The Association Between Oral Soft Tissue Lesions and Psychological Stress in HIV+ Adults

BY

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THESIS

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LIST OF ABBREVIATIONS

AIDS        Acquired Immune Deficiency Syndrome
ANOSIM      Analysis of Similarity
ANS         Autonomic Nervous System
AZT         Azidothymidine
BIOM        Biological Observation Matrix
CDC         Centers for Disease Control
CES-D       The Center for Epidemiologic Studies Depression Scale
CRC         Clinical Research Center
CTQ         Childhood Trauma Questionnaire
DNA         Deoxyribonucleic Acid
GI          Gastrointestinal
HAART       Highly Active Antiretroviral Therapy
HIV         Human Immunodeficiency Virus
HHV         Human Herpes Virus
HPA         Hypothalamic-Pituitary-Adrenal
HPV         Human Papilloma Virus
HSV         Herpes Simplex Virus
IRB         Institutional Review Board
IRIS        Immune Reconstitution Inflammatory Syndrome
KS          Kaposi’s Sarcoma
LGE         Linear Gingival Erythema
MMWR        Mortality and Morbidity Weekly Report
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<tr>
<th>Abbreviation</th>
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<tr>
<td>NHL</td>
<td>Non-Hodgkins Lymphoma</td>
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<td>NIH</td>
<td>National Institutes of Health</td>
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<tr>
<td>NMDS</td>
<td>Non-Metric, Multi-Dimensional Scaling</td>
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<td>NNRTI</td>
<td>Non-nucleoside Reverse Transcriptase Inhibitors</td>
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<tr>
<td>NRTI</td>
<td>Nucleoside Reverse Transcriptase Inhibitor</td>
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<td>NUG</td>
<td>Necrotizing Ulcerative Gingivitis</td>
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<td>NUP</td>
<td>Necrotizing Ulcerative Periodontitis</td>
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<tr>
<td>OHL</td>
<td>Oral Hairy Leukoplakia</td>
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<td>OPC</td>
<td>Oral Pharyngeal Candidiasis</td>
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<tr>
<td>OTU</td>
<td>Operational Taxonomic Units</td>
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<tr>
<td>PBL</td>
<td>Plasmablastic Lymphoma</td>
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<tr>
<td>PBS</td>
<td>Phosphate-Buffered Saline</td>
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<tr>
<td>PCL-C</td>
<td>PTSD Checklist-Civilian Version</td>
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<tr>
<td>PCP</td>
<td><em>Pneumocystis carinii</em> pneumonia</td>
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<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<td>PNS</td>
<td>Peripheral Nervous System</td>
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<td>PSS</td>
<td>Perceived Stress Scale</td>
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<tr>
<td>PTSD</td>
<td>Post Traumatic Stress Disorder</td>
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<td>RAS</td>
<td>Recurrent Apthous Stomatitis</td>
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<tr>
<td>rRNA</td>
<td>Ribosomal Ribonucleic Acid</td>
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<td>UIC</td>
<td>University of Illinois at Chicago</td>
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SUMMARY

This study is the result of an interdisciplinary collaboration between oral and mental health researchers. The primary aim of this study is to investigate a possible association between oral lesions and psychological stress in people living with the human immunodeficiency virus (HIV).

Oral soft tissue lesions were some of the first signs described in the emerging immune disease that came to be known as Acquired Immune Deficiency Syndrome (AIDS). Thirty-four years have passed since the first cases of Pneumocystis carinii pneumonia (PCP) and mucosal candidiasis were reported in the Mortality and Morbidity Weekly Report (MMWR) published by the CDC (CDC, 1981). Since that time, pharmacological advances have transformed HIV/AIDS from a lethal diagnosis into a chronic, manageable condition. Despite tremendous advances in pharmacological treatment of HIV, oral lesions continue to represent a source of morbidity to many people infected with HIV. A growing body of evidence has shown HIV+ people are also disproportionately affected by psychological trauma and depression, and that these mental health conditions are correlated to a worse prognosis for their HIV infection (Chida & Vedhara, 2009). In this novel study, we propose to explore a possible and previously unreported association between oral manifestations of HIV and measures of psychological stress in HIV disease.

