP is found in jejunum and it has been proposed as a good biomarker to measure epithelial cell integrity, particularly in celiac patients.[23]

In the event of intestinal damage, I-FABP is leaked into systemic circulation and elevated levels indicate intestinal epithelial damage;[2] reference values for healthy individuals are considered 2 ng/mL or less in circulation.[24] It can be measured in plasma and urine implementing an Enzyme Linked Immunosorbent Assay (ELISA). Therefore, I-FABP has emerged as a possible non-invasive marker for evaluating gut integrity loss and intestinal inflammation.[25]

Effects of HIV on the Gastrointestinal Tract

The gastrointestinal (GI) tract mucosa is considered one of the most active and important tissues of the immune system. The anatomical, histological and physiological characteristics of the GI mucosa provide a barrier against microorganisms. The enterocyte is the basic unit of the GI mucosa, serving as the single layer of cells for absorption of nutrients, water, and protection from external pathogens. Therefore, preserving the integrity of the GI mucosa is an essential task for the human body and the immune system.[26]

Studies have shown that People Living with HIV (PLWH) have histological abnormalities in the lamina propia of the enterocyte suggesting the occurrence of pathologic processes induced by HIV. These abnormalities are mainly due to the infiltration of lymphocytes, reduction and atrophy of the villus, and enlargement of the intestinal crypts. The histological changes can be observed in the early stages of the infection provoking the characteristic GI symptoms of HIV infection, such as diarrhea,

increased intestinal permeability, intestinal inflammation, bloating, malabsorption of bile acids and of vitamin B_{12}. [26–29]

HIV has been shown to directly affect the enterocytes due to the action of its accessory protein Transactivation of transcription (Tat) that seems to inhibit the uptake of glucose into the enterocyte. In addition, the envelope glycoprotein gp120, promotes calcium accumulation inside the cell affecting the ionic homeostasis, [30,31] and activates the intestinal immune system with the release of pro-inflammatory cytokines such as TNF-α causing apoptosis, and interferon (IFN)-γ, interleukins (IL-12, IL-8 and IL-6), which promote microbial translocation.[32,33]

The local immune activation of pro-inflammatory cytokines leads to gastrointestinal CD4+ T destruction across the intestine and represents one of the major sites for HIV replication.[34,35] Studies have shown that CD4+ T cells in the GI tract are 10-fold more frequently infected by HIV than are the cells in the blood, suggesting that the GI tract is one of the largest HIV targets and an HIV reservoir in the human body.[4,26,36–38] The loss of CD4+ T cells in the GI lamina propia, promotes a massive depletion of T helper 17 (Th17) cells in the intestine that are responsible for the intestinal homeostasis, mucosal regeneration and integrity.[39,40]

CD4+ T cells are the preferred target for HIV. These T cells are located in greater amounts in the GI tract and lymphoid tissue than in peripheral blood. A study investigated CD4+ T cells depletion in peripheral blood, lymphoid tissue and GI tract of naïve PLWH and healthy controls. Blood samples to assess Peripheral Blood Mononuclear Cells (PBMCs), inguinal lymphoid tissue, ileal Peyer's patches and lamina propia samples from the terminal ileum through biopsies were collected. There was a

profound CD4+ T cells depletion in naïve PLWH in early stages of the disease.[41] Also, it was shown that the depletion was greatly observed in a subtype of CD4+ T cells that expresses the C-C chemokine receptor type five (CCR5) which were mainly found in the GI tract. The CCR5 is a co-receptor of the CD4+ T cell that facilitates the entry of the HIV inside the cell. The HIV's gp120 membrane protein attaches to the CCR5 protein due to the tyrosine and acidic residues of the N-terminal, forming a strong chemokine binding that ultimately promotes the entry of the HIV into the host cell.[42] Thus, CCR5 CD4+ T cell can be considered a marker for CD4+ T cell depletion in the GI tract.[41] In fact, a decrease in intestinal CD4+ T cells (less than 200 cells/ μl) has been linked to the incidence of diarrhea and opportunistic infections in PLWH.[43]

Effects of Antiretroviral Therapy in the Gastrointestinal Tract

Antiretroviral Therapy (ART) is considered the first line of treatment for HIV infection. ART is able to reduce viral load to undetectable levels in peripheral blood creating an opportunity for CD4+ T cell synthesis, and therefore, decreasing mortality and morbidity among PLWH.[44] It may be expected that the beneficial effects of ART appear in other tissues besides peripheral blood. Previous data demonstrated that ART was capable of ameliorating GI symptoms associated with HIV enteropathy in early stages of the infection.[45] Studies examining long term effects of ART on GI CD4+ T cell reconstitution by immunohistochemistry and flow cytometry techniques, however, demonstrated that CD4+ T cell in the GI had a slower rate of reconstitution than

peripheral blood cells. Also, poor reconstitution of CD4+ T cell in the GI tract promoted immune activation and debilitated the mucosal repair system.[46–48]

The mechanism behind poor CD4+ T cell reconstitution in the GI tract has not been elucidated completely. One possible explanation may be that viral replication still occurs at low levels in GI tract, preventing rapid CD4+ T cells reconstitution. A study analyzing viral replication in rectal biopsies of PLWH found that CD4+ T cells continued to produce the virus even after long term exposure to ART.[49] It has been contemplated that although the intestinal tract has a vast supply of blood, the apical surface of the epithelial cells in both small and large intestines exhibit multi-drug-resistance proteins or "toxin pumps" that decrease the bioavailability of the ART in the intestine allowing the virus to replicate at low but continuous levels.[26,50]

Another possible explanation for the poor CD4+ T cell reconstitution in the GI tract is the ongoing local inflammation. Local inflammation promotes fibrotic deposition of collagen in the Peyer's patch damaging the physical structure of the cell and making CD4+ T cell reconstitution difficult.[26,43] Therefore, long-term effects of ART on GI tract produce poor CD4+ T cell reconstitution when comparing to the recovery in the peripheral blood. Thus, even in patients, who comply with ART and have undetectable HIV viral load in their blood, the intestinal CD4+ T cells recovery is incomplete, bacterial translocation continues, and promotes immune activation, diminishing ability of the intestinal mucosal to be repaired.[46–48] As many as 30% of PLWH on ART fail to reconstitute CD4+ T cells in the GI tract, despite having undetectable viral load in the peripheral blood; these patients have been labeled "immunological non-responders" (INRs) with faster HIV disease progression.[43]

Recently, some researchers have raised a concern about the effects of ART on gut microbiome as another possible mechanism that contributes to HIV enteropathy. It has been proposed that protease inhibitor-based regimens pose the most dangerous effect to the microbiome because this type of ART induces greater intestinal epithelium damage, Microbial Translocation (MT), immune activation and inflammation than other regimes such as nonnucleoside transcriptase inhibitors and integrase strand transfer inhibitors.[51–53]

Effects of HIV on Microbiome

Microbiome or microbiota is a term related to all population of microorganisms that colonize a particular area in the body; the term not only includes bacteria but also other microorganism such as viruses, fungi, archaea and protozoans [54,55]

One of the critical roles of the microbiota in the intestine is the digestion of insoluble fiber and synthesis of important metabolites such as short-chain fatty acids that serve as energy for the enterocytes, which is important for immunomodulation.[54] Also, the microbiota enhance the connection between the intestinal tissue and the immune system, specifically in the GALTs.[56] The composition and diversity of the gut microbiota is influenced by numerous factors such as age, diet, geography, antibiotic use and chronic diseases, including HIV infection.[54,55]

The microbiome diversity appears to be reduced during HIV infection.[57,58] Studies have shown stools and mucosal biopsy samples of PLWH on ART and those who are ART naïve to have greater abundance of *Prevotella* and reduced amount of *Bacteroides.*[39,54,56,59] *Prevotella* belongs to the phyla Proteobacteria that also includes

pathogenic microorganism such as *Shigella*, *Salmonella* and *Helicobacter*, *Pseudomonas*, and *Acinetobacter* that lead to the development of diarrhea, opportunistic infections and production of Lipopolysaccharide (LPS). These bacteria play a major role in MT, and immune activation during HIV-infection.[57,59–61] On the other hand, *Bacteroides* derived from the phyla Bacteroidaceae, including *Lactobacillus ssp*, offer anti-inflammatory protection in the gut, and have been found to be reduced in PLWH.[39,56,60,62,63]

Microbial Translocation in HIV infection

It has been well established that humans and the microbiome have a symbiotic relationship based on mutually beneficial interactions that maintain both the homeostasis of humans and of the microbiome.[64] The combination of reduced intestinal CD4+ T cells and alterations in the microbiome lead to MT during HIV infection. Translocation of bacteria is defined as the passage or transport of the microflora or its products from the GI tract into the systemic circulation without causing bacteremia.[65,66]

The transport of the bacterial products follows a complex mechanism where they have to cross the luminal mucosa, immunoglobulin A and the epithelial barrier, escape from the macrophages in the GI lamina propia, and finally elude liver-mediated clearance by the liver sinusoidal endothelial cells, Kupffer cells, and liver associated lymphocytes.[64] The causes of MT are diverse, these include: low level of immunoglobulin A and enterotoxins from pathogenic species such as *Vibrio, Escherichia, Salmonella, Helicobacter, Clostridia* and some viruses, including HIV, that generates disruption of the gut integrity.[64]

The harmful effects of the HIV enteropathy is highlighted by the extensive depletion of the CD4+ T cells in the GI tract, poor mucosal reconstitution, physical destruction of the epithelial barrier, increased permeability and local inflammation, which pose a risk for MT and immune activation.[32–35,67,68] Studies have demonstrated that products of MT, specifically LPS in the circulation induce T-cell activation which is considered a hallmark in predicting clinical outcomes of HIV infection.[69–73] These findings were based on an early research conducted by Funderburg *et al.*[69] who assessed the effects of microbial Toll-like receptors (TLR) ligands on T-cell activation in healthy individuals (n=20). It has been established that TLR ligands recognize MT products in the systemic circulation as demonstrated in an *in vitro* study, with PBMC's exposed to either plate-bound-anti-CD3 monoclonal antibodies or TLR ligands. TLR ligands induced T-cell activation, particularly CD4+ and CD8+ T cells and TLR ligand exposure promoted CD4+ T cell apoptosis. It was also concluded that TLR are important sensors for innate immune response in healthy controls.[69] This was the first study that proposed a model mechanism behind MT and the pathogenesis of HIV infection.

Markers of Microbial Translocation

Microbial translocation can be measured by direct markers including LPS and bacterial DNA or RNA fragments, and indirect makers such as Lipopolysaccharide Binding Protein (LBP) and Endogenous endotoxin-core antibodies (EndoCAb).[66] Once these by-products of MT are in the systemic circulation, they induce inflammatory responses by stimulating a number of cell-surface receptors, including nucleotide-binding

oligomerization domain 1 (NOD1), NOD2, Toll-like receptor 2 (TLR2), TLR4, TLR5, TLR6 and TLR9, and soluble CD14 (sCD14), which are expressed in many cell types of the innate immune system.[74]

The innate immune system cells, monocytes, macrophages and dendritic cells, become activated by the products of MT, initiating a cascade of pro-inflammatory cytokines such as interleukin 1-β (IL-1β), IL-6, TNF-α and type I interferons.[74]

Lipopolysaccharide (LPS) is the most important component of the outer membrane in Gram-negative bacteria and a strong stimulator of the innate immune system. LPS is composed of a poly or oligosaccharide that is attached to outer bacterial membrane by a specific carbohydrate lipid moiety, lipid A, considered the factor that stimulates the immune system[75] and has been suggested as the major marker for MT, as it activates the cellular complex of LPS binding-protein (LBP), sCD14 and TLR4 (TLR4*MD-2 complex) that initiates inflammatory response.[66,75]

Efforts have been made in the scientific community to establish the relationship among MT markers during HIV infection. In this regard, one study investigated the correlation between MT markers in PLWH (n=18) with controlled viral load and exposed to ART. Blood samples were collected to assess LPS, LBP, sCD14 and 16S rRNA at baseline and different time points.

Researchers found a significant association between LPS and sCD14 ($r=0.407$, $P<0.001$) and LPS and LBP ($r=0.260$, $P=0.042$). These findings indicated that LPS correlates well with other biomarkers of MT, LBP, and immune activation, and it serves as a good marker for MT during HIV infection.[76]

Immune Activation in HIV infection

Immune activation is defined as a phenomenon characterized by complex processes that stimulate and activate the immune system creating constant inflammation.[77] During the course of certain diseases, including HIV, acute inflammation persists, and generates chronic inflammation with continuous activation of cells and cytokines, which can be stimulated for years under low-grade inflammation or immune activation.[78]

HIV infection activates both innate and adaptive immune responses, and therefore, immune activation. Once HIV has entered to the human body, it activates the innate response from dendritic and natural killer cells. It had been found that the loss of dendritic cells causes decrease in Interferon alpha (INF-α) production, an important cytokine, is due to increased viral load during HIV infection.[71,79] In addition, natural killer cells protect the body from HIV replication by eliminating HIV infected cells, which provoke cytokine liberation, particularly IFN-γ, TNF-α and β-chemokines (CCL3, CCL4 and CCL5). [80]

Simultaneously, the adaptive immune response is triggered, especially with the activation of CD4+ T and CD8+ T cells. Helper T cells or CD4+ T cells are the preferred target of HIV. Once HIV infects CD4+ T cell, it induces apoptosis probably due to the action of the virus proteins gp120, Tat, Nef and Vpu, causing massive loss of these cells.[71,77] The CD4+ T cells provoke the release of a numerous cytokines that can determine disease progression. Pro-regulatory cytokines, (Th1 type) including interleukin 2 (IL-2), IL-12 and IFN-γ, up-regulate HIV virus replication in infected cells while pro-inflammatory cytokines (Th2 type) such as IL-4, IL-10, IL-5, IL-3 induces more

inflammation during HIV infection.[78] It has been suggested that during HIV infection there is an imbalance between cytokines, with an overproduction of Th2 type and underproduction of Th1 type contributing to immune activation.[81] The constant stimulation and activation of the CD4+ T cells and cytokines by HIV, induces the expression of CCR5 receptors that predispose more CD4+ T cells to become infected, creating a vicious cycle, in which infection causes immune activation and leads to a massive CD4+ T cell depletion.[82]

Besides the cytopathic effects of HIV on CD4+ T cells, there are additional consequences including proliferation, expansion and death of non-infected CD4+ T cells that creates an increased T-cell turnover, and ultimately the collapse or exhaustion of the regenerative capacity of the entire immune system.[83,84] The high T-cell turnover is directly associated with the level of viremia and CD4+ T cell loss,[85] which are the hallmarks for HIV disease progression in the absence of ART.[86] Studies show that immune activation and inflammation persist even after long-term ART exposure and sustained viral suppression, which eventually promotes a rapid disease progression.[87–89]

Markers of Immune Activation

Soluble CD14 (sCD14) is a co-receptor localized in peripheral blood monocyte and tissue macrophages. Once LPS is in the systemic circulation, CD14+ monocyte/macrophages release sCD14 to be linked to the LPS molecule and initiate inflammatory response; sCD14 also works in conjunction with LBP and TLR4 to activate the TLR4*MD-2 complex and induce the inflammatory response.[64,73] sCD14 can be

measured in plasma and it correlates very well with LPS.[66] It has been suggested that sCD14 can also be activated by other factors such as TLR ligand flagellin and inflammatory cytokines including IL-6 and IL-1β indicating that sCD14 is a marker of monocyte activation that is not exclusively due to LPS stimulation.[90] Studies have shown that increased levels of sCD14 may be associated with chronic diseases among PLWH including preclinical atherosclerosis,[91] hypertension,[92] and hepatitis C,[93] as well as neurocognitive impairment.[94] In addition, this biomarker was found to be associated with 6-fold increased risk of all-cause mortality among PLWH.[74]

Soluble CD163 (sCD163) is considered another marker for monocyte activation and innate immune response associated with the hemoglobin scavenger receptor.[95] Studies have suggested that high sCD163 levels are present in tissue macrophages indicating not only immune activation but tissue inflammation during HIV infection;[96] also, high sCD163 levels had been associated with neurocognitive impairment,[97] cardiovascular diseases[98] and mortality among PLWH.[99]

Soluble CD27 (sCD27) is considered to be a marker for T cells and the adaptive immune response[95] during HIV infection.[100] Its structure consists of a glycoprotein that belongs to pro-inflammatory cytokine TNF receptor family and it is presented on the surface of some T cells.[95] Levels of sCD27 correlate with IgG concentrations among PLWH. Even in patients on ART, elevated levels of sCD27 continue[101] and are associated with the development of neoplastic diseases such as non-Hodgkin lymphoma in PLWH on ART.[95]

Cytokines are a group of proteins secreted by the immune system, mainly T cells and macrophages. The main purpose for cytokines is to facilitate cell signaling, cell to

cell communication in immune response and the migration of cells to sites of inflammation.[102] Cytokine is considered a general term that groups lymphokine (cytokine made by lymphocytes), chemokines (cytokine with chemotactic effects that attracts leukocytes to site of inflammation), monokines (cytokines made by monocytes) and interleukin (cytokines made by one leukocyte that acts on other leukocytes).[102] Cytokines have a cascade effect and can act synergistically as messengers of the immune system. Cytokines can be classified as pro-inflammatory and anti-inflammatory. Pro-inflammatory cytokines are usually produced by macrophages in response to inflammation.[102]

Markers of Inflammation

Interleukin 6 (IL-6) is produced by monocytes, macrophages and T-lymphocytes in response to inflammation. It is involved in T cell growth, proliferation and differentiation and it acts in conjunction with IL-1β and TNF-α to enhance inflammatory response. It is also believed that IL-6 promotes HIV replication[103,104] since IL-6 is abnormally high during HIV infection causing detrimental consequences to the host.[105]

Interleukin 17 (IL-17) is produced by Th17 cells and helps to regulate the inflammatory response by activating macrophages and neutrophils to improve their recruitment and survival.[106,107] Studies revealed that IL-17 levels are particularly high during HIV infection;[106] however, in another study it was demonstrated that the levels of IL-17 in the GI tract were decreased due to depletion of Th17 cells and CD4+ T cells when the virus infects the GI tract.[26] Low levels of IL-17 promote bacterial infections,

MT, and ultimately it is considered a strong predictor for immune activation and HIV disease progression.[64,108]

Interleukin 22 (IL-22) is produced by Th22 cells and plays a critical role in preserving the innate immunity in the intestine, protecting from bacterial infections and inflammation, as well as improving intestinal epithelium repair.[109,110] A study demonstrated that sigmoid IL-22-producing T cells and Th22 cells were severely depleted during chronic HIV infection, epithelial integrity was compromised, and MT was increased.[111]

Tumor Necrosis Factor alpha (TNF-α) is a protein secreted by many cells of the immune system including monocytes, macrophages, T and B cells, NK cells among others. TNF-α is a potent inflammatory cytokine that has 2 main receptors: soluble TNF receptor 1 and TNF receptor 2 that inhibit the effects of the TNF-α.[104] During HIV infection, TNF-α stimulates viral replication, therefore, plasma levels in untreated PLWH are high and are highly correlated with disease progression.[103,104,112]

The acute phase C-reactive protein is a well-known marker for inflammation and tissue damage that is the consequence of acute and chronic infections. CRP levels change in response to other pro-inflammatory cytokines such as IL-1 and IL-6.[113] Studies have demonstrated that high sensitive CRP (hs-CRP) levels were particularly higher in PLWH when compared with healthy population and that hs-CRP is a good inflammatory marker in HIV disease and shows the risk for other comorbidities, including cardiovascular diseases.[114,115]

Microbial translocation and immune activation in HIV disease progression

Immune activation is considered of the best predictors to assess HIV disease progression. It has been demonstrated the MT induces massive immune activation in PLWH.[65] In an earlier study, researchers examined the relationship between MT and immune activation in PLWH (n=205) and uninfected participants (n=47). Blood samples were collected to assess LPS using Limulus *Amebocyte* assay. Soluble CD14 and Interferon alpha (IFN-α) were determined with ELISA assays as markers of immune activation. In this study, PLWH had significantly higher levels of plasma LPS than uninfected participants ($P<0.001$) indicating the presence of microbial products in systemic circulation. Immune activation was found higher in PLWH with elevated plasma sCD14 levels ($P<0.0001$) than uninfected participants; there was a positive correlation between LPS and sCD14 ($r=0.3$, $P=0.001$) and LPS and IFN-α ($r=0.624$, $P<0.0001$) indicating that MT induces immune activation in PLWH.[116]

In another study, Dinh *et al.*[117] evaluated microbiota composition, MT and systemic inflammation in PLWH on ART with undetectable viral load (n=21) and healthy controls (n=16). Stool and blood samples were collected. Phylogenetic diversity, MT markers including Endotoxin core immunoglobulin M (EndoCAb IgM), sCD14 and LPS were measured with ELISA and Limulus *Amebocyte* assays respectively, plasma 16S rRNA gene levels were determined with quantitative real-time PCR; immune activation markers: IFN-γ, interleukin 1-β (IL-1β), interleukin-6 (IL-6), TNF-α and hs-CRP were measured. This study found that PLWH had predominantly Probacteria, Enterobacteriales and Enterobacteriacea counts and decreased *Rikenellaceae* and *Alistipes* compared to healthy controls indicating a microbiome dysbiosis in PLWH. Plasma sCD14 levels were

significantly elevated in PLWH when compared to healthy controls (P<0.001); EndoCAb levels were significantly reduced in PLWH (P<0.001) since this molecule binds LBP increasing LPS levels. High LPS levels were found in PLWH, however, it was not significant (P=0.08), as well as high plasma 16S rRNA levels. As expected, PLWH had immune activation with elevated IL-1β and TNF-α (P=0.02 and P=0.03, respectively). The products of MT induced immune activation as LPS were positively correlated with IL-6 (P<0.001), TNF-α (P<0.001) and hs-CRP (P=0.01) in PLWH despite ART use and viral suppression.[117]

Findings from other studies in PLWH with long exposure to ART showed that MT improved CD4+ T cell reconstitution and promoted immune activation.[87,118,119] This suggests that MT and immune activation could be independent factors that promote damage of the GI tract during HIV infection despite ART and viral replication.

Immune activation has been identified as one of the factors that increase morbidity and mortality among PLWH regardless of viral suppression and ART use. Constant low levels of virus replication from reservoirs, particularly GI tract, are kept despite ART use. Co-infection with other viruses such as hepatitis C exponentially enhances immune activation during HIV infection.[72] This creates a vicious cycle where gut barrier immunity is compromised by the HIV promoting MT and immune activation, and ultimately disease progression.

Hunt *et al*.[120] assessed immunological predictors of mortality during HIV infection in a case-control study. Participants were recruited from the Longitudinal Study of the Ocular Complications of AIDS (LSOCA) a multicenter cohort of PLWH who started ART treatment with AIDS diagnosis. PLWH who died (not to be known

accidental) within 12 months of ART exposure (n=64) were matched to 2 controls (total number of controls 128) by duration of ART, CD4+ T cell levels, age, sex and prior opportunistic infections. Plasma and PBMC's samples were taken from prior visits and analyzed. Researchers discovered that I-FABP and zonulin-1 (gut integrity biomarkers), sCD14, soluble TNF-1 receptor, hs-CRP and D-dimer levels all strongly predicted mortality, even after adjusting for CD4+ T cell levels (all $P<0.001$).[120] These findings support the idea of the need to further examine the damage to the gut barrier integrity and MT during HIV infection and ART use.

Substance Abuse

Cocaine use

Cocaine is an alkaloid derived from the leaves of the coca plant *Erythroxylum coca.* It is a potent vasoconstrictor and powerful stimulant of the brain which when abused leads to several medical complications. Indeed, the effects of cocaine on the brain, particularly the inhibition of the dopamine re-uptake, make cocaine one of the most addictive and abused illicit drugs.[121] Therefore, the National Institute of Drug Abuse classified cocaine as a Schedule II drug with high potential for abuse.[122] Cocaine can be used orally, intranasal, intravenously and by inhalation. Cocaine users use cocaine in two chemical forms: water-soluble hydrochloride salts, a powder that can be injected or snorted; and the water-insoluble cocaine base, which is mixed with sodium bicarbonate and water, and then heated to produce what is commonly called crack.[122]

Cocaine use and Gut integrity

Several lines of evidence have shown that cocaine abuse poses detrimental effects for GI tract including weight loss, malnutrition, anorexia and reduced blood supply to enterocytes.[123–125] The exact mechanisms behind the action of cocaine and deterioration of the GI tract have not been fully elucidated.

However, studies suggested that cocaine has the ability to dysregulate the expression of the tight junction proteins in the intestinal epithelium, promoting gut integrity damage and hyperpermeability.[124] A study conducted with rodents demonstrated that alteration in gut microbiome seemed to potentiate sensitivity to cocaine reward and locomotor effects, proposing a connection between gut microbiome and altered behavioral responses to cocaine.[126]

A recent study with mice demonstrated that intraperitoneal administration of cocaine created a microbiome dysbiosis and depletion of bacteria responsible for producing short-chain fatty acids, key elements that provide energy to the enterocytes and promotes microbiome diversity.[127] Also, cocaine generated an inflammatory response in the gut due to the release of pro-inflammatory cytokines (IL-18 and IL-1β).

Furthermore, *in vivo* and *in vitro* analyses demonstrated that cocaine upregulated the expression of an important tight junction protein (claudin-2) promoting intestinal permeability in the gut.[127] This finding suggests that cocaine abuse poses dangerous effects for gut integrity, intestinal inflammation and microbiome.

Cocaine use and HIV disease progression

Epidemiological studies have demonstrated that active illicit drug use, particularly cocaine, is associated with poor adherence to ART, reduced virologic and immunologic control, HIV disease progression and mortality.[128–130]

Cocaine and its biological forms are one of the drugs of choice among PLWH. In a study analyzing the illicit drug patterns among PLWH in 8 U.S. cities, it was found that 40% of participants had heavy alcohol consumption or crack-cocaine use in the preceding 12 months.[131] Since cocaine use is highly prevalent among PLWH, several studies have been conducted to examine the effects of cocaine use on HIV disease progression.

Cocaine has the ability to reduce CD4+ T count and increases HIV viral load in PLWH. Baum *et al*.[130] conducted a prospective, longitudinal study to evaluate the effects of cocaine use on HIV disease progression in PLWH on ART (n=222). Blood was collected every 6 months for 30 months to assess CD4+ T cell count and viral load, urine toxicology and socio-demographic data were also collected. Crack-cocaine users were 2.14 times more likely to have a decline in CD4+ T cell count (≤200 cells/ml), independent of ART use, than -cocaine non-users (95% CI: 1.08-4.25, P=0.029). Viral load was significantly elevated among crack users (P=0.037) regardless of ART use. Researchers concluded that crack-cocaine use promotes decline in CD4+ T cells count and reduced medication adherence, which ultimately accelerates HIV disease progression.[130]

Many studies have tried to elucidate mechanisms behind the toxic effects of cocaine in CD4+ T cells.[121,132–134] Cocaine seems to support HIV replication because it facilitates the virus entry by upregulating the expression of the co-receptors CXCR4 and

CCR5 on the membrane of the CD4+ T cells.[135,136] Cocaine also enhances later steps in HIV viral replication by inhibiting cellular miRNA "miR-125b" in CD4+ T cells, which is responsible for inhibiting the HIV replication cycle.[137] It has been shown that cocaine induces CD4+ T cell apoptosis in cell culture studies due to an increase in oxidative stress, particularly in the reactive oxygen species (ROS).[138] In addition, cocaine has been postulated as a potent immunosuppressant that alters the function of the cells of the immune system.

Cocaine affects thymic endocrine function in PLWH, which plays a vital role on immune recovery, by reducing the thymulin activity even in the presence of ART and HIV viral load, contributing to the progression of the disease.[139] Another study demonstrated that cocaine users were 75 times more likely to have low thymulin activity (OR= 74.7, 95% CI: 1.59- 3519.74, P= 0.002) and that thymulin activity was inversely associated with cocaine use (β= -0.908, 95% CI: -1.704, -0.112, P= 0.026) independent of ART use.[139] This study suggested another possible mechanism that may predicts HIV disease progression.

Cocaine use and microbiome during HIV infection

As discussed previously, cocaine has been associated with important gastrointestinal disturbances including diarrhea, nausea, vomiting and anorexia that alter the microbiome, as well as the use of antibiotic therapy.[140] In addition, lifestyle pattern, dietary choices, and nutritional status may affect the microbiome. [140] A cross-sectional study investigated microbiome composition, MT, immune activation and nutritional

status in drug users living with HIV (n=32) from the CARE study. Participants were grouped into 4 arms: (1) PLWH, (2) healthy controls, (3) PLWH cocaine users and healthy controls who used cocaine. Stool samples were collected to evaluate microbiome alterations and diversity by 16SrRNA gene pyrosequencing. Blood was collected to analyze markers of MT and immune activation. Dietary data were collected by 24-hour recall. It was found that cocaine users had relative abundance of phyla *Bacteroides* and *Firmucutes* suggesting a dysbiosis in the microbiome. As expected, sCD14 and TNF-α were significantly higher in PLWH (P=0.01 and P=0.03, respectively). Cocaine users had a lower Healthy Eating Index when compared to cocaine non-users.[140] This study suggests that cocaine use may cause alterations in microbiome, increased MT and immune activation among PLWH.

Alcohol abuse

It has been well established that alcohol and its metabolites provoke disruption of intestinal tissue homeostasis, intestinal inflammation, changes in microbiome, bacteria overgrowth and destabilization of the local immune system.[59,141–147] One of the most devasting effect of alcohol in the intestine is the ability to destroy epithelial cell wall causing trans-epithelial permeability, and the spaces between the epithelial cells (paracellular permeability), which are represented by the tight junctions, cytoskeleton and proteins.[148,149] These events lead to cellular damage, destruction of gut integrity and increased permeability, allowing passage of large molecules, including LPS, through intestinal barrier,[150,151] ultimately causing MT.

Studies have reported that PLWH are twice more likely to consume alcohol than healthy individuals.[152] Hazardous alcohol drinking increases viral replication and production of pro-inflammatory cytokines in PLWH.[153] It is not surprising that alcohol and its metabolites worsen the already disrupted GI tract in PLWH, contributing to the ongoing local inflammation, MT and immune activation. Alcohol appears to induce apoptosis in T cells through downregulation of the Vitamin D receptor (VDR),[154–157] proposing a link between vitamin D deficiency and alcohol abuse in the context of HIV infection.

Vitamin D

Vitamin D is a fat-soluble steroid derived from 7-dehydrocholesterol, a cholesterol precursor.[158,159] Vitamin D has two major forms that are important for human health, ergocalciferol or D_2 synthesized from ergosterol in plant sources, particularly fungi, and cholecalciferol or D_3 derived from cholesterol in animal products.[158–160] The main source of vitamin D_3 in humans (80-90%)[161] is made in the skin upon sun exposure due to the irradiation of 7-dehydrocholesterol to pre-vitamin D_3 at UVB wavelengths of 290-320nm, with a further isomerization to create vitamin D_3.[162,163]

The remaining source of vitamin D (10-20%)[161] is obtained through dietary sources including: fish, eggs, oily fish (pink salmon, sardines, Mackerel), fortified foods (milk, dairy products and breakfast cereals). Vitamin supplements are available in D_2 and D_3 forms.[161,163]

Vitamin D Metabolism

The active forms of Vitamins D_2 (ergocalciferol) and D_3 (cholecalciferol) are synthetized by the action of enzymatic hydroxylation process, which occurs mainly in liver.[163] In the liver, vitamin D_2 and D_3 are converted into 25-hydroxivitamin D_2 ($25[OH]D_2$) and 25-hydroxivitamin D_3 ($25[OH]D_3$),[160,161] respectively due to the action of 25-hydroxylases, which belong to the cytochrome P450 group.[163] In the kidneys, 25-hydroxivitamin D is converted to its biologically active form 1,25-dihydroxyitamin D_2 ($1,25[OH]D_2$) or D_3 ($1,25[OH]D_3$) (calcitriol) by 1α-hydroxylase.[161,163] The rate of conversion depends upon circulating parathyroid hormone concentrations. Once $1,25[OH]D_3$ (calcitriol) is available, serum calcium and phosphate levels are regulated via the control of calcium absorption in the intestine, renal resorption of phosphate, and release of calcium from bones.[162,163]

A small proportion of vitamin D is transported in blood as "free" steroid and access the cells via simple diffusion. The remaining vitamin D in blood is attached to Vitamin D-binding protein (DBP).[158,160] The DBP has strong affinity for 25[OH]D, and a weak affinity for 1,25[OH]D (biologically active form).[159] However, $1,25[OH]_2D$ has high affinity for VDR which provides access to gene transcription system inside cell.[159] Importantly, vitamin D can also enter the cell throughout VRD localized in membrane to execute its non-genomic effects such as modulation of growth factors and cytokines.[164] VDR is found in many tissues and cells, especially in intestine, kidney, and immune cells, which makes vitamin D a regulatory agent in the innate and adaptive immune systems.[165,166]

Also, the effects of vitamin D in combination with VDR on the immune response is seen in other cells, including intestinal epithelial cells,[167] demonstrating a role of Vitamin D in gut integrity.

Vitamin D and Gut integrity

In vitro studies demonstrate that optimal levels of vitamin D_3 is capable of decreasing intestinal permeability and preserve gut integrity.[168,169] One of the mechanism of the protective role of vitamin D in the intestine is explained by signaling of VDR.[170] It seems that activation of VDR in the intestine regulates the intestinal Paneth cells which act as antimicrobial host defense in the pathogenesis of Chron's disease.[171,172] In addition, vitamin D_3 induces the expression of occudin, zonula occludens (ZO) ZO-1, ZO-1 and vinculin, which represent the major protein family expressed in intestinal tight junction, that are in charge of sealing spaces among intestinal cells, representing the key element in gut integrity.[173–176]

Several studies have shown the association of vitamin D and VDR in Intestinal Bowel Disease (IBD).[177] Vitamin D deficiency have been reported in patients with IBD, and VDR protein was reduced in IBD and colitis-associated colon cancer patients.[178,179] Therefore, adequate levels of vitamin D and subsequent expression of intestinal VDR are essential to control intestinal homeostasis.

Studies suggested that vitamin D status is related to the function and composition of the microbiome.[180,181] Vitamin D_3 is able to modify the composition of the intestinal

microbiome in patients with IBD.[182] Importantly, VDR regulate the immune response to the microbiome and control microbiota dysbiosis.[183]

Vitamin D Deficiency in HIV infection

Vitamin D deficiency is considered one of the major public health issues worldwide in all age groups;[184–187] even in regions with appropriate UV radiation, and in industrialized countries where typically vitamin D is supplemented.[159,184] Data from the National Health and Nutritional Examination Survey (NHANES) showed that vitamin D deficiency and insufficiency were found in 33.5% and 71.1% of the US population respectively in 2013-2014, and the prevalence was higher in disadvantage minorities.[188]

Numerous studies have suggested that vitamin D deficiency is linked not only to bone disorders but to chronic diseases including cardiovascular disease,[161] chronic kidney disease,[189] type II diabetes,[190] metabolic syndrome, cancer and infectious diseases, including HIV and hepatitis C infections.[159,191–193]

Epidemiological studies have shown that prevalence of vitamin D deficiency among PLWH ranges between 70-85%.[193,194] PLWH present similar risk factors for vitamin D deficiency as healthy people including: gender (females have higher risk), limited sunlight exposure, black ethnicity, advanced age, poor dietary vitamin D intake, obesity, cardiovascular and renal disorders, diabetes mellitus, metabolic syndrome, hepatic steatosis, and alcohol consumption.[159,193,195–198] However, PLWH have additional risk factors for vitamin D deficiency due to the action of HIV itself.[159] It has been well established that HIV promotes chronic inflammation and massive immune

activation,[81,199] and persons with vitamin D deficiency show an increase in pro-inflammatory cytokines, such as IL-6 and TNF-α,[200] and monocyte activation, which are considered key elements for HIV disease progression.