This study was designed with a primary aim to investigate if an association exists between oral soft tissue lesions and psychological stress in HIV+ subjects. The null hypothesis tested was the following: there is no association between oral soft tissue
lesions and psychological stress in HIV subjects. A secondary aim of this study was designed to test whether the oral microbiome mediated the postulated association between oral soft tissue diseases and psychological stress.

In an ongoing study at the University of Illinois at Chicago (UIC) Department of Psychiatry, 29 participants (mean age, 35.3 years; 65% male; 93% African American) were given a thorough oral soft tissue examination, and oral soft tissue lesions were recorded. Demographic variables, mood, trauma, and stress questionnaires were also measured. Independent t-tests were conducted to examine the association between having an oral soft tissue lesion and the measures of mood, trauma, and stress. Given the small sample sizes, Cohen’s d effect sizes were calculated (small effect = 0.2; medium effect = 0.5; large effect = 0.8).

Based on the oral examination, 10 out of 29 HIV-infected individuals presented with oral soft tissue lesions. Among the 10 individuals with oral lesions, the most common lesions were oral candidiasis (23%) and oral warts (23%). Compared to individuals without oral soft tissue lesions, individuals with oral soft tissue lesions were found to have a statistically significant (p>0.05) increase in depressive symptoms and emotional neglect. No significant differences in the oral bacterial microbiome could be detected when comparing subjects with and without oral lesions. The oral microbiome did not differ either when comparing subjects with higher and lower levels of psychological stress.

This study is the first report of an association between oral soft tissue lesions, an objective clinical sign of defective immunity, and psychological stress in HIV. The
significance of this finding should be explored further in the context of a possible bidirectional interaction between mental health and immunity in people living with HIV.
1 INTRODUCTION

1.1 Background

Human Immunodeficiency virus (HIV) is the pathogenic agent responsible for Acquired Immune Deficiency Syndrome (AIDS). The disease was first reported as Pneumocystis pneumonia in 5 patients in Los Angeles, California in 1981 (CDC, 1981). HIV was not isolated until 1983 (R. C. Gallo et al., 1983). Early in the epidemic, signs of HIV infection were only recognized late into the AIDS disease progression when patients presented with critical opportunistic infections suggestive of a severe immune deficiency that could not be explained otherwise. Subsequent retrospective analysis revealed additional AIDS cases from 1978-81, with the earliest evidence of AIDS in North America dating back to 1977 (Robbins et al., 2003). In the past 34 years, HIV/AIDS has become a worldwide epidemic—more than 25 million people have died of the disease, approximately 35.3 million are living with HIV, and there were 2.3 million new infections in 2012 (Joint United Nations Programme on HIV/AIDS 2013).

The most current Centers for Disease Control (CDC) data for the United States estimate 1.1 million people in the United States are living with HIV (95% CI 1,117,800–1,178,500) (CDC, 2012). Approximately 18% of people infected with HIV in the United States (207,000 people) are unaware of their HIV+ serostatus (CDC, 2012). The 13-24 age group has the largest proportion of undiagnosed HIV infection with an estimated 60% undiagnosed (CDC, 2012). Undiagnosed people are at greater risk of spreading HIV due to increased viral load in body fluids as well as continued high-risk behavior (Miller, Rosenberg, Rutstein, & Powers, 2010). Risk of infection to both healthcare providers and sexual partners of HIV+ individuals is
decreased by adherence to a viral suppressive Highly Active Antiretroviral Therapy (HAART) regimen (Del Romero, Castilla, Hernando, Rodriguez, & Garcia, 2010; Reynolds et al., 2011).

Since HIV was first recognized, soft tissue lesions of the mouth and pharynx have been shown to correlate to degrees of immune suppression associated with HIV infection (CDC, 1981; L. Patton, 2014). Lesions of the mouth are considered some of the earliest signs of immune suppression as well as markers for disease progression or treatment failure in both treated and non-treated HIV infection (Coogan, Greenspan, & Challacombe, 2005; Nokta, 2008; Sroussi & Epstein, 2007). HIV-associated oral soft tissue lesions can be associated with significant morbidity including “significant oral pain, diminished nutritional intake, and wasting” (Nokta, 2008). The presence of oral lesions is also a source for an increased amount of HIV particles in the saliva, which has the potential to increase the transmission of the virus (Bolscher et al., 2002).