Chronic inflammation initiated by HIV alters the activity of 1α-hydroxylase in the kidneys, which is the enzyme responsible for the conversion of 25(OH)D to the biologically active form $1,25(OH)_2D$, causing a reduced production of the vitamin and promoting deficiency.[159,193,201]

HIV-infected T cells have been shown to have attenuated expression of VRD which impairs vitamin D effects.[157,202] Also, ART use, particularly Protease Inhibitors (e.g. Reyataz, Prezista, Rezolsta, Kaletra, Evotaz) lower the conversion of 25(OH)D to 1,25(OH)D,[193,203] and non-nucleoside reverse transcriptase inhibitors (e.g. Sustiva, Intelence, Viramune, Edurant) increase 25(OH)D catabolism.[204] Conjointly, comorbidities, infectious complications, hospitalizations and drug use by PLWH lead to limited sunlight exposure, poor nutritional status, and reduced vitamin D intake, contributing to risk of vitamin D deficiency.[193,205,206]

Vitamin D Levels: Measurements and Cut-Off Points

Measurement of 25(OH)D in serum or plasma is considered the fastest and most accurate way to assess levels of vitamin D in body.[159] Quantification of 25(OH)D is preferred over $1,25(OH)_2D$ due to lack of standardization methods (linearity, specificity, the effect of anticoagulants).[207] Also, 25(OH)D reflects vitamin D produced in the skin and obtained from food and supplements,[208] and it has a long circulating half-life of 15

days, whereas $1,25(OH)_2D$ has a very short half-life of 15 hours and serum concentrations are tightly regulated by parathyroid hormone, calcium and phosphate.[209]

Levels of $1,25(OH)_2D$ do not decrease until a severe vitamin D deficiency occurs.[210–212] In fact, a group of experts from the Vitamin D External Quality Assessment Scheme (DEQAS), who participated in the Vitamin D Standardization Program, recommended the use of the standardized laboratory measurement of 25(OH)D.[213]

There are two established methods to estimate vitamin D levels: competitive immunoassays, including protein-binding and radioimmunoassay, which measure total levels of 25(OH)D without differentiating $25(OH)D_2$ and $25(OH)D_3$; and those based on high-performance liquid chromatography and direct detection with liquid chromatography tandem-mass spectrometry (LC-MS/MS).[185,214] The last two are highly sensitive and allows for the quantification of both $25(OH)D_2$ and $25(OH)D_3$. However, they are difficult to perform and require specialized technology.[215] Vitamin D levels are commonly expressed in nanogram per milliliter (ng/mL) or nanomole/liter (nmol/L). According to the Endocrine Society Clinical Practice Guidelines, adequate levels of vitamin D in adults are considered to be >30ng/mL (75nmol/L), insufficient levels 21-29 ng/mL (52.5-72.5 nmol/L) and deficient levels at <20 ng/mL (50nmol/L).[185,216]

Vitamin D Deficiency, MT and Immune activation in HIV infection

Several studies have elucidated the relationship between vitamin D deficiency and MT. Garcia-Alvarez *et al.*[217] conducted a cross-sectional study to assess the association between 25(OH)D levels and MT as measured by the bacterial 16S ribosomal RNA in

120 people living with HIV/HCV and found that 15% of the participants were vitamin D deficient, while 77% were vitamin D insufficient. Low plasma levels of 16S ribosomal RNA were found in 66.7% of participants with optimal levels of 25(OH)D. Also, low plasma levels of CCL7, an inflammatory marker, were found in participants with optimal levels of 55(OH)D suggesting that optimal vitamin D status was associated with low levels of MT and inflammation in this population.[217]

Similarly, Missailidis *et al*.[218] investigated the association between vitamin D status, MT and inflammation in Caucasian men living with HIV (n=97) and controls (n=30) in Sweden. In this cross-sectional study, researchers did not find association between vitamin D levels and MT. However, PLWH had higher levels of immune activation (sCD14) and inflammation (hs-CRP) ($P<0.001$ for both) than HIV negative controls. Vitamin D deficient participants living with HIV had higher levels of IL-6 than PLWH with adequate levels of vitamin D ($P=0.003$).[218]

Additional cross-sectional studies have focused on investigating link between vitamin D status and inflammatory markers in PLWH. It was suggested that in PLWH on ART, higher 25(OH)D levels were inversely correlated with levels of TNF-α ($r=-0.20$, $P=0.04$), and adiponectin ($r=0.30$, $P=0.002$).[219] In addition, another study demonstrated that PLWH who had vitamin D deficiency had higher levels of inflammation as measured by IL-6 ($P<0.01$), TNF-α ($P=0.03$), and increased immune activation as measured by $CD14^{dim}CD16+$ ($P<0.01$) and CX3CR1+ monocytes ($P<0.001$) than PLWH with insufficient and sufficient levels of vitamin D.[200]

Summary

Gut integrity is essential for the function and protection of the intestine. A chronic alteration of the factors that modulate gut integrity can cause intestinal permeability, MT and inflammation. One of the factors that can disrupt gut integrity is HIV infection. The intestine is considered an important target for HIV because of the large presence of CD4+ T cells in this area. In fact, studies have shown that CD4+ T cells in the GI tract are 10-fold more frequently infected by HIV than are the cells in the blood, suggesting that the GI tract is one of the prominent HIV targets and reservoir in the human body.[26,36–38] The deleterious effects of the HIV enteropathy are highlighted by the extensive depletion of the CD4+ T cells in the GI tract, poor mucosal reconstitution, physical destruction of the epithelial barrier, increased permeability, inflammation and microbiome dysbiosis, which pose a greater risk for MT and immune activation.[32–35,57,58,67,68]

Immune activation has been identified as one of the factors that increase morbidity and mortality among PLWH, regardless of viral suppression and ART use. Constant low levels of virus replication from reservoirs, particularly GI tract, continue despite ART use, and enhance immune activation during HIV infection.[72] This creates a vicious cycle where gut barrier immunity is compromised by the HIV promoting MT and immune activation, and ultimately facilitating disease progression.

Cocaine is associated with poor adherence to ART, reduced virologic and immunologic control, HIV disease progression and mortality.[128–130] In addition, cocaine promotes dysbiosis in the microbiome, promotes MT and immune activation in PLWH.[140]

Vitamin D deficiency is highly prevalent among PLWH, especially in those with comorbidities, infectious complications, hospitalizations and drug use, leading to limited sunlight exposure, poor nutritional status, and reduced vitamin D intake, therefore, contributing to risk of vitamin D deficiency.[193,205,206] Importantly, it has been suggested that beyond its classical roles in calcium homeostasis, vitamin D_3 is capable of decreasing intestinal permeability and preserve gut integrity.[168,169]

Some studies have suggested association of vitamin D deficiency and high levels of MT, immune activation and inflammation in PLWH.[200,217–219] However, these studies were done with predominantly Caucasians men and illicit drug use was not investigated. Gut integrity was not assessed in the context of vitamin D deficiency or insufficiency and dietary intake data were not collected.

The role of cocaine in gut integrity, MT, inflammation and immune activation in the context of vitamin D deficiency or insufficiency has not been examined. The evidence shows that vitamin D is an essential nutrient for immunity and gut integrity, and its deficiency is highly prevalent among PLWH. Therefore, it is critical to assess relationships among gut integrity, MT and immune activation and inflammation in cocaine users living with HIV, who exhibit vitamin D deficiency or insufficiency.

Building on the findings of others, our findings may help to elucidate the potential mechanisms involved and be translated in the future into clinical recommendations for the management of HIV, drug addiction and gut health to ultimately ameliorate the continuous immune activation and inflammation observed in PLWH.

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CHAPTER III: ASSOCIATIONS BETWEEN MICROBIAL TRANSLOCATION, IMMUNE ACTIVATION AND VITAMIN D STATUS IN DRUG USERS LIVING WITH HIV

Abstract

Background. The human immunodeficiency virus (HIV) promotes Microbial Translocation (MT), affects the integrity of gastrointestinal mucosa and induces immune activation even with effective Antiretroviral Therapy (ART) use. Cocaine use may accelerate these processes in People Living with HIV (PLWH). Vitamin D plays protective role in antimicrobial host defense and its deficiency is associated with intestinal diseases. Vitamin D deficiency is highly prevalent among PLWH and may be affecting immunity as well as gut integrity. The purpose of this study was to examine associations between MT, immune activation and vitamin D status in the context of cocaine abuse among PLWH.

Methods. Participants were selected from the ongoing Miami Adult Studies in HIV (MASH) cohort. Cocaine use was assessed by self-report, urine screen and blood metabolites. Blood samples were collected to assess MT levels (LPS), immune activation levels (sCD14, sCD163 and sCD27), gut integrity damage (I-FABP) and vitamin D status. Data on socio-demographic characteristics, anthropometrics, diet and disease progression were collected. Descriptive statistics, independent t-test, multiple linear and logistic regressions were conducted to analyze the data.

Results. A total of 100 PLWH, 66 cocaine non-users and 34 cocaine users had a mean age was 53.37±6.88 years old. Most participants were male (51%), and African Americans (66%). All participants were on ART and had controlled viral load (<200 copies/mL). Half of the participants were considered vitamin D deficient (≤20 ng/mL) and 32% had insufficient levels (21-29 ng/mL). Cocaine users had higher levels of MT than non-users (P<0.001). Cocaine use was a significant predictor for both LPS (P=0.047) and immune activation markers sCD163 and sCD27 (P=0.002 and P=0.03, respectively). Cocaine use and vitamin D deficiency independently predicted high levels of MT (LPS ≥ median 0.63 EU/mL) (P<0.001 and P=0.043, respectively). Cocaine users also were 6.66 times more likely to exhibit high MT levels than non-users (OR: 6.658 95% CI: 2.429, 18.250; P<0.001)

Conclusion. Cocaine use and impairment of vitamin D status were associated with MT and immune activation in PLWH despite ART use and viral suppression. Therefore, it is imperative to develop effective interventions that target lifestyle modifications, optimal vitamin D status and mitigation of drug abuse, to more effectively decrease MT and ultimately reduce immune activation among PLWH.

Key words. Microbial translocation, immune activation, cocaine, vitamin D

Introduction

People Living with HIV (PLWH) exhibit pathological processes that affect the Gastrointestinal Tract (GI). One of the main features of the HIV in the GI tract is massive destruction of the intestinal CD4+ T cell.[1,2] Studies have shown that CD4+ T cells in the GI tract are 10-fold more frequently infected by HIV than are the cells in the blood, suggesting that the GI tract is one of the biggest HIV targets and reservoir in the human body.[3–7] The loss and poor reconstitution of CD4+ T cells in the GI tract promote disruption of intestinal homeostasis, mucosal regeneration and gut integrity despite Antiretroviral Therapy (ART).[7–11]

Studies have demonstrated that the microbiome diversity appears to be reduced in HIV infection.[12–15] It is not surprising that the combination of reduced intestinal CD4+ T cells and alterations in the microbiome lead to Microbial Translocation (MT) in PLWH. Translocation of bacteria is defined as the passage or transport of the microflora or its products from the GI tract into the systemic circulation without causing bacteremia.[16,17] Several lines of evidence have confirmed that PLWH present products of MT, specifically Lipopolysaccharide (LPS), in the circulation inducing immune activation, which is considered a hallmark in predicting clinical outcomes of HIV infection.[18–22] Immune activation has been identified as one of the factors that increase morbidity and mortality among PLWH regardless viral suppression and ART use.

Constant low levels of virus replication from reservoirs, particularly in the GI tract, are kept despite ART use, and it exponentially enhances immune activation in HIV infection.[21] This creates a vicious cycle where gut barrier immunity is compromised by the HIV promoting MT and immune activation, and ultimately disease progression.

Epidemiological studies have demonstrated that active illicit drug use, particularly cocaine, is associated with poor adherence to ART, reduced virologic and immunologic control, HIV disease progression and mortality.[23–25] Cocaine abuse pose deleterious effects for GI tract including weight loss, malnutrition, anorexia, reduced blood supply to enterocytes and dysbiosis of microbiome among PLWH.[26–28] In Miami, cocaine seems to be one of the illicit drugs of choice among PLWH. In a study analyzing the illicit drug use among PLWH in 8 U.S. cities, it was found that 40% of participants had heavy alcohol consumption or crack-cocaine use in the preceding 12 months.[29] Cocaine users frequently mix the drug with alcohol to enhance and prolong its effect.[30]

Vitamin D deficiency is also highly prevalent among PLWH; the prevalence rate ranges between 70-85%.[31,32] PLWH present similar risk factors for vitamin D deficiency as the general population including: gender (females have higher risk), limited sunlight exposure, black ethnicity, advanced age, poor dietary vitamin D intake, obesity, cardiovascular and renal disorders, diabetes mellitus, metabolic, hepatic steatosis, and alcohol consumption.[31,33–37] However, PLWH have additional risk factors for vitamin D deficiency due to action of HIV itself[33] and ART.[38] It has been well established that HIV promotes chronic inflammation and massive immune activation,[39,40] and individuals with vitamin D deficiency show an increase in pro-inflammatory cytokines, such as IL-6 and TNF-a,[41] and monocyte activation, which are considered key elements for HIV disease progression.

Several studies have tried to elucidate the relationship between vitamin D deficiency and MT among PLWH. A study found that optimal vitamin D levels were associated with low levels of MT and inflammation PLWH.[42] However, another study did

not find significant associations between vitamin D status and MT among PLWH.[43] Contradictions in the literature may be due to methodological differences. However, the interplay between cocaine use, MT, immune activation and vitamin D status has not been investigated. The purpose of this study is to examine the associations between MT, immune activation and vitamin D status in the context of cocaine abuse among PLWH.

Methods

Study Participants. Participants for this cross-sectional study were selected from the ongoing Miami Adult Studies in HIV (MASH) cohort. The MASH cohort is a longitudinal study examining the impact of substance abuse on HIV infection, HIV/HCV co-infection, and comorbidities with a focus on liver disease. The MASH cohort in Miami is unique, because it has a large number of non-Hispanic Blacks, Hispanics, women, and non-injector drug users, with predominantly heterosexual HIV- transmission etiology. The Inclusion Criteria for this study were to be HIV mono-infected adults, age 21 and older, having undetectable HIV viral load <200 copies/ml, CD4+ T cell count >200 cells/μl, and compliant with ART use (95%) and clinic visits for at least 6 months. Participants were also positive for cocaine use by questionnaire, blood metabolites and/or urine drug screen. The Exclusion Criteria included co-infection with hepatitis B or C, detectable HIV viral load, CD4+ T cell count<200 cells/μl, diagnosis of inflammatory bowel disease, pregnancy, hazardous alcohol use (AUDIT score >8) and current vitamin D supplementation. After applying these criteria, a total of 100 participants were eligible for this study.

Laboratory analyses. Plasma samples were collected at the baseline visit and used to assess LPS for MT, sCD14, sCD163, sCD27 for immune activation, and vitamin D levels. LPS was measured using the Pierce Chromogenic Endotoxin Quant Kit by Thermo Fisher Scientific (Rockford, IL, USA) according to manufacturer's instructions with the following modifications: samples were diluted 1:10 with endotoxin-free water to avoid interference with background color and preheated to 70°C for 15 minutes prior to analyses to inactivate plasma proteins. Triplicates were assessed for each sample. Immune activation markers were measured by using the analyte-specific bead-based Luminex multiplex immunoassays (EMD, Millipore Corporation). I-FABP, a measure of gut integrity damage, was assessed using the Human FABP2/I-FABP Immunoassay by R&D Systems (Minneapolis, MN, US) according to manufacturer's instructions with a sample dilution of 1:5. Quantification of 25(OH)D levels were measured in 25µl of sample by the 25-Hydroxy Vitamin D^{s} Enzyme Immunoassay by Immunodiagnostic Systems Ltd (Bolton, UK) according to manufacturer's instructions. According to the Endocrine Society Clinical Practice Guidelines, adequate levels of vitamin D in adults are considered to be >30ng/mL (75nmol/L), insufficient levels 21-29 ng/mL (52.5-72.5 nmol/L) and deficient levels at <20 ng/mL (50nmol/L).[44] For all assays, the coefficient of variation or CV% was calculated and CV's greater than 10% were reanalyzed.

Substance use. Cocaine use was assessed by self-report (use in the past 30 days) questionnaire, blood metabolite and urine drug screen (American Bio Medical®). Alcohol use was evaluated by the Alcohol Use Disorders Identification Test (AUDIT).

Anthropometric Assessments and Dietary Intake Data. Standing height (meters), weight (kg) and Body Mass Index (kg/m^2) were assessed from parent study. Dietary intake data were assessed by one 24-hour recall. Food models and portion size prompts were used. Dietary intake data were analyzed using a software program (NutriBase 9 Cybersoft).

Disease Progression and Medication History. Markers of disease progression (CD4+ T cell count and HIV viral load) were collected from the patient's medical chart, with participant written authorization.

Statistical Analyses

Descriptive statistics including mean, standard deviation, median, interquartile range, standard error and percentages were used to describe the data. Independent T-test and Mann-Whitney test were conducted to compare mean differences between cocaine users and non-users. Variables including HIV viral load, CD4+ T cell count, sCD14, sCD163 and sCD27 did not exhibit a normal distribution, therefore, they were log transformed. Pearson correlation was implemented to evaluate association among biomarkers of MT and immune activation. Multiple linear regression models were implemented to analyze associations between MT, immune activation, vitamin D status and cocaine use. The linear multivariable regression analysis also included a measure to check for multicollinearity known as variance inflation factor (VIF). All models had a VIF <5, therefore, multicollinearity was ruled out. Analyses were adjusted for potential confounding factors including age, gender, CD4+ T count, HIV viral load, obesity, seasonality of sunlight exposure (spring, summer, fall and winter). Logistic regression models included dependent MT marker at the following cut-offs: LPS ≤ or ≥ median. An

alpha less than 0.05 was considered significant. All statistical analyses were performed using SPSS version 23.

Results

Participant Characteristics

A total of 100 PLWH were selected for this study. The non-cocaine group had a greater proportion of participants (66%, n=66), than cocaine users (34%, n=34). For the two groups, mean age was 53.37±6.88 years old. Cocaine users were older than non-users, however, this difference was not statistically significant (P=0.338). Most participants were male (51%), and African American (66%). Participants had a mean duration of known HIV diagnosis of 17.21±7.28 years. All participants were on ART and had controlled viral load (<200 copies/mL) with a median of 48.5 (Interquartile Range IQR: 20-307) copies/ml. Cocaine users had higher AUDIT score than non-users (3.12±2.50 vs. 2.03±1.96 respectively, P=0.031), indicating greater alcohol use. Among all participants, 19% used marijuana, 11% used opioids, 1% used heroin and 2% used fentanyl (data not shown). Participants had a mean BMI of 28.70±5.76 kg/m^2 which classify them as overweight and obesity was found in 37% of participants.

Regarding vitamin D status, 50% of participants were considered deficient (≤20 ng/ml), 32% insufficient (21-29 ng/ml) and 18% sufficient (>30 ng/ml). There were not differences between cocaine users and non-users in terms of vitamin D deficiency or insufficiency. Most of the samples were collected during winter (47%) and fall (27%) seasons. Cocaine users had higher levels of MT than non-users (0.72±0.12 vs. 0.53±0.24 respectively, P<0.001). Also, cocaine users had higher levels of sCD14, sCD163, sCD27,

however, these differences did not reach statistical significance. Cocaine users had higher levels of I-FABP than non-users (2866.97±798.06 vs. 2337.56±1072.95, P=0.014) indicating greater intestinal damage. Mean of vitamin D levels did not differ between groups (Table 1).

Correlations

A Pearson product-moment correlation was run to determine the linear relationship between continuous variables. There was a positive correlation between BMI and CD4 + T cell count (r = 0.201, P =0.046), BMI and sCD14 levels (r =0.220, P=0.035), I-FABP and sCD27 levels (r = 0.213, P=0.048), sCD14 and sCD163 levels (r= 0.216, P=0.038), sCD14 and sCD27 levels (r = 0.344, P=0.001). There was a negative correlation between BMI and age (r = -0.231, P =0.021), a trend was observed for I-FABP levels and BMI (r = -0.198, P =0.05) and, vitamin D and I-FABP levels (r = -0.198, P=0.05).

Association between Cocaine use and Microbial Translocation

Multiple regression analysis was carried out to investigate whether cocaine use, age, gender, CD4+ T cell count, HIV viral load, BMI and gut integrity damage were associated with MT levels. The results of the regression analysis indicated that the model explained 65.3% of the variation and that the model was significant with F (7,13) = 3.501, P =0.025. Cocaine use was significantly associated with LPS levels (β=0.196; 95% CI= 0.003, 0.390; P=0.047). In addition, gender (male) (β=-0.313; 95% CI= -0.552, -0.075; P=0.014) and lower log transformed CD4+ T cell count (β=-0.359; 95% CI= -

0.637, -0.081; P=0.021) were significantly associated with LPS levels after controlling for age, log HIV viral load, BMI and gut integrity (Table 3).

Table 4 showed that among cocaine users, 73.5% exhibited LPS levels ≥ median 0.63 EU/mL. Also, having LPS levels ≥ median 0.63 EU/mL were significantly associated with gut integrity damage (P=0.02). None of the parameters for vitamin D levels, BMI and HIV disease progression were significantly associated with median levels of LPS.

Logistic regression analysis indicated that cocaine use and vitamin D deficiency were significantly associated with LPS ≥ median 0.63 (IQR:0.48-0.73) EU/mL (P<0.001 and P=0.043, respectively) after controlling for age, gender, obesity and winter season. The model correctly classified 68% of cases. In addition, cocaine users had 6.66 times higher odds to exhibit LPS levels above the median than non-users (OR: 6.658 95% CI: 2.429, 18.250; P<0.001). In addition, vitamin D deficient participants had 2.58 times higher odds to exhibit LPS levels ≥ median 0.63 EU/mL than participants with sufficient levels (OR: 2.58, 95% CI: 1.032, 6.470; P=0.043) (Table 5).

Association between Cocaine use and Immune activation

A multiple regression was conducted to investigate whether cocaine use, age, gender, CD4+ T cell count, HIV viral load, MT and gut integrity damage were associated with immune activation. The results of the regression analysis indicated that the model explained 82.6% of the variation and that the model was significant with F (7,11) = 7.441, P=0.002. Cocaine use was significantly associated with sCD163 levels (β=0.873; 95% CI= 0.399, 1.347; P=0.002). Age, gender (male) and lower CD4+ T cell count were

significantly associated with sCD163 levels (β=-0.056; 95% CI= -0.094, -0.018; P=0.038, β=-0.645; 95% CI= -1.221, -0.069; P=0.031 and β=-1.238; 95% CI= -1.937, -0.538; P=0.002, respectively). In addition, levels of LPS and I-FABP were significantly associated with sCD163 levels (β=1.352; 95% CI= 0.890, 1.937; P<0.001 and β=0.697; 95% CI= 0.218, 1.175; P=0.008, respectively) (Table 6). For 1-unit increase in LPS levels there was a 1.352 increase in sCD163 levels, indicating that MT promotes immune activation in our participants.

An additional multiple regression was carried out to investigate whether cocaine use, age, gender, CD4+ T cell count, HIV viral load, MT and gut integrity were associated with another biomarker of immune activation (sCD27). The results of the regression analysis indicated that the model explained 71.1% of the variation and that the model was significant with F (7,10) = 3.507, P =0.036. Cocaine use was significantly associated with sCD27 levels (β=0.477; 95% CI= 0.056, 0.899; P=0.030). Gender (male) and lower CD4+ T cell count were significantly associated with sCD27 levels (β=-0.819; 95% CI= -1.398, -0.241; P=0.010 and β=-0.002; 95% CI= -0.003, -0.001; P=0.007, respectively). In addition, levels of LPS levels were significantly associated with sCD27 levels (β=0.716; 95% CI= 0.302, 1.131; P=0.003) after controlling for age, gender, and HIV viral load (Table 7).

For 1-unit increase in LPS levels there was a 0.716 increase in sCD27 levels, indicating that MT promotes immune activation in our participants. No significant association was found between cocaine use, LPS and sCD14 levels.

Associations among Microbial Translocation, Immune activation, Cocaine use and Vitamin D status

Vitamin D deficiency

In participants who exhibited vitamin D deficiency, those who used cocaine (n=15) had higher levels of MT than those who did not use cocaine (t=-2.412 df=47 P=0.02). No statistical differences were found for the immune activation markers.

A multiple regression was conducted to investigate whether cocaine use, age, gender, obesity, winter season and smoking may significantly predict MT levels in vitamin D deficient participants. The results of the regression analysis indicated that the model explained 43% of the variation and that the model was significant with F (6,42) = 2.414, P =0.043. Cocaine use was significantly associated with LPS levels (β=0.162; 95% CI= 0.005, 0.318; P=0.043) in vitamin D deficient participants. In addition, gender (male) was significantly associated with LPS levels (β=-0.177; 95% CI= -0.315, -0.039, P=0.013) after controlling for age, obesity, seasonality (winter) and smoking (Table 8).

Vitamin D insufficiency

In participants who had vitamin D insufficiency, Mann Whitney test revealed that those who used cocaine (n=11) had higher levels of MT than participants who did not use cocaine (P=0.022). No statistical differences were found for the immune activation markers. A multiple regression was carried out to investigate whether cocaine use, age, gender, obesity, winter season and smoking could significantly predict MT levels in vitamin D insufficient participants. The results of the regression analysis indicated that

the model explained 38.6% of the variation and that the model showed a trend towards significance with F (6,23) = 2.414, P =0.05. Cocaine use was the only significant predictor for LPS levels (β=0.234; 95% CI= 0.068, 0.399; P=0.008) among vitamin D insufficient participants after controlling for age, obesity, winter season and smoking (Table 9).

Dietary Intake Characteristics in Study Participants

Table 10 describes the dietary intake characteristics of participants. The 2015-2020 USDA Dietary Guideline Recommendations for Americans[45] were implemented to compare total fat % of caloric intake (20-35% caloric intake), cholesterol (<300 mg/d), fiber (25.2-30.8g/d) and vitamin D intake (600 IU/d). We calculated the proportion of participants exceeding these recommendations. All participants exceeded recommendations for cholesterol intake (331.90 ± 317.43 mg/d) but no differences were observed between cocaine and non-cocaine users (P=0.887). For total fat % calories (20-35%) 46% participants exceeded recommendation, indicating that a pattern for a high fat diet was reported. All participants had inadequate intake for dietary fiber (14.66±11.99 gr/d) and vitamin D (18.55±35.18 IU/d) but no differences were observed between cocaine and non-cocaine users (P=0.458 and P=0.723, respectively). Interestingly, a great proportion of participants (67%) did not consume vitamin D as reported in the 24-hour recall.

Discussion

We examined associations between MT, immune activation and vitamin D status in the context of cocaine abuse among PLWH. Cocaine users had higher levels of MT and I-FABP than non-users. We also demonstrated that cocaine was a significant predictor for MT levels even after controlling for age, gender, HIV viral load, CD4+ T cell count, BMI and compromised gut integrity. However, this effect was not seen for markers of immune activation (sCD14, sCD163 and sCD27) and vitamin D levels. Our findings contradict the only study published so far investigating the effects of cocaine on microbiome and MT in PLWH.[28] This may be due to methodological differences in Volpe *et al*.[28] study, since they had a smaller sample size (n=32), large proportion of males (26/32), duration of ART use was not evaluated and cocaine use was self-reported, which could affect the ability of the study design to detect any difference in terms of MT.[28]

In addition, we showed that lower CD4+ T cell count was a significant predictor for MT which support the premise of the deleterious effects of HIV on the GI immune system.[46,47] It is important to mention that all our participants were on stable ART and controlled viral load (<200 copies/mL) which indicates that HIV continues to damage the GI tract even when it is controlled in blood, promoting MT, probably due to poor CD4+ T cell control and reconstitution in the intestine.[7,10,11,21]

Most of the cocaine users (73.5%) had high levels of MT [LPS ≥median of 0.63 (IQR:0.48-0.73) EU/mL]. Participants that had higher levels of MT also had higher levels of gut integrity damage when compared to those low levels of MT (2874.94±1334.18 vs. 2320.12±953.29 pg/mL, P=0.020) supporting previous findings about the role of gut

integrity damage and MT on HIV/SIV infections of humans and rhesus macaques.[48] During HIV infection, there is a massive loss of CD4+ T cells in the GI lamina propia. This loss also affects a subtype of CD4+ T cells called T helper 17 (Th17) in the intestine, which are responsible for the intestinal homeostasis, mucosal regeneration and integrity,[8,9] creating an environment for damage of gut integrity, increased intestinal permeability and, consequently, MT.

A novel finding from this study was that cocaine users were 6.66 times more likely to exhibit high MT levels than non-users, which indicates the damaging effect of cocaine in the GI mucosa, promoting intestinal inflammation and ultimately high levels of MT. The exact mechanism behind the impact of cocaine use in the intestine has not been completely elucidated. However, past studies have suggested that cocaine has the ability to dysregulate the expression of the tight junction proteins in the intestinal epithelium promoting damage on gut integrity and hyperpermeability, which generates gut inflammation due to the release of pro-inflammatory cytokines (IL-18 and IL-1b).[49,50]

Our analyses demonstrated that cocaine, MT levels, damaged gut integrity and lower CD4 T cell count drive immune activation, marked by higher levels of sCD163 and sCD27, indicating that cocaine use and HIV seemed to compromise gut integrity, promote MT and, therefore, immune activation despite ART use and viral suppression. To the best of our knowledge, this is the first study demonstrating these associations.

Regarding vitamin D status, half of our participants were vitamin D deficient and one third were insufficient. These prevalence rates are comparable to the ones reported by other cohort studies showing a high prevalence of vitamin D deficiency in PLWH in the US.[35,51]

Our cohort is comprised of a high proportion of African Americans and Hispanics and almost half were obese, which represents a risk factors for vitamin D deficiency.[31]

We demonstrated that in participants with impaired vitamin D status, those who used cocaine had higher MT levels than non-users and that cocaine use was a significant predictor for MT levels but not for immune activation, which suggest that even with significant MT, the immune response is compromised in this population. To the best of our knowledge, this is the first study showing that cocaine use is associated with MT when vitamin D status is impaired.

Also, we showed that individuals that had vitamin D deficiency were 2.58 times more likely to exhibit high MT levels than participants who did not have deficiency. Our finding is supported by Garcia-Alvarez *et al.*[42] in which lower levels of MT were associated with sufficient levels of vitamin D in PLWH.[42] On the other hand, our data conflict with results showed by Missailidis *et al.*[43] in which no association was found between vitamin D deficiency and MT or immune activation in PLWH.[43] This conflict may be due to differences in methodology, including a small proportion of participants with vitamin D deficiency (9%), vitamin D insufficiency was not considered, 25% of participants were taking vitamin D supplements (400 to 1400 IU/day), and socio-demographic characteristics of study participants, as this study was conducted in Sweden.[43] In our study, 50% of participants had vitamin D deficiency and were mostly African Americans.

Our dietary data demonstrated that PLWH seemed to follow a dietary pattern characterized by a high fat, high cholesterol and low fiber diet. These findings support

previous studies showing that PLWH had an increase of total fat, saturated fat and cholesterol and low fiber intake,[52–55] posing a risk for the development of chronic conditions, including cardiovascular disease, obesity and inflammation. Our participants had inadequate vitamin D intake and a great proportion of them did not consume vitamin D at all. This may explain, in part, why half of our participants were vitamin D deficient. Our findings suggest that personalized dietary interventions that focus on healthy eating and physical activity may be beneficial in the management of HIV.

Our study had some limitations. The cross-sectional design does not allow us to establish causation. Immune activation markers were available for only 85 study participants. Dietary intake data were assessed by one 24-hour recall. The analysis of a 24-hour food recall was limited by important factors such as the interviewee's memory, concepts of food portion size, and current versus historical food consumption.

The results of this study need to be interpreted with caution due to the specific geographical, socioeconomic and ethnical composition of study participants, as this study included mostly minorities living in poverty, but on stable ART and controlled viral load. However, this study provides important clinical evidence that supports the development of strategies to ameliorate cocaine use and vitamin D deficiency among PLWH.

Conclusion

Cocaine abuse and vitamin D deficiency are relevant issues among PLWH. Our data suggested that cocaine abuse and impaired vitamin D status are associated with MT and immune activation in PLWH despite ART use and viral suppression. Therefore, there is a critical need to develop effective interventions that target lifestyle modifications,

optimal nutrition and counseling on drug abuse, to more effectively decrease MT and ultimately immune activation among PLWH.

Table 1. Participants Characteristics

Characteristics	Total (n=100)	Cocaine Non-users (n=66)	Cocaine Users (n=34)	P value
Age, years	53.37±6.88	52.89±7.38	54.29±5.77	0.338
Gender (male)	51(51)	35 (35)	16 (16)	0.674
Race/Ethnicity				
African American	66 (66)	38 (38)	28 (28)	0.011
Hispanic	29 (29)	23 (23)	6 (6)	0.103
White	28 (28)	24 (24)	4 (4)	0.100
Other	6 (6)	4 (4)	2 (2)	0.603
CD4+ T (cells/μL)	630.08±344.07	628.80±298.12	632.64±426.63	0.963
HIV VL (copies/mL)	48.50 (20-307)	59 (31.5-140.25)	40 (26.5-136)	0.497
Duration known HIV (years)	17.21±7.28	18.01±7.23	15.64±7.14	0.150
AUDIT score	2.4±2.19	2.03±1.96	3.12±2.50	0.031
Smoking (yes)	47 (47)	24 (24)	23 (23)	0.300
BMI (kg/m^2)	28.70±5.76	28.89±5.82	28.33±5.71	0.648
Obesity (BMI>30kg/m^2)	37 (37)	24 (24)	13 (13)	0.511
LPS (EU/mL)	0.59±0.23	0.53±0.24	0.72±0.12	<0.001
sCD14 (pg/mL)	1038.47±424.2	1021.81±402.55	1080.73±480.7	0.552
sCD27 (pg/mL)	7.95±2.94	7.70±2.52	8.58±3.81	0.291
sCD163 (pg/mL)	635.71±418.52	613.25±431.73	692.74±385.01	0.393
I-FABP pg/mL	2515.83± 1072.95	2337.56±1072.95	2866.97±798.06	0.014
Vitamin D ng/mL	20.97 (16.96-27.84)	19.80 (16.68-26.60)	23.07 (19.23-29.59)	0.087
Vitamin D Status				
Deficient ≤20 ng/mL	50 (50)	35 (35)	15 (15)	0.527
Insufficient 21-29 ng/mL	32 (32)	21 (21)	11 (11)	1.000
Sufficient ≥30 ng/mL	18 (18)	10 (10)	8 (8)	0.411
Seasonality				
Winter	43 (43)	30 (30)	13 (13)	0.529
Fall	27 (27)	18 (18)	9 (9)	1.000
Spring	22 (22)	14 (14)	8 (8)	0.803
Summer	8 (8)	4 (4)	4 (4)	0.439

Bold indicates statistical significance at P<0.05. Data for continuous variables are summarized as mean ± standard deviation and median (interquartile range IQR) with statistical comparison using T test and Mann-Whitney. Categorical variables are summarized as count n (%) to test for proportion using Chi square. HIV VL: HIV Viral Load; BMI: body mass index; LPS: Lipopolysaccharide; sCD14: soluble CD14; sCD27: soluble CD27; sCD163: soluble CD163; I-FABP: Intestinal Fatty Acid Binding Protein.