Despite the tremendous advances in the pharmacological treatment of HIV, great variability exists in individual response to treatment. While much of this variability has not been thoroughly explained, some have proposed that psychological stress can play a role in the variability of response to treatment (Chida & Vedhara, 2009). Meta analysis of the pertinent literature has concluded that certain personality types, coping styles, and/or psychological distress have a tendency to have a more significant association with a diminished HIV disease prognosis (Chida & Vedhara, 2009). Therefore, it is reasonable to speculate that as oral lesions are a measure of HIV disease progression, they may be associated with markers of psychological stress. To our knowledge, there has been no publication reporting an
prevalence of oral soft tissue lesions in HIV. The present study addresses this gap of knowledge.

1.2 Specific Aims

The primary aim of this study was to investigate a possible association between oral soft tissue lesions and psychological stress in HIV+ subjects. Additionally, a second aim was to investigate if alterations in the bacterial oral microbiome mediate this association.

1.3 Null Hypothesis

The null hypothesis tested in the primary aim was the following: there is no association between oral soft tissue lesions and psychological stress in HIV subjects. The second aim was investigated by testing two null hypotheses. First, there is no association between oral soft tissue lesions and a reduction in the diversity of the oral microbiome. Second, there is no association between increased levels of psychological stress and a reduction of diversity of the oral microbiome.

1.4 Literature Review

1.4.1 HIV and Oral Soft Tissue Lesions

There are seven main oral soft tissue lesions associated with HIV infection: oral pharyngeal candidiasis (OPC), oral hairy leukoplakia (OHL), non-Hodgkin’s lymphoma (NHL), human papilloma virus (HPV)-associated lesions, oral Kaposi’s sarcoma (KS), necrotizing ulcerative gingivitis (NUG), necrotizing ulcerative periodontitis (NUP), necrotizing stomatitis, and marginal linear erythema (Gottlieb et al., 1981; Mataftsi, Skoura, & Sakellari, 2011; Nokta, 2008; Saini, 2011). Before the introduction of effective anti-retroviral medications, OHL, KS, and OPC were seen when the CD4+ cell count dropped below 400 cells/mL: major aphthous ulcers. NUP.
NUG, and necrotizing stomatitis were seen when the CD4+ cell counts dropped below 200 cells/mL (Leigh, Shetty, & Fidel, 2004). Oral KS, esophageal candidiasis, lymphoma, and chronic herpes simplex ulcers are also used to mark the transition to AIDS and are known as AIDS-defining illnesses (Leigh et al., 2004).

Oral Pharyngeal Candidiasis is the most common opportunistic infection of the oral mucosa in HIV+ patients and is experienced by 50-90% of patients at some point during the course of their disease (Nokta, 2008; Owotade & Patel, 2014). The first AIDS cases described in the US all presented with mucosal candidiasis (CDC, 1981). OPC is most often caused by an abnormal overgrowth of a commensal fungus (i.e. Candida albicans) and it is a sign of dysfunctional immunity (Leigh et al., 2004). In the oral-facial region, pathogenic yeast infections most often present as angular chelitis, erythematous candidiasis, and pseudomembranous candidiasis. Less commonly, Candida infection presents as esophageal candidiasis, which is a landmark feature of HIV infection and is classified as an AIDS-defining illness (Cassone & Cauda, 2012). It is thought that adverse changes in the host’s oral environment allow overgrowth of Candida organisms and cause pathology rather than a change in the pathogenicity of the fungus itself (Owotade & Patel, 2014). For example, it was proposed that HIV-mediated depletion of the Th17 subset of CD4+ cells results in the loss of the host’s ability to prevent commensal candida from invading the oral mucosa (Cassone & Cauda, 2012). With the introduction of HAART, and consequent increase in CD4+ T-cell count, a decrease in the incidence of oral candidiasis was documented (Nokta, 2008). Paradoxically the incidence is also seen to increase in some patients after beginning HAART in what is known as immune reconstitution syndrome (Nokta, 2008).
Oral hairy leukoplakia (OHL) is a lesion of the oral mucosa originally described in HIV infection. OHL was first recognized early in the HIV pandemic and served as an objective clinical sign of HIV disease and progression (D. Greenspan et al., 1984). OHL is an overgrowth of oral epithelial cells caused by human herpes virus 4 (Epstein-Barr virus). It does not require treatment but rather is an indication of HIV-mediated immune suppression or HAART failure (Nokta, 2008).