Table 2. Pearson Correlation Coefficients

Variable	Age	CD4+	BMI	I-FABP	LPS	sCD14	sCD163	sCD27	Vit. D
Age	1								
CD4+	-.09	1							
BMI	-.23*	.20*	1						
I-FABP	.06	-.01	-.19*	1					
LPS	-.06	.06	-.17	.14	1				
sCD14	.01	-.08	.22*	-.03	-.01	1			
sCD163	-.11	-.03	.19	-.00	-.08	.21*	1		
sCD27	.18	-.15	-.10	.21*	-.17	.34*	.41*	1	
Vit. D	.13	.02	.04	-.19*	-.05	.06	.05	.15	1

*Correlation is significant at the 0.05 level (2-tailed).

Table 3. Multiple Linear Regression Analysis Predicting Association Between Cocaine Use And Microbial Translocation

Variable	β	SE	95% CI	P-value
Age	-0.012	0.008	-0.029, 0.006	0.170
Gender (male)	-0.313	0.111	-0.552, -0.075	0.014
Log transformed CD4+ T cell count (cells/μL)	-0.359	0.129	-0.637, -0.081	0.015
Log transformed HIV viral load (copies/mL)	0.076	0.056	-0.044, 0.197	0.193
BMI (kg/m^2)	-0.015	0.007	-0.029, 0.002	0.059
Cocaine use (yes)	0.196	0.089	0.003, 0.390	0.047
Log transformed I-FABP (pg/mL)	0.171	0.103	-0.053, 0.394	0.123

Bold indicates statistical significance at P<0.05

Table 4. Differences Between Median Groups Of Lps And Cocaine Use, Gut Integrity And Vitamin D Levels

Variable	LPS ≤0.63 (EU/mL)	LPS ≥0.63 (EU/mL)	P-value
Cocaine use (yes) (%)	26.5	73.5	<0.001
I-FABP (pg/mL)	2320.125 ± 953.293	2874.947±1334.180	0.020
Vitamin D (ng/mL)	24.320 ± 7.907	22.256 ± 8.190	0.205
BMI (kg/m^2)	29.290 ± 5.518	28.091 ± 5.990	0.300
CD4+ T cell count (cells/μL)	609.330±325.752	652.130± 364.678	0.540
HIV viral load (copies/mL)	83.630±74.578	81.43± 82.090	0.950

Bold indicates statistical significance at P<0.05

Table 5. Logistic Regression Analyses Predicting the Likelihood of High Microbial Translocation (LPS ≥ 0.63 EU/mL) in Study Participants

Variable	β	SE	OR	95% CI OR	P-value
Age	-0.037	0.034	1.180	0.277, 0.963	0.277
Gender (male)	-0.104	0.475	0.902	0.356, 2.286	0.496
Cocaine (yes)	1.896	0.514	6.658	2.429, 18.250	<0.001
Obesity	-0.088	0.521	0.915	0.330, 2.541	0.865
Vitamin D deficiency	0.949	0.468	2.584	1.032, 6.470	0.043
Season Winter	0.513	0.465	1.670	0.671, 4.154	0.713

Bold indicates statistical significance at P<0.05

Table 6. Multiple Linear Regression Analysis of The Effect of Cocaine Use and Microbial Translocation on Immune Activation (Log Transformed sCD163)

Variable	β	SE	95% CI	P-value
Age	-0.056	0.017	-0.094, -0.018	0.008
Gender (male)	-0.645	0.262	-1.221, -0.069	0.031
Log transformed CD4+ T cell count (cells/μL)	-1.238	0.318	-1.937, -0.538	0.002
Log transformed HIV viral load (copies/mL)	0.011	0.147	-0.313, 0.336	0.940
Cocaine use (yes)	0.873	0.215	0.399, 1.347	0.002
Log transformed LPS (EU/mL)	1.352	0.210	0.890, 1.815	<0.001
Log transformed I-FABP (pg/mL)	0.697	0.217	0.218, 1.175	0.008

Bold indicates statistical significance at P<0.05

Table 7. Multiple Linear Regression Analysis on The Effect of Cocaine Use and Microbial Translocation on Immune Activation (Log Transformed sCD27)

Variable	β	SE	95% CI	P-value
Age	-0.019	0.017	-0.057, 0.019	0.289
Gender (male)	-0.819	0.260	-1.398, -0.241	0.010
CD4+ T cell count (cells/μL)	-0.002	0.001	-0.003, -0.001	0.007
HIV viral load (copies/mL)	-0.001	0.002	-0.005, 0.003	0.725
Cocaine use (yes)	0.477	0.189	0.056, 0.899	0.030
Log transformed LPS (EU/mL)	0.716	0.186	0.302, 1.131	0.003
Log transformed I-FABP (pg/mL)	0.399	0.199	-0.044, 0.843	0.073

Bold indicates statistical significance at P<0.05

Table 8. Multiple Linear Regression Analysis Predicting Association Between Cocaine Use and Microbial Translocation in Vitamin D Deficient Participants

Variable	β	SE	95% CI	P-value
Age	-0.002	0.005	-0.011, 0.007	0.656
Gender (male)	-0.177	0.068	-0.315, -0.039	0.013
Cocaine use (yes)	0.162	0.078	0.005, 0.318	0.043
Obesity (BMI>30kg/m^2)	-0.045	0.072	-0.190, 0.101	0.541
Season Winter	0.070	0.068	-.067, 0.207	0.307
Smoking (yes)	0.032	0.073	-0.114, 0.179	0.658

Bold indicates statistical significance at P<0.05

Table 9. Multiple Linear Regression Analysis of The Effect of Cocaine Use and Microbial Translocation in Vitamin D Insufficient Participants

Variable	β	SE	95% CI	P-value
Age	-0.011	0.006	-0.022, 0.001	0.072
Gender (male)	-0.140	0.080	-0.306, 0.026	0.093
Cocaine use (yes)	0.234	0.080	0.068, 0.399	0.008
Obesity (BMI>30kg/m^2)	-0.122	0.093	-314, 0.07	0.202
Season Winter	0.068	0.073	-0.083, 0.220	0.362
Smoking (yes)	-0.100	0.082	-0.270, 0.070	0.236

Bold indicates statistical significance at P<0.05

Table 10. Dietary Intake Characteristics

Nutrient	Recommended Range*	Total (n=100)	Cocaine non-users (n=66)	Cocaine users (n=34)	P-value
Calories (cal)	1800-2200	1991.2±1233.3	2017.8±1191.7	1937.8±1329.9	0.763
Protein (gr)	46-56	88.4±96.4	92.43±112.18	80.39±53.15	0.470
Carbohydrate (gr)	130	242.5±172.3	248.83±175.93	230.06±166.90	0.612
Total Fat (gr)	-	75.9±51.0	74.8±46.1	78.1±60.4	0.783
Total Fat intake 35%**	20-35	46	60.9	39.1	0.142
Cholesterol (mg)	300	341.9±317.4	338.9±343.1	347.8±263.46	0.887
Dietary Fiber (gr)	25.2-30.8	14.6±11.9	15.0±11.9	13.3±12.1	0.458
Vitamin D (IU)	600	18.5±35.1	19.4±36.2	16.8±33.3	0.723
Inadequate Vitamin D intake***	-	67 (67)	44 (44)	23 (23)	0.473

* Recommended ranges based on adults who consume 1800-2200 kcals daily. Variations exits by age, sex and activity level.
**Participants with high fat diet (>35% Calories from Total Fat Intake)
***Participants with inadequate Vitamin D intake (0 IU/day)

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CHAPTER IV: MICROBIAL TRANSLOCATION, COCAINE USE AND OBESITY ARE ASSOCIATED WITH INFLAMMATION AMONG DRUG USERS LIVING WITH HIV

Abstract

Background. HIV is considered a chronic inflammatory disease. Immune activation and inflammation persist even after long-term ART exposure and sustained viral suppression. Cocaine abuse facilitates inflammation and disease progression in People Living with HIV (PLWH). Persistent systemic inflammation has been associated with impaired vitamin D status among PLWH. The purpose of this study was to examine associations between inflammation and vitamin D status in the context of cocaine abuse among PLWH.

Methods. One hundred cocaine users and non-users living with HIV were selected from the ongoing Miami Adult Studies in HIV (MASH) cohort. Cocaine use was assessed by self-report, urine screen and blood metabolites. Blood samples were collected to assess markers of inflammation (hs-CRP, IL-6 and TNF-α), immune activation (sCD14 and sCD163), microbial translocation (LPS), and vitamin D status. Data on socio-demographic characteristics, anthropometrics and disease progression were collected by the MASH cohort. Descriptive statistics, independent t-test, multiple linear regression models, and logistic regressions were conducted to analyze the data.

Results. A total of 100 participants consisted of 66 cocaine non-users and 34 cocaine users. The mean age of the participants was 53.37±6.88 years old. Most participants were

male (51%), and African Americans (66%). All participants were on ART and had controlled viral load (<200 copies/mL). Half of the participants were considered vitamin D deficient (≤20 ng/mL) and 32% insufficient (21-29 ng/mL). Overall, participants had a mean hs-CRP levels of 3.70±3.57 mg/dL. Inflammation, defined as hs-CRP >3mg/dL was found in 41% of all participants. LPS was a significant predictor of inflammation (P=0.033) in all participants. In cocaine users, alcohol use was associated with higher hs-CRP levels (P=0.037), and BMI was a significant predictor for inflammation (OR:1.256 95% CI: 1.013, 1.557; P=0.038). No association was found between vitamin D status and systemic inflammation in either the cocaine users or non-users.

Conclusion. Inflammation was associated with MT, cocaine use and higher BMI among PLWH despite ART use and viral suppression. Despite widespread vitamin D deficiency, vitamin D status was not associated with inflammation. There is a critical need to develop effective approaches that target drug abuse and obesity among PLWH.

Key words. Inflammation, cocaine, vitamin D, HIV

Introduction

Immune activation is defined as a phenomenon characterized by complex processes that stimulate and activate the immune system to generate constant inflammation.[1] During the course of HIV disease, low grade chronic inflammation develops with continuous activation of immune cells and cytokines, which can be stimulated for years.[2] It has been suggested that during HIV infection there is an imbalance between cytokines resulting in an overproduction of pro-inflammatory Th2 type and underproduction of Th1 type contributing to immune activation and inflammation.[3] Immune activation and inflammation persist even after long-term ART exposure and sustained viral suppression in the GI tract, affecting the ability of the immune system to reconstitute CD4+ T cell in this system.[4–6] It has been proposed that persistent systemic inflammation in people living with HIV (PLWH) with suppressed viral load is associated with the damage of the gastrointestinal mucosa and microbial translocation (MT).[7,8]

Several studies have shown that products of MT, specifically Lipopolysaccharide (LPS), is present in the circulation of PLWH induce inflammation and immune activation, and predict clinical outcomes of HIV infection.[9–13] Therefore, immune activation and inflammation has been identified as key determinants that increase morbidity and mortality among PLWH regardless viral suppression and ART exposure.[13,14]

Epidemiological studies have also demonstrated that illicit drug use, particularly cocaine, is associated with poor adherence to ART, reduced virologic and immunologic control, systemic inflammation, HIV disease progression and mortality.[15–18] Cocaine

reduces CD4+ T cell count and increases HIV viral load[17] potentially through its action on thymic endocrine function, preventing immune recovery.[19]

Vitamin D deficiency is highly prevalent among PLWH, ranging between 70-85%.[20,21] PLWH present similar risk factors for vitamin D deficiency as healthy, un-infected individuals, including gender (females have higher risk), limited sunlight exposure, black ethnicity, advanced age, poor dietary vitamin D intake, obesity, cardiovascular and renal disorders, diabetes mellitus, metabolic, hepatic steatosis, and alcohol consumption.[20,22–26] It has been well established that HIV promotes chronic inflammation and massive immune activation,[3,27] and individuals with vitamin D deficiency show an increase in pro-inflammatory cytokines, such as IL-6 and TNF-a,[28] and monocyte activation, which are considered key elements for HIV disease progression.

Other studies have tried to elucidate the relationship between vitamin D deficiency and inflammation among PLWH. One study found that optimal vitamin D levels were associated with low levels of MT and inflammation among PLWH.[29] In addition, PLWH with vitamin D deficiency exhibit high levels of systemic inflammation as measured by IL-6 and TNFα.[28] However, a different study did not find significant associations between vitamin D status and other inflammatory biomarkers including highly sensitive C reactive protein (hs-CRP), IL-17, IL-4 and IL-10.[30] Contradictions in the literature may be due to methodological dissimilarities. However, the interplay between cocaine use, inflammation and vitamin D status has not been adequately investigated. The purpose of this study is to examine the associations between inflammation and vitamin D status in the context of cocaine abuse among PLWH.

Methods

Study Participants. Participants for this cross-sectional study were selected from the ongoing Miami Adult Studies in HIV (MASH) cohort. The MASH cohort is a longitudinal study investigating the impact of substance abuse on HIV and HCV infection, HIV/HCV co-infection, and comorbidities. The MASH cohort in Miami is unique because it has a large number of non-Hispanic Blacks, Hispanics, women, and non-injecting drug users, with predominantly heterosexual HIV- transmission etiology. The Inclusion Criteria for this study were to be HIV mono-infected adults, age 21 and older, having undetectable HIV viral load <200 copies/ml, CD4+ T cell count >200 cells/μl, and compliant with ART use (95%) and clinic visits for at least 6 months. Participants were also positive for cocaine use by questionnaire, blood metabolites and/or urine toxicology. The Exclusion Criteria included co-infection with hepatitis B or C, detectable HIV viral load, CD4+ T cell count<200 cells/μl, diagnosis of inflammatory bowel disease, pregnancy, hazardous alcohol use (AUDIT score >8) and current vitamin D supplementation. After eligibility criteria, the samples and data of the first 100 participants who were eligible for this study were utilized.

Laboratory analyses. Plasma samples were collected at the baseline visit and used to assess inflammatory markers including IL-6, TNFα and hs-CRP, marker of MT, immune activation (sCD14 and sCD163) and vitamin D levels. Inflammatory and immune activation markers were measured using the analyte-specific bead-based Luminex multiplex immunoassays (EMD, Millipore Corporation). Marker concentrations were calculated using a standard curve derived from the known reference concentration

supplied by the manufacturer. Concentrations obtained below the sensitivity limit of detection (LOD) of the method were recoded to the mid-point between zero and the LOD for that analyte for statistical comparisons. Levels of hs-CRP were assessed by LabCorp®. Inflammation was defined as hs-CRP >3 mg/dL, based on the Centers for Disease Control and Prevention (CDC)/American Heart Association guidelines.[31]

Quantification of 25(OH)D levels were measured in 25ul of sample by the 25-Hydroxy Vitamin D^{s} Enzyme Immunoassay by Immunodiagnostic Systems Ltd (Bolton, UK) according to manufacturer's instructions. According to the Endocrine Society Clinical Practice Guidelines, adequate levels of vitamin D in adults are considered to be >30ng/mL (75nmol/L), insufficient levels 21-29 ng/mL (52.5-72.5 nmol/L) and deficient levels at <20 ng/mL (50nmol/L).[32] Microbial translocation marker, LPS, was measured using the Pierce Chromogenic Endotoxin Quant Kit by Thermo Fisher Scientific (Rockford, IL, USA) according to manufacturer's instructions with the following modifications: samples were diluted 1:10 with endotoxin-free water to avoid interference with background color and preheated to 70°C for 15 minutes prior to analyses to inactivate plasma proteins. Triplicates were assessed for each sample. For all assays, the coefficient of variation or CV% was calculated and CV's greater than 10% were reanalyzed.

Substance use. Cocaine use was assessed by self-report (use in the past 30 days) questionnaire, blood metabolites and urine drug screen (American Bio Medical®). Alcohol use was evaluated by the Alcohol Use Disorders Identification Test (AUDIT).

Anthropometric Assessments. Standing height (meters), weight (kg) and Body Mass (kg/m^2) Index were collected from parent study's database.

Disease Progression and Medication History. Markers of disease progression (CD4+ T cell count and viral load) were collected from the patient's medical chart with the participant's written authorization.

Statistical Analyses

Descriptive statistics including mean, standard deviation, median, interquartile range (IQR), standard error and percentages were used to describe the data. Independent T-test and Mann-Whitney test were conducted to compare mean differences between cocaine users and non-users. The variable HIV viral load and CD4+ T cell count did not exhibit a normal distribution; therefore, it was log transformed. Cytokine (IL-6 and TNF-α) concentrations were positively skewed due to high proportions of values under the limit of detection (LOD); these were replaced with the LOD minus a decimal. Pearson correlations were implemented to evaluate association among biomarkers of inflammation, immune activation and HIV disease progression. Multiple linear regression models were implemented to analyze associations between inflammation, vitamin D status and cocaine use. The linear multivariable regression analysis also included a measure to check for multicollinearity known as variance inflation factor (VIF). All models had a VIF <5, therefore multicollinearity was ruled out. Analyses were adjusted for potential confounding factors including age, gender, CD4+ T count, HIV viral load, AUDIT score and smoking.

Logistic regression models included dependent variable hs-CRP at the following cut-offs: hs-CRP <3mg/dL or ≥3mg/dL. An alpha less than 0.05 was considered significant. All statistical analyses were performed using SPSS version 23.

Results

Participant Characteristics

A total of 100 PLWH were selected for this study. The proportion of cocaine non-user was greater (66%, n=66), than of cocaine users (34%, n=34). For the two groups, mean age was 53.37±6.88 years old. Cocaine users were older than non-users, however, this difference was not statistically significant (P=0.338). Most participants were male (51%), and African American (66%). Participants had a mean duration of known HIV diagnosis of 17.21±7.28 years. All participants were on ART and had controlled viral load (<200 copies/mL) with a median of 48.5 (Interquartile range: 20-307) copies/mL. Among all participants, 19% used marijuana, 11% used opioids, 1% used heroin and 2% used fentanyl (data not shown). Cocaine users had higher AUDIT score than non-users (3.12±2.50 vs. 2.03±1.96 respectively, P=0.031), indicating greater alcohol use. Participants had a mean BMI of 28.70±5.76 kg/m^2 which classify them as overweight; obesity was found in 37% of participants.

In regard to vitamin D status, 50% of participants were considered deficient (≤20 ng/mL), 32% insufficient (21-29 ng/mL) and 18% sufficient (>30ng/mL). The majority of samples were collected during winter (47%) and fall (27%) seasons. Cocaine users had higher levels of MT than non-users (0.72±0.12 vs. 0.53±0.24, P<0.001). Also, cocaine

users had higher levels of sCD14 and sCD163, however, these differences did not reach statistically significant. Vitamin D levels did not differ between groups (Table 1).

Overall, participants had a mean hs-CRP levels of 3.70±3.57 mg/dL, which meets the criteria for inflammation, defined as hs-CRP ≥ 3mg/dL. Inflammation was found in 41% of all participants. There were no statistical significance differences in terms of IL-6 and TNF-α between cocaine users and non-users.

Correlations

A Pearson product-moment correlation was run to determine the linear relationship between continuous variables. There was a positive correlation between BMI and CD4+ T cell count (r = 0.201, P =0.046), BMI and sCD14 levels (r =0.220, P=0.035), BMI and hs-CRP levels(r = 0.225, P=0.028), and sCD14 and TNF-α levels (r = 0.260, P=0.019), indicating that BMI was associated with both inflammation and immune activation. There was an inverse correlation between BMI and age (r = -0.231, P =0.021). Vitamin D levels were not correlated with inflammatory markers.

Association among Cocaine use, Microbial Translocation and Vitamin D status with Inflammation

A logistic regression model was conducted to evaluate associations among cocaine use, MT and vitamin D status with inflammation (hs-CRP ≥ 3mgdL). The model showed that LPS was a significant predictor of inflammation (P=0.033) after controlling for age, gender, CD4+ T cell count and cocaine use. The model correctly classified

64.6% of cases. Vitamin D deficiency did not show a significant association with inflammation (Table 3).

Table 4 showed that participants who exhibited inflammation (hs-CRP ≥ 3mg/dL) had higher BMI (P=0.045), higher IL-6 levels (P=0.049) and immune activation as measured by sCD163 levels (P=0.024) when compared with participants who did not exhibit inflammation (hs-CRP<3 mg/dL). In addition, there were no statistical differences between hs-CRP groups in terms of vitamin D levels.

Association among HIV, Substance Use and Inflammation in Cocaine users living with HIV

Multiple regression analysis was carried out to investigate whether HIV disease progression markers and substance use predict inflammation in cocaine users. The results of the regression indicated that the model explained 92.7% of the variation and that the model was significant with F (6,8) = 17.940, P =0.049. Age was significantly associated with hs-CRP levels (β=0.774; 95% CI= 0.147, 1.401; P=0.034). In addition, AUDIT score was significantly associated with hs-CRP levels (β=1.158; 95% CI= 0.175, 2.141; P=0.037) after controlling for gender, CD4+ T cell count, log transformed HIV viral load and smoking. For 1-unit increase in AUDIT score, there was a 1.158 increase in hs-CRP levels in cocaine users living with HIV (Table 5).

Logistic regression analyses indicated that BMI was significantly associated with inflammation as measured by hs-CRP ≥ 3mg/dL in cocaine users (P=0.038) after controlling for age, log transformed CD4+ T cell count, HIV viral load <20 copies/mL,

and smoking. The model correctly classified 69.7% of cases. In addition, for 1-unit increase in BMI, there was a 1.256 increase in inflammation in cocaine users [Odds Ratio (OR): 1.256 95% CI: 1.013, 1.557; P=0.038] (Table 6). Vitamin D status, including deficiency and insufficiency, was not associated with inflammatory markers in our participants.

Discussion

We examined the associations between inflammation, MT and vitamin D status in the context of cocaine abuse among PLWH. Our participants exhibited a mean hs-CRP of 3.70±3.57 mg/dL which meets criteria for inflammation, and over 40% had high levels of inflammation. Cocaine users had higher levels of hs-CRP than non-users, however, this difference did not reach statistical significance. Nevertheless, we observed that inflammation was prevalent in our participants, despite ART use and viral suppression. This is supported by other studies showing residual immune activation and inflammation among PLWH even after the introduction of ART.[4,6,33–35] Over the past decade, evidence has shown that HIV infection is a chronic inflammatory disease that has features similar to those of other inflammatory non-infectious conditions.[36]

We found that participants who exhibit inflammation (hs-CRP ≥ 3mg/dL) had higher BMI, IL-6 and sCD163 levels when compared to those who did not exhibit inflammation. Moreover, levels of MT were associated with inflammation in our participants, which confirms the findings of other studies in PLWH on ART and with undetectable viral load.[37–39] It has been well established that damaged gut integrity in HIV infection is a major driver for MT. This process allows the passage of products of

MT, such as LPS, into systemic circulation, initiating a strong immune response and chronic inflammation.[10,12,35,40–42] Although, cocaine use was not directly associated with inflammation in our study, we demonstrated that cocaine users had higher MT levels than non-users (Chapter 3); this could indicate the possible role of cocaine in damaging gut integrity, promotion of MT, and ultimately causing systemic inflammation.[43,44]

We further analyzed cocaine users living with HIV. We found that aging and alcohol use were associated with hs-CRP levels. This finding corroborates previous association of substance use, particularly alcohol and cocaine, with inflammation among PLWH.[45] Alcohol and cocaine are often used together,[46] and this combination provides an additive effect, which causes immune and neurological toxicity and dependence severity.[47]

In addition, we showed that higher BMI independently predicted inflammation (hs-CRP ≥ 3mg/dL) in cocaine users living with HIV. This is a novel finding that indicates that cocaine use and higher BMI among PLWH provide a synergistic effect that not only promotes systemic inflammation and HIV disease progression, but it potentiates the risk for non-AIDS related conditions, particularly cardiovascular diseases (CVD). Most importantly, our finding that higher BMI was associated with inflammation in cocaine users living with HIV, and that these are 1.256 times more likely to have high inflammation than cocaine users with lower BMI, may be of clinical relevance for the prevention of excessive weight gain in this population. In our cohort, cocaine users living with HIV had a mean BMI of 28.33±5.71 kg/m^2, which classified most of them as overweight and 38.2% were obese, which is comparable with the overweight and obesity rates in the general US population.[48]

It has been suggested that in the general population, cocaine use promotes high levels of hs-CRP, promotes coronary vasoconstriction, high blood pressure which may increase the risk for CVD events.[49] Studies also showed significant association between cocaine use and obesity with increased risk for CVD events among PLWH.[50–53] This identifies PLWH who use cocaine as a vulnerable population for sustained inflammation caused by HIV itself,[1] and weight gain, which may increase risks for CVD and other non-AIDS co-morbidities.

It is important to mention that cocaine use was not associated with other inflammatory markers including IL-6 and TNF-α. Other studies, however, have shown that cocaine use was associated with levels of IL-6 and TNF-α, which are considered inflammatory markers.[54,55] These discrepancies may be due to that in our study, we had a high proportion of values under levels of detection for IL-6, and TNF-α, and that the inflammatory processes associated with HIV were ameliorated by controlled viral load in our participants.

In our study, vitamin D status was not associated with inflammatory biomarkers and the rate of vitamin D deficiency did not differ between cocaine users and non-users. This finding supports some studies in the literature showing no association between vitamin D status and inflammation among PLWH.[30,56,57] In fact, Hoffman *et al*.[56] demonstrated that even after vitamin D supplementation (2,000 IU for 12 weeks) in those who were insufficient, the inflammatory biomarkers, hs-CRP and TNF-α did not show a significant improvement.[56] On the other hand, different studies showed a positive association between vitamin D status and inflammation among PLWH.[28,29,58] These studies, however, were conducted on PLWH on ART and ART-naïve, and some did not

include individuals with controlled viral load, making direct comparisons with our study difficult. Also, discrepancies may be due to methodological differences, including, but not limited, to sample size, cross-sectional study design, and socio-demographic characteristics of participants.

Our study had limitations. The cross-sectional design does not allow us to establish causation. Our analysis of cytokines (IL-6 and TNF-α) was restricted by large proportions of undetectable values, which may have affected findings and were only available for 85 study participants. The results of this study need to be interpreted with caution because of the specific geographical and ethnical characteristics of our cohort. In addition, our population, despite living in poverty, were on stable ART and had suppressed viral load. However, this study provides important clinical evidence that supports the development of strategies to ameliorate cocaine abuse and obesity among PLWH.

Conclusion

Our data showed that MT was independently associated with inflammation in PLWH. In addition, high BMI combined with cocaine use were associated with inflammation in PLWH despite ART use and viral suppression. Vitamin D status was not associated with inflammation in our participants. Therefore, there is a need to develop effective strategies focused on lifestyle modifications, including weight management and counseling on drug abuse, to more effectively decrease systemic inflammation, and ultimately, reduce incidence of chronic inflammatory diseases common in PLWH

Table 1. Participant Characteristics

Characteristics	Total (n=100)	Cocaine Non-users (n=66)	Cocaine Users (n=34)	P value
Age, years	53.37±6.88	52.89±7.38	54.29±5.77	0.338
Gender (male)	51(51)	35 (35)	16 (16)	0.361
Race/Ethnicity				
African American	66 (66)	38 (38)	28 (28)	0.011
Hispanic	29 (29)	23 (23)	6 (6)	0.103
White	28 (28)	24 (24)	4 (4)	0.100
Other	6 (6)	4 (4)	2 (2)	0.603
CD4+ T cells/μL	630.08±344.07	628.80±298.12	632.64±426.63	0.963
HIV Viral Load copies/ml	48.50 (20-307)	59 (31.5-140.25)	40 (26.5-136)	0.497
Duration of known HIV (years)	17.21±7.28	18.01±7.23	15.64±7.14	0.150
AUDIT score	2.4±2.19	2.03±1.96	3.12±2.50	0.031
Smoking (yes)	47 (47)	24 (24)	23 (23)	0.300
BMI, kg/m^2	28.70±5.76	28.89±5.82	28.33±5.71	0.648
Obesity (BMI>$30kg/m^2$)	37 (37)	24 (24)	13 (13)	0.511
LPS EU/mL	0.59±0.23	0.53±0.24	0.72±0.12	<0.001
sCD14 (pg/mL)	1038.47±424.20	1021.81±402.55	1080.73±480.73	0.552
sCD163 pg/mL	635.71±418.52	613.25±431.73	692.74±385.01	0.393
hs-CRP mg/dL	3.70±3.57	3.59±3.64	3.95±3.49	0.638
hs-CRP ≥ 3mg/dL	41 (41)	25 (25)	16 (16)	0.251
IL-6 pg/mL	0.7 (6.6-50.8)	24.8 (8.5-55.5)	4.9 (3.3-28.3)	0.453
TNF-α pg/mL	8.02 (5.76-11.44)	8.31 (5.78-11.44)	7.48 (3.05-11.47)	0.445
Vitamin D ng/mL	20.97 (16.96-27.84)	19.80 (16.68-26.60)	23.07 (19.23-29.59)	0.087
Vitamin D Status				
Deficient <20 ng/mL	50 (50)	35 (35)	15 (15)	0.527
Insufficient 21-29 ng/mL	32 (32)	21 (21)	11 (11)	1.000
Sufficient >30 ng/mL	18 (18)	10 (10)	8 (8)	0.411
Seasonality				
Winter	43 (43)	30 (30)	13 (13)	0.529
Fall	27 (27)	18 (18)	9 (9)	1.000
Spring	22 (22)	14 (14)	8 (8)	0.803
Summer	8 (8)	4 (4)	4 (4)	0.439

Bold indicates statistical significance at P<0.05. Data for continuous variables are summarized as mean ± standard deviation and median (interquartile range IQR) with statistical comparison using T test and Mann-Whitney, respectively. Categorical variables are summarized as count n (%) to test for proportions using Chi square. BMI: body mass index; LPS: Lipopolysaccharide; sCD163: soluble CD163; hs-CRP: highly sensitive C-reactive protein; IL-6: interleukin 6; TNF-α: tumor necrosis factor- alpha.

Table 2. Pearson Correlation Coefficients

Variable	Age	CD4+	BMI	IL-6	TNF-α	Hs-CRP	sCD14	Vit. D
Age	1							
CD4+	-.099	1						
BMI	-.231*	.201*	1					
IL-6	.027	.014	.053	1				
TNF-α	-.001	-.158	-.062	-.109	1			
Hs-CRP	.111	.096	.225*	.206	-.142	1		
sCD14	.013	-.081	.220*	.060	-.260*	.077	1	
Vit. D	.131	.029	.043	-.044	.142	.021	.067	1

*Correlation is significant at the 0.05 level (2-tailed).

Table 3. Logistic Regression Analysis of The Effect Demographics, Cocaine Use, Microbial Translocation, HIV And Vitamin D Status on Inflammation (Hs-CRP≥ 3 mg/dL)

Variable	β	SE	OR	95% CI OR	P-value
Age	0.015	0.032	1.015	0.952, 1.081	0.651
Gender	0.103	0.489	1.109	0.426, 2.890	0.832
Log transformed CD4+ T cell count (cells/μL)	0.483	0.489	1.621	0.621, 2.227	0.324
Cocaine (yes)	0.810	0.519	2.247	0.813, 6.211	0.119
LPS (EU/mL)	2.347	1.104	0.096	0.011, 832	0.033
Vitamin D deficiency	0.489	0.447	1.631	0.681, 3.915	0.273

Bold indicates statistical significance at P<0.05

Table 4. Differences Between Hs-CRP Groups By Cocaine Use, Immune Activation, BMI and Vitamin D Levels

Variable	Hs-CRP <3 (mg/dL)	Hs-CRP ≥ 3 (mg/dL)	P-value
Cocaine use (yes) (%)	30.5	39	0.251
CD4+ T cell count (cells/μL)	586.53 ± 251.81	642.88 ± 392.102	0.403
HIV viral load (copies/mL)	64.89 ± 46.22	80.45 ± 73.35	0.571
BMI (kg/m^2)	27.38 ± 5.02	29.64 ± 5.87	0.045
IL-6 (pg/mL)	10.59 ± 24.17	27.34 ± 47.86	0.049
sCD163 (pg/mL)	535.00 ± 355.63	726.63 ± 429.08	0.024
Vitamin D (ng/mL)	22.93 ± 7.57	23.72 ± 8.77	0.637

Bold indicates statistically significant at P<0.05

Table 5. Multiple Linear Regression Analysis of The Effect of Alcohol Consumption and Age on Inflammation in Cocaine Users Living with HIV

Variable	β	SE	95% CI	P-value
Age	0.774	0.146	0.147, 1.401	0.034
Gender	-2.807	1.592	-9.656, 4.043	0.220
CD4+ T cell count (cells/μL)	0.007	0.002	-0.003, 0.017	0.085
Log transformed HIV viral load	1.664	0.729	-1.491, 4.778	0.153
AUDIT score	1.158	0.228	0.175, 2.141	0.037
Smoking	-0.893	1.976	-9.393,7.607	0.695

Bold indicates statistically significant at P<0.05

Table 6. Logistic Regression Analysis of The Effect of BMI, HIV and Smoking on Inflammation (Hs-CRP ≥ 3 mg/dL) in Cocaine Users Living with HIV

Variable	β	SE	OR	95% CI OR	P-value
Age	0.119	0.081	1.126	0.961, 1.319	0.142
Log transformed CD4+ T cell count (cells/μL)	0.495	0.629	1.641	0.323, 8.329	0.550
HIV viral load <20 copies/mL	0.781	0.947	2.184	0.343, 13.913	0.408
Smoking	0.275	0.947	0.759	0.119, 4.855	0.771
BMI (kg/m^2)	0.228	0.110	1.256	1.013, 1.557	0.038

Bold indicates statistically significant at P<0.05

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CHAPTER V: GUT INTEGRITY, INTESTINAL INFLAMMATION AND VITAMIN D STATUS IN DRUG USERS USERS LIVING WITH HIV

Abstract

Background. The gastrointestinal tract is profoundly affected by HIV, which damages the gut, decreasing its integrity and causing intestinal inflammation regardless of Antiretroviral Therapy (ART) use and virologic control. Cocaine abuse seems to accelerate these processes in People Living with HIV (PLWH), who also manifest a high prevalence of vitamin D deficiency. These factors associate with lifestyle and the disease not only compromised immunity but also gut integrity in this population. The purpose of this study was to evaluate associations between gut integrity damage, intestinal inflammation and vitamin D status in the context of cocaine abuse among PLWH.

Methods. One hundred cocaine users and non-users living with HIV were selected from the ongoing Miami Adult Studies in HIV (MASH) cohort. Cocaine use was assessed by self-report, urine screen and blood metabolites. Blood samples were collected to assess gut integrity damage levels (I-FABP), intestinal inflammation (IL-17 and IL-22), MT levels (LPS), and vitamin D status. Data on socio-demographic characteristics, anthropometrics, dietary intake and disease progression were collected. Descriptive statistics, independent t-test, multiple linear and logistic regressions were conducted to analyze the data.

Results. A total of 100 participants consisted of 66 cocaine non-users and 34 cocaine users. For the two groups, mean age was 53.37±6.88 years old. Most participants were male (51%), and African Americans (66%). All participants were on ART and had suppressed viral load (<200 copies/ml). Half of the participants were considered vitamin D deficient (≤20 ng/ml) and 32% insufficient (21-29 ng/ml). Cocaine users had higher levels of I-FABP than non-users (P=0.014). Cocaine use and a high fat diet were significant predictors for high levels of damaged gut integrity [I-FABP ≥ median 2460.43 (IQR:1813.63-2995.61) pg/ml, P=0.043 and P=0.016 respectively] but not vitamin D status. Cocaine users were 3.04 times more likely to exhibit high gut integrity damage than non-users (OR: 3.038 95% CI: 1.038, 8.894; P=0.043). Cocaine users with intestinal inflammation, had 3.43 times higher odds to exhibit high levels of gut integrity damage than cocaine users without intestinal inflammation (OR: 3.43 95% CI: 1.077, 10.934; P=0.037).

Conclusion. Cocaine use and high fat diets were independently associated with gut integrity damage and intestinal inflammation in PLWH despite ART use and viral suppression. Our study provided preliminary data for the development of future research focusing on dietary interventions for gut health and counseling on drug to more effectively decrease intestinal damage, and ultimately chronic inflammation among PLWH.

Key words. Gut integrity damage, intestinal inflammation, cocaine, vitamin D

Introduction

The intestinal barrier is considered a complex anatomical structure that separates the internal milieu from the luminal intestine.[1,2] This structure provides a physical barrier with a cellular component for the protection and function of the intestine. Intestinal epithelium is a key element of the intestinal barrier that plays a central role in intestinal immunity.[3–5] Preserving gut integrity is a fundamental task for the intestinal epithelium, and it is regulated by tight junction structures, diet quality, short-chain fatty acids, prebiotics, probiotics and the microbiome.[2]

The negative effects of the HIV enteropathy were highlighted by the extensive depletion of the CD4+ T cells in the gastrointestinal (GI) tract, poor mucosal reconstitution, physical destruction of the epithelial barrier, increased permeability and local inflammation. These factors pose a greater risk for microbial translocation (MT), immune activation and chronic inflammation.[6–11]

Studies have demonstrated that active illicit drug use, particularly cocaine, is associated with poor adherence to ART, reduced virologic and immunologic control, HIV disease progression and mortality.[12–14] Cocaine abuse poses harmful effects for the GI tract, including malnutrition, anorexia, reduced blood supply to enterocytes and dysbiosis of microbiome among People Living with HIV (PLWH).[15–17] Epidemiological data have shown that prevalence of vitamin D deficiency among PLWH ranges between 70-85%.[18,19] *In vitro* studies demonstrated that vitamin D is capable of decreasing intestinal permeability and preserve gut integrity via signaling of Vitamin D Receptors (VDR) in the intestine.[20–22]

In relation to intestinal damage, Intestinal Fatty Acid Binding Protein (I-FABP) has been proposed as a reliable marker of gut integrity damage among PLWH. Studies have revealed that adults living with HIV had increased I-FABP levels in the circulation inducing MT, immune activation, and increased mortality in this population.[23–30] Massive depletion of CD4+ T cells and gut integrity during HIV, promotes intestinal inflammation affecting IL-17 and IL-22-producing cells, which are responsible for mucosal immunity and preservation of intestinal homeostasis.[6,31] However, association between gut integrity damage, intestinal inflammation and vitamin D status in the context of cocaine abuse has not been investigated yet. The purpose of this study was to evaluate those association.

Methods

Study Participants. Participants for this cross-sectional study were selected from the ongoing Miami Adult Studies in HIV (MASH) cohort. The MASH cohort is a longitudinal study investigating the impact of substance abuse on HIV infection, HIV/HCV co-infection, and comorbidities with a focus on liver disease. The MASH cohort in Miami is unique because it has a large number of non-Hispanic Blacks, Hispanics, women, and non-injector drug users, with predominantly heterosexual HIV-transmission etiology. The Inclusion Criteria for this study were to be HIV mono-infected adults, age 21 and older, having undetectable HIV viral load <200 copies/ml, CD4+ T cell count >200 cells/μl, and compliant with ART use (95%) and clinic visits for at least 6 months. Participants were also positive for cocaine use by questionnaire, blood

metabolites and/or urine toxicology. The Exclusion Criteria included co-infection with hepatitis B or C, detectable HIV viral load, CD4+ T cell count<200 cells/µl, diagnosis of inflammatory bowel disease, pregnancy, hazardous alcohol use (AUDIT score >8) and current vitamin D supplementation. After eligibility criteria, a total of 100 participants were eligible for this study.

Laboratory analyses. Plasma samples were collected at the baseline visit and used to assess I-FABP for gut integrity damage, cytokines IL-17 and IL-22 for intestinal inflammation, LPS for MT, and vitamin D levels. I-FABP was measured using the Human FABP2/I-FABP Immunoassay by R&D Systems (Minneapolis, MN, US) according to manufacturer's instructions with a sample dilution of 1:5. LPS was measured using the Pierce Chromogenic Endotoxin Quant Kit by Thermo Fisher Scientific (Rockford, IL, USA) according to manufacturer's instructions with the following modifications: samples were diluted 1:10 with endotoxin-free water to avoid interference with background color and preheated to 70°C for 15 minutes prior to analyses to inactivate plasma proteins. Triplicates were assessed for each sample. Intestinal inflammation markers were measured by using the analyte-specific bead-based Luminex multiplex immunoassays (EMD, Millipore Corporation). Quantification of 25(OH)D levels were measured in 25µl of sample by the 25-Hydroxy Vitamin D^{s} Enzyme Immunoassay by Immunodiagnostic Systems Ltd (Bolton, UK) according to manufacturer's instructions. According to the Endocrine Society Clinical Practice Guidelines, adequate levels of Vitamin D in adults are considered to be >30ng/mL (75nmol/L), insufficient levels 21-29 ng/mL (52.5-72.5 nmol/L) and deficient levels at

<20 ng/mL (50nmol/L).[32] For all assays, the coefficient of variation or CV% was calculated and CVs greater than 10% were reanalyzed.

Substance use. Cocaine use was assessed by self-report (use in the past 30 days) questionnaire, blood metabolite and urine drug screen (American Bio Medical®). Alcohol use was evaluated by the Alcohol Use Disorders Identification Test (AUDIT).

Anthropometric Assessments and Dietary Data. Standing height (meters), weight (kg) and Body Mass (kg/m^2) Index were assessed from the parent study datasets. Dietary intake data were assessed by one 24-hour recall. Food models and portion size prompts were used. Dietary intake data were analyzed in a software program (NutriBase 9 Cybersoft).

Disease Progression and Medication History. Markers of disease progression (CD4+ T cell count and viral load) were collected from the patient's medical chart with participants' written authorization.

Statistical Analyses

Descriptive statistics including mean, standard deviation, median, interquartile range (IQR), standard error and percentages were used to describe the data. Independent T-test was conducted to compare mean differences between cocaine users and non-users. Variables HIV viral load and CD4+ T cell count did not exhibit a normal distribution; therefore, it was log transformed. Our data showed that IL-17 and IL-22 levels had high proportions of values under the limit of detection (LOD). For the purpose of these analyses, we replaced these values with the LOD minus a decimal. In the case of IL-17,

low values were considered at <1.99 pg/mL and for IL-22, low values were considered at <0.01pg/mL

Pearson correlations were implemented to evaluate association among biomarkers of gut integrity and intestinal inflammation. Logistic linear regression models were implemented to analyze associations between gut integrity damage, cocaine use and vitamin D status. Analyses were adjusted for potential confounding factors including age, gender, CD4+ T count and BMI. Logistic regression models included dependent variable as high levels of gut integrity damage at the following cut-offs: I-FABP ≤ or ≥ median. An alpha less than 0.05 was considered significant. All statistical analyses were performed using SPSS version 23.

Results

Participant Characteristics

A total of 100 PLWH were selected for this study. The proportion of cocaine non-users was greater (66%, n=66), than that of cocaine users (34%, n=34). For the two groups, mean age was 53.37±6.88 years old. Cocaine users were older than non-users, however, this difference was not statistically significant (P=0.338). Most participants were male (51%), and African American (66%). Participants had a mean duration of known HIV diagnosis of 17.21±7.28 years. All participants were on ART and had suppressed viral load (<200 copies/mL) with a median of 48.5 (Interquartile range: 20-307) copies/mL Among all participants, 19% used marijuana, 11% used opioids, 1% used heroin and 2% used fentanyl (data not shown).

Cocaine users had higher AUDIT score than non-users (3.12±2.50 vs. 2.03±1.96 respectively, P=0.031), indicating greater alcohol use among cocaine users. Participants had a mean BMI of 28.70±5.76 kg/m^2 which classified most of them as overweight and 37% were obese. In regard to vitamin D status, 50% of participants were considered deficient (≤20 ng/ml), 32% insufficient (21-29 ng/ml) and 18% sufficient (>30ng/ml). Most of the samples were collected during winter (47%) and fall (27%) seasons.

Cocaine users had higher levels of I-FABP than non-users (2866.97±798.06 vs. 2337.56±1072.95 respectively, P=0.014) indicating greater gut integrity damage. Cytokines IL-17 and IL-22 are considered biomarkers for intestinal inflammation; there were no differences between cocaine users and non-users in regard to intestinal inflammation. However, for both IL-17 and IL-22, most of the participants exhibited low levels, which indicates the presence of inflammation in the intestine (IL-17: 89.3% and IL-22: 86.9%; Table 1). Low levels of these cytokines indicate that IL-17 and IL-22-producing cells were affected by ongoing inflammation in the gut, which may continue to promote disruption of the mucosa immunity and integrity.[33–36] In addition, cocaine users had higher levels of MT than non-users (0.72±0.12 vs. 0.53±0.24 respectively, P<0.001). Vitamin D levels did not differ between groups (Table 1).

Correlations

A Pearson product-moment correlation was run to determine the linear relationship between continuous variables. There was a positive correlation between BMI and CD4 + T cell count (r = 0.201, P =0.046), IL-17 and IL-22 levels (r =0.585,

P<0.001). There was an inverse correlation between BMI and age (r = -0.231, P =0.021). Although not statistically significant, there was a trend for inverse correlations between I-FABP levels and BMI (r = -0.198, P =0.05), IL-17 and CD4+ T cell count (r=-0.211, P=0.05) and, vitamin D and I-FABP levels (r = -0.198, P=0.05) (Table 2).

Association between Cocaine use and High Levels of Gut Integrity Damage

Logistic regression analyses indicated that cocaine use was significantly associated with high levels of gut integrity damage (I-FABP ≥ median 2460.43 pg/mL) (P=0.042) after controlling for age, gender, CD4+ T cell count, vitamin D levels and participants with intestinal inflammation (IL-22 levels under LOD). The model correctly classified 59.3% of cases. In addition, cocaine users had 3.04 times higher odds to exhibit high levels of gut integrity damage than non-users (OR: 3.039 95% CI: 1.039, 8.890; P=0.042) (Table 3). Analysis were conducted with vitamin D status, for both deficiency and insufficiency, however, these were not significantly associated with high levels of gut integrity damage in our participants (data not shown).

Table 4 showed that among cocaine users, 42.9% exhibited high levels of gut integrity damage [I-FABP ≥ median 2460.43 (IQR: 1813.63-2995.61) pg/mL]. Also, among individuals who had high levels of MT [LPS levels ≥ median 0.63 (IQR:0.48-0.73) EU/mL], 59.2% exhibited high levels of gut integrity damage. These findings indicated that cocaine use posed an important threat for gut integrity contributing with increased intestinal permeability and high levels of MT. None of the parameters for vitamin D levels, BMI and HIV disease progression were significantly associated with high levels of gut integrity damage.

Association between Cocaine use and Intestinal Inflammation

Intestinal inflammation was assessed by IL-17 and IL-22 levels. Low levels of these cytokines indicate that IL-17 and IL-22-producing cells were impaired probably due to HIV enteropathy. Most of our participants exhibited low levels of IL-17 and IL-22 (89.3% and 86.9%, respectively) (Table 1). In participants who exhibited low levels of IL-22 (<0.01 pg/mL), cocaine use was significantly associated with high levels of gut integrity damage (I-FABP ≥ median 2460.43 pg/mL) (P=0.037). The model correctly classified 59.2% of cases. In addition, cocaine users with low levels of IL-22, had 3.42 times higher odds to exhibit high levels of gut integrity damage than cocaine users with levels of IL-22 >0.01 pg/mL (OR: 3.424 95% CI: 1.076, 10.900; P=0.037) (Table 5). This association remained significant after adjusting for age, gender, CD4+ T cell count, BMI and vitamin D levels. Analysis were conducted with vitamin D status, for both deficiency and insufficiency, however, these were not significantly associated with high levels of gut integrity damage (data not shown).

Dietary Intake Characteristics in Study Participants

Table 6 describes the dietary intake characteristics of participants. The 2015-2020 USDA Dietary Guideline Recommendations for Americans[37] were implemented to compare total percent of fat from caloric intake (<35% caloric intake), cholesterol (<300 mg/d), fiber (28g/d) and vitamin D intake (600 IU/d). We calculated the proportion of participants exceeding these recommendations. All participants exceeded recommendations for cholesterol intake (331.90 ± 317.43 mg/d) but no differences were observed between cocaine and non-cocaine users (P=0.887). For total percent of calories

from fat (≥35%) 46% of participants exceeded recommendation, indicating that a pattern for a high fat diet was reported. All participants had inadequate intake of fiber (14.66±11.99 gr/d) and vitamin D (18.55±35.18 IU/d) but no differences were observed between cocaine and non-cocaine users (P=0.458 and P=0.723, respectively). Interestingly, a great proportion of participants did not consume vitamin D as reported in the 24-hour recall.

Associations between Gut Integrity Damage and Dietary Fat Intake

Logistic regression analyses indicated that a high fat diet (total fat ≥35% calories) was significantly associated with high levels of gut integrity damage [I-FABP ≥ median 2460.43 (IQR:1813.63-2995.61) pg/mL] after controlling for age, gender, total fat intake and obesity. The model correctly classified 58.2% of cases. In addition, participants who followed a high fat diet (total fat ≥35% calories) had 3.38 times higher odds to exhibit high levels of gut integrity damage than those who did not follow a high fat diet (OR: 3.38 95% CI: 1.256, 9.123; P=0.016) (Table 7).

Discussion

We examined associations between gut integrity, intestinal inflammation and vitamin D status in the context of cocaine abuse among PLWH. Our data suggested that our participants showed similar I-FABP levels to the ones reported in others studies conducted in PLWH, indicating great damage to the gut integrity.[27,30,38] Cocaine users had higher levels of I-FABP than non-users, showing greater gut integrity damage in cocaine users. However, this effect was not seen for markers of intestinal inflammation (IL-17 and IL-22) and vitamin D levels.

We also showed that cocaine was a significant predictor for high I-FABP levels even after controlling for age, gender, CD4+ T cell count and vitamin D levels. To the best of our knowledge, this is the first study showing an association between cocaine use and gut integrity damage among PLWH. It is important to mention that all our participants were on stable ART, suppressed viral load (<200 copies/mL) and did not report hazardous drinking (AUDIT score >8), which implies that cocaine is a key player for destabilizing gut integrity in PLWH. Studies have reported that cocaine has the ability to dysregulate the expression of the tight junction proteins in the intestinal epithelium, promoting gut integrity damage, hyperpermeability, intestinal inflammation and disruption of the microbiome.[39–41] Our findings suggest that in addition to deleterious effects of HIV on the GI tract, cocaine seems to be an important factor that aggravates the ongoing inflammation and damage to the gut integrity in HIV infection.

A unique finding from this study was that cocaine users were 3.04 times more likely to have high damage to the gut integrity (I-FABP ≥ median 2460.43 pg/mL) than non-users. This may indicate the harmful influence of cocaine in the GI mucosa that promotes destruction of tight junctions, creating a “leaky” gut that allows the passage of microbial products into systemic circulation.

Participants with high damage to the gut integrity (I-FABP ≥ median of 2460.43 pg/mL) also had high levels of MT (LPS ≥ median of 0.63 EU/mL) supporting previous findings on the role of HIV and SIV on the damage to the gut integrity and MT in humans and rhesus macaques.[24] During HIV infection, there is a massive loss of CD4+ T cells in the GI lamina propia. This loss also affects a subtype of CD4+ T helper 17 cells

(Th17) in the intestine, which are responsible for the intestinal homeostasis, mucosal regeneration and integrity [42,43] creating an environment for gut integrity damage, increased intestinal permeability and MT.

In regard to vitamin D status, half of our participants were vitamin D deficient and one third were insufficient. These rates are comparable to the ones reported by other cohort studies showing a high incidence of vitamin D deficiency in PLWH in the US.[44,45] Our cohort is mostly comprised of African Americans and Hispanics and almost half of the cohort was overweight ,which is a risk for vitamin D deficiency.[18] However, vitamin D status, deficiency or insufficiency, was not associated with high levels of gut integrity damage (I-FABP ≥ median 2460.43 pg/mL) in our participants. This finding suggests that cocaine use is an important and significant factor for high levels of gut integrity damage in our cohort.

Additionally, we showed that cocaine use was significantly associated with higher levels of gut integrity damage in participants who exhibited intestinal inflammation (low levels of IL-22), and that these individuals were 3.43 times more likely to exhibit high gut integrity damage than individuals who did not have intestinal inflammation. Several lines of evidence have suggested that intestinal CD4+ T cells that produce IL-17 and IL-22 are severely compromised during HIV infection, provoking intestinal inflammation, MT, immune activation and ultimately disease progression.[24,46–49] In our study, we showed that most of our participants exhibited intestinal inflammation supporting the idea that the depletion of CD4+ T cell decreases IL-17 and IL-22 production. Most importantly, this is the first study suggesting that cocaine use worsen the increased gut

integrity damage during intestinal inflammation among PLWH. This findings contribute to the existing evidence that supports targeting drug abuse,[50] Th17 producing cells and gut microbiome as potential areas for reducing HIV reservoir in the intestine, and for HIV remission and future potential cure.[51,52] Our data do not support that impairment of vitamin D status (deficiency or insufficiency) may be a significant factor for high levels of gut integrity damage in our participants.

Our dietary data showed that PLWH in Miami seemed to follow a dietary pattern characterized by a high fat, high cholesterol diet. These findings support previous studies showing that PLWH had an increase of total fat, saturated fat and cholesterol and low fiber intake[53–56] posing a risk for the developments of chronic conditions, including cardiovascular disease, obesity and inflammation. Our participants had inadequate vitamin D intake and a great proportion of them did not consume vitamin D at all. This study suggests that personalized dietary interventions that focus on healthy eating and physical activity may be beneficial in the management of HIV.

Individuals who followed a high fat diet seemed to have higher levels of gut integrity damage. Our study is among the first to show associations between diet and damage to the gut integrity in PLWH. In another study, researchers did not find associations between high fat diet and damage to the gut integrity.[56] Differences may be due to methodological approaches, including dietary intake assessment and socio-demographics characteristics of study participants. Nevertheless, a growing body of evidence comprised of animal and human studies have shown the effects of high fat diet on gut integrity.[57] In this regard, a study conducted in obese mice found that a high fat

diet seemed to disrupt gut integrity promoting increased permeability[58] via the activation of mast cells in the intestinal mucosa. These cells are responsible for the release of pro-inflammatory cytokines in the intestine[59], including TNF-α, which promotes destruction of tight junction structures leading to gut integrity damage and increased permeability.[60] Another study conducted in mice fed with a high fat diet suggested that the expression of tight junction proteins (claudin-1, claudin-3 and occluding) were reduced and gut microbiome was affected, posing ideal conditions for intestinal permeability and inflammation.[61,62]

Our study has limitations. The cross-sectional design does not allow us to establish causation. Intestinal inflammation markers (IL-17 and IL22) were available only for 85 study participants. Dietary intake data were assessed by one 24-hour recall. The analysis of a 24-hour food recall is limited by important factors such as the interviewee's memory, concepts of food portion size, and current versus historical food consumption. The results of this study need to be interpreted with caution due to the specific conditions surrounding the study such as socioeconomics, ethnic distribution and geography, as this study included only people on stable ART and controlled viral load. This study, however, provides important clinical evidence that supports the development of strategies to ameliorate and prevent cocaine abuse and vitamin D deficiency among PLWH.

Conclusion

Our data demonstrated that cocaine abuse and a high fat diet are independently associated with high levels of gut integrity damage and intestinal inflammation in PLWH despite

ART use and viral suppression. Our study provided preliminary data for the development of future research focusing on dietary interventions for gut health and counseling on drug to more effectively decrease intestinal damage, and ultimately chronic inflammation among PLWH.

Table 1. Participants Characteristics

Characteristics	Total (n=100)	Cocaine Non-users (n=66)	Cocaine Users (n=34)	P value
Age, years	53.37±6.88	52.89±7.38	54.29±5.77	0.338
Gender (male)	51(51)	35 (35)	16 (16)	0.361
Race/Ethnicity				
African American	66 (66)	38 (38)	28 (28)	0.011
Hispanic	29 (29)	23 (23)	6 (6)	0.103
White	28 (28)	24 (24)	4 (4)	0.100
Other	6 (6)	4 (4)	2 (2)	0.603
CD4+ T (cells/μL)	630.08±344.07	628.80±298.12	632.64±426.63	0.963
HIV Viral Load (copies/mL)	48.50 (20-307)	59 (31.5-140.25)	40 (26.5-136)	0.497
Duration of known HIV (years)	17.21±7.28	18.01±7.23	15.64±7.14	0.150
AUDIT score	2.4±2.19	2.03±1.96	3.12±2.50	0.031
Smoking (yes)	47 (47)	24 (24)	23 (23)	0.300
BMI (kg/m^2)	28.70±5.76	28.89±5.82	28.33±5.71	0.648
Obesity (BMI>30kg/m^2)	37 (37)	24 (24)	13 (13)	0.511
I-FABP pg/mL	2515.83± 1072.95	2337.56±1072.95	2866.97±798.06	0.014
IL-17 pg/mL	4.01±7.94	6.12±15.77	1.92±0.11	0.084
IL-22 pg/mL	0.11±0.32	0.11±0.43	0.09±0.218	0.813
Participants with low levels IL-17 pg/mL*	89.3 (75)	86.2 (50)	96.2 (25)	0.164
Participants with low levels IL-22 pg/mL*	86.9 (73)	87.9 (51)	84.6 (22)	0.460
LPS (EU/mL)	0.59±0.23	0.53±0.24	0.72±0.12	<0.001
Vitamin D ng/mL	20.97 (16.96-27.84)	19.80 (16.68-26.60)	23.07 (19.23-29.59)	0.087
Vitamin D Status				
Deficient ≤20 ng/mL	50 (50)	35 (35)	15 (15)	0.527
Insufficient 21-29 ng/mL	32 (32)	21 (21)	11 (11)	1.000
Sufficient ≥30 ng/mL	18 (18)	10 (10)	8 (8)	0.411
Seasonality				
Winter	43 (43)	30 (30)	13 (13)	0.529
Fall	27 (27)	18 (18)	9 (9)	1.000
Spring	22 (22)	14 (14)	8 (8)	0.803
Summer	8 (8)	4 (4)	4 (4)	0.439

Bold indicates statistical significance at P<0.05. Data for continuous variables are summarized as mean ± standard deviation and median (interquartile range IQR) with statistical comparison using T test and Mann-Whitney, respectively. Categorical variables are summarized as count n (%) to test for proportions using Chi square. BMI: body mass index; I-FABP: Intestinal Fatty Acid Binding Protein; IL-17: interleukin 17; IL-22: interleukin 22; LPS: Lipopolysaccharide. *Indicates proportion of participants with low levels of IL-17 and IL-22.

Table 2. Pearson Correlation Coefficients

Variable	Age	CD4+	AUDIT	BMI	I-FABP	LPS	IL-17	IL-22	Vit. D
Age	1								
CD4+	-.09	1							
AUDIT	-.06	-.15	1						
BMI	-.23*	.20*	.13	1					
I-FABP	.07	-.01	.08	-.17**	1				
LPS	-.06	.06	.04	-.17	.14	1			
IL-17	.08	-.21**	.03	-.07	-.09	.06	1		
IL-22	.13	-.17	.17	-.07	-.03	-.13	.58*	1	
Vit. D	.13	.02	.07	.04	-.19**	-.05	-.02	.05	1

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation showing a trend P=0.05.

Table 3. Logistic Regression Analyses Predicting The Likelihood of High Levels of Gut Integrity Damage (I-FABP ≥ Median 2460.43 pg/mL) in Study Participants

Variable	β	SE	OR	95% CI OR	P-value
Age	0.044	0.034	1.045	0.978, 1.116	0.197
Gender	-0.259	0.529	0.772	0.274, 2.177	0.625
Log transformed CD4+ T cell count (cells/μL)	0.310	0.571	1.363	0.445, 4.174	0.588
Cocaine (yes)	1.111	0.548	3.038	1.038, 8.894	0.043
Vitamin D levels (ng/mL)	-0.035	0.032	0.966	0.907, 1.028	0.277
Participants with low levels IL-22 (pg/mL) *	1.063	0.774	2.896	0.635, 13.198	0.169

Bold indicates statistical significance at P<0.05
*Participants who exhibit IL-22 levels under Limit of Detection (LOD)

Table 4. Differences Between Median Groups of I-FABP and Cocaine Use, Microbial Translocation and Vitamin D Levels

Variable	I-FABP (≤ 2460.43 pg/ml)	I-FABP (≥ 2460.43 pg/ml)	P-value
Cocaine use (yes) (%)	25.5	42.9	0.049
CD4+ T cell count (cells/μL)	642.6 ± 390.99	616.77 ± 289.584	0.708
HIV viral load (copies/mL)	98.78 ± 75.12	70.77 ± 80.23	0.414
BMI (kg/m^2)	29.42 ± 5.58	27.95 ± 5.89	0.204
IL-17 (pg/mL)	5.79 ± 14.32	3.84 ± 12.1	0.503
IL-22 (pg/mL)	0.12 ± 0.34	0.09 ± 0.41	0.738
LPS (≥0.63 EU/mL) (yes) (%) *	40.8	59.2	<0.001
Vitamin D (ng/mL)	23.72 ± 8.55	22.87 ± 7.62	0.603

Bold indicates statistically significant at P<0.05
*LPS above the median (>0.63 EU/ml)

Table 5. Logistic Regression Analyses Predicting The Likelihood of High Levels of Gut Integrity Damage (I-FABP ≥ 2460.43 pg/mL) in Participants with Intestinal Inflammation (Il-22 <0.01 pg/mL)

Variable	β	SE	OR	95% CI OR	P-value
Age	0.027	0.035	1.028	0.959, 1.101	0.443
Gender	0.087	0.591	1.091	0.343, 3.473	0.883
Log transformed CD4+ T cell count (cells/μL)	0.514	0.611	1.671	0.504, 5.541	0.401
Cocaine (yes)	1.233	0.591	3.430	1.077, 10.934	0.037
Vitamin D (ng/mL)	-0.008	0.036	0.992	0.926, 1.064	0.831
BMI (kg/m^2)	-0.041	0.045	0.960	0.879, 1.049	0.370

Bold indicates statistical significance at P<0.05

Table 6. Dietary Intake Characteristics

Nutrient	Recommended Range*	Total (n=100)	Cocaine non-users (n=66)	Cocaine users (n=34)	P-value
Calories (cal)	1800-2200	1991.20±1233.32	2017.87±1191.7	1937.84±1329.9	0.763
Protein (gr)	46-56	88.42±96.45	92.43±112.18	80.39±53.15	0.470
Carbohydrate (gr)	130	242.57±172.35	248.83±175.93	230.06±166.90	0.612
Total Fat (gr)	-	75.90±51.07	74.80±46.12	78.12±60.48	0.783
Total Fat intake >35%**	20-35	46	60.9	39.1	0.142
Cholesterol (mg)	300	341.90±317.43	338.93±343.11	347.84±263.46	0.887
Dietary Fiber (gr)	25.2-30.8	14.66±11.99	15.03±11.96	13.394±12.12	0.458
Vitamin D (IU)	600	18.55±35.18	19.42±36.28	16.81±33.32	0.723
Inadequate Vitamin D intake***	-	67 (67)	66.7 (44)	69.7 (23)	0.473

* Recommended ranges based on adults who consume 1800-2200 kcals daily. Variations exits by age, sex and activity level.
**Participants with high fat diet (>35% Calories from Total Fat Intake)
***Participants with inadequate Vitamin D intake (0 IU/day)

Table 7. Logistic Regression Analysis of Dietary Factors Predicting High Gut Integrity Damage Levels in Study Participants

Variable	β	SE	OR	95% CI OR	P-value
Age	0.014	0.032	1.014	0.952, 1.081	0.657
Gender	-0.056	0.440	0.946	0.399, 2.241	0.899
Participants with high fat diet*	1.220	0.506	3.386	1.256, 9.123	0.016
Total fat (gr)	-0.009	0.005	0.991	0.981, 1.001	0.085
Obesity	-0.619	1.846	0.539	0.206, 1.405	0.206

Bold indicates statistically significant at P<0.05
*Participants with high fat diet (>35% Total Fat Intake)

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CHAPTER VI: SUMMARY OF CONCLUSION AND IMPACT ON PRACTICE

This study investigated the association of gut integrity, microbial translocation (MT), immune activation, inflammation and vitamin D status in the context of cocaine abuse among People Living with HIV (PLWH).

It has been well stablished that HIV destroys the intestinal CD4+ T cells causing a major disruption in the intestinal immune system characterized by local inflammation and destruction of the gut integrity. These events create an environment where the ability of the intestine to protect its integrity is compromised, generating a "leaky gut" that allows the passage of microbial products into systemic circulation, a process called microbial translocation (MT). The presence of MT products in the systemic circulation activates the immune system and contributes to the ongoing inflammation caused by HIV despite Antiretroviral Therapy (ART) and viral suppression.[1–9]

Illicit drug abuse, particularly cocaine, is prevalent among PLWH.[10] Cocaine poses harmful effects to the immune system, gut microbiome, and promotes HIV disease progression and mortality among PLWH.[11–14] However, the impact of cocaine use on gut integrity, MT, immune activation and inflammation has not been fully investigated. Only one study has shown disruption of the microbiome but no association with MT.[14]

To the best of our knowledge, our study is the first to demonstrate the impact of cocaine use on MT, immune activation, inflammation and gut integrity among PLWH. Our data revealed that cocaine users had higher levels of MT than non-users, and that cocaine use was a significant predictor for markers of MT (LPS) and immune activation (sCD163 and sCD27). We showed that cocaine users were 6.66 times more likely to

exhibit high MT levels than non-users, which were associated with intestinal inflammation and ultimately high levels of MT. These findings suggest the damaging effect of cocaine in the Gastrointestinal (GI) mucosa.

Although all our participants were on stable ART and virally suppressed, we found that lower CD4+ T cells were associated with MT levels, which support the premise of the continuous destructive effects of HIV on the GI immune system and poor reconstitution of CD4+T cells despite of ART use.[8,9,15–17]

Our analyses suggested that MT was a significant predictor of systemic inflammation in our participants, an association that had been established previously in other studies in PLWH on ART and viral supression.[18–20] It has been recognized that damage to the gut integrity during HIV infection is a major driver for MT, immune activation and chronic inflammation.[6,21–25] Although, cocaine use was not directly associated with inflammation in our study, we showed that cocaine users had higher MT levels than non-users; this could indicate the possible role of cocaine in the gut integrity, promotion of MT, and ultimately systemic inflammation.[26,27]

This study also revealed that among cocaine users living with HIV, alcohol use was associated with inflammation. This finding agrees with previous evidence showing an association of alcohol and cocaine with inflammation among PLWH.[28] Alcohol and cocaine are often used together,[29] and this combination produces a synergistic effect that increases the time and severity of immune and neurological toxicity, as well as the effects on dependence.[30]

Additionally, our findings indicated that an increase on BMI independently predicted inflammation in cocaine users living with HIV. This is a novel finding that

suggests that cocaine and weight gain provide a cooperative effect that not only promotes systemic inflammation and HIV disease progression, but it may increase the risk for non-AIDS related conditions, such as cardiovascular disease.

Our data suggested that cocaine users living with HIV also had higher I-FABP levels than non-users, which indicated greater damage of the gut integrity in this population. Cocaine was an independent predictor of high levels of gut integrity damage, and cocaine users were 3.04 times more likely to exhibit high levels of gut integrity damage than non-users. Evidence has shown that cocaine has the ability to dysregulate the expression of the tight junction proteins in the intestine, promoting the damage of the gut integrity, increased permeability, intestinal inflammation and disruption of the microbiome.[26,27,31] Our findings suggest that in addition to the deleterious effects of HIV on the GI tract, cocaine use seems to be an important factor in exacerbating the ongoing inflammation and in damaging gut integrity in HIV infection.

It is noteworthy that many of our participants had intestinal inflammation as measured by low levels of cytokines IL-17 and IL-22. Several studies have suggested that intestinal CD4+T cells that produce IL-17 and IL-22 are severely compromised during HIV infection, provoking intestinal inflammation, MT, immune activation and ultimately disease progression.[32–36]

In those participants who had intestinal inflammation, cocaine use was a significant predictor for high levels of gut integrity damage. To the best of our knowledge, this is the first study showing an association between cocaine use and damage of the gut integrity during intestinal inflammation among PLWH. This finding supports the importance of targeting drug abuse,[37] intestinal inflammation and gut

microbiome as prospective targets for reducing HIV reservoir in the intestine, and achieving remission and cure.[38,39]

In regard to vitamin D status, we found that half of our participants were deficient and one third were insufficient. These prevalence rates are comparable to the ones shown by other studies demonstrating a high prevalence of vitamin D deficiency among PLWH in the US.[40,41] Our cohort is comprised of African Americans and Hispanics and almost half of the cohort was overweight, all of which represent risk factors for vitamin D deficiency.[42]

In those participants who were vitamin D deficient and insufficient, those who used cocaine had higher MT levels than non-users. Importantly, cocaine use was associated with MT levels in both vitamin D deficient and insufficient participants, indicating that cocaine is a significant driver for MT when vitamin D status is impaired. This effect, however, was not observed for immune activation, inflammation and gut integrity damage.

The results of our study suggested that cocaine is an independent factor that aggravates gut integrity and intestinal inflammation and drives MT and immune activation among PLWH. Cocaine is one of the most addictive illicit drugs. In this regard, a very interesting study conducted in mice exposed to cocaine, demonstrated that disruption of the microbiome may influence behavioral responses, particularly the rewarding and sensitizing properties of cocaine via the gut-brain axis.[31]

To the best of our knowledge, there is only one study conducted in humans that reported that individuals who use cocaine exhibit depletion of the gut microbiome.[14] These important pieces of evidence suggest the possibility for the GI tract, particularly

gut microbiome, to be an important target for the prevention or treatment of drug addiction.

Studies have demonstrated that cocaine users living with HIV do not follow an optimal diet, and have higher incidence of food insecurity and alcohol consumption than cocaine non-users.[14,43,44] Our dietary data showed that cocaine users living with HIV exceeded dietary recommendations for total % of calories from fat and cholesterol, and had poor fiber intake. These are considered key nutrients for both disruption and maintenance of gut integrity and microbiome.[45–48]

Therefore, there is an imperative need to tailor programs that focus on effective counseling on drug abuse and lifestyle modification. Nutrition interventions focused on gut health may help to prevent and ameliorate gut integrity damage, disruption of gut microbiome and associated inflammation in a vulnerable population such as cocaine users living with HIV. Further research is needed to continue examining the association of drug abuse and intestinal health as a key area to overcome some of the many challenges for HIV management, which are to reduce chronic inflammation and HIV disease progression, in order to achieve better control of the condition.

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CHAPTER VII: FUTURE RESEARCH

The results from this study suggested that cocaine use was a major driver for disruption of gut integrity, microbial translocation, immune activation and inflammation among People Living with HIV (PLWH). Thus, the association among cocaine use, gut integrity, microbial translocation, immune activation and inflammation in a longitudinal study with a larger sample size may provide a better understanding of the impact of substance abuse on HIV disease progression. More studies are needed to elucidate the mechanisms behind the harmful effects of cocaine on microbial translocation and intestinal inflammation among PLWH, and for identifying potential targets for treatment. In addition, further clinical trials focused on gut health, gut microbiome restoration and cocaine abuse are warranted to provide clinically meaningful data that can be translated into future recommendations for HIV management and behavioral and clinical interventions.

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