Kaposi’s sarcoma (KS) was the malignancy most commonly associated with HIV/AIDS, although the incidence of KS in the post-HAART era has dropped dramatically in industrialized countries (Nokta, 2008). While KS lesions can be found in many tissues in the body, approximately 50% of AIDS-associated KS have oral involvement (Levine & Tulpule, 2001). Human Herpes Virus 8 (HHV8) is the etiologic agent of AIDS-associated KS, and the oral cavity is an important site for replication, transmission, and pathogenesis of HHV8 (Nokta, 2008). KS is listed as an AIDS-defining illness and is historically associated with low CD4+ T-cell counts, KS has also been reported even in patients with high CD4-cell counts (Sroussi, Villines, Epstein, Alves, & Alves, 2006).

Periodontal lesions associated with HIV disease include linear gingival erythema (LGE), necrotizing ulcerative periodontitis (NUP), necrotizing ulcerative gingivitis (NUG), and necrotizing stomatitis (Nokta, 2008). LGE was one of the first oral lesions associated with AIDS and typically presents in patients with a CD4+ count less than 200 cells/cc (Nokta, 2008). Clinically, it presents as a red band along the free gingival margin of the anterior teeth, and the etiologic agent is thought to be a species of candida (Mataftsi et al., 2011). NUP and NUG are aggressive infections of the periodontal structures that cause rapid destruction of those structures (Mataftsi et al., 2011). The data concerning the association between NUP, NUG and CD4+
and/or viral load have been conflicting, with some studies reporting both NUP and NUG as having a positive predictive value for immune suppression and others reporting no predictive value (Mataftsi et al., 2011). In HIV+ patients properly responding to a HAART regimen, the incidence and severity of HIV-associated periodontal diseases (LGE, NUP, NUG, and necrotizing stomatitis) is believed to be similar to what is observed in healthy non-HIV infected patients (Ryder, 2002).

Non-Hodgkins lymphoma (NHL) in HIV+ patients typically presents as plasmablastic lymphoma (PBL) in the oral cavity and is a distinct lymphoma related to HIV infection (Delecluse et al., 1997). NHL of the oral cavity is an AIDS defining illness and presents in the mouth as soft tissue masses with or without ulceration (Nokta, 2008). There is an increased incidence of PBL in patients on long-term HAART, and the prognosis is very poor; most patients die within the first year after diagnosis (Nokta, 2008; Thirlwell, Sarker, Stebbing, & Bower, 2003).

Oral warts are one of several lesions including OPC, OHL, and salivary gland disease that have not had a significant reduction in incidence in the post-HAART era (D. Greenspan, Canchola, MacPhail, Cheikh, & Greenspan, 2001; D. Greenspan et al., 2004; L. L. Patton et al., 2013). “HPV-associated oral warts have a prevalence of 0.5% in the general population, occur in up to 5% of HIV-seropositive subjects, and in up to 23% of HIV-seropositive subjects on highly active antiretroviral therapy” (Feller et al., 2011). While the occurrence of oral warts is temporally related to starting HAART, it is debated if oral warts are part of immune reconstitution syndrome (Lilly et al., 2005; Tsang & Samaranayake, 2010). They persist after starting HAART, and several groups have found a positive association between the presence of oral warts and the length of time on HAART or time since HIV diagnosis (Anaya-Saavedra et al., 2013; Sroussi et al., 2006). While oral warts are benign and
not associated with significant morbidity, there is some evidence suggesting they could help explain the increased incidence of head and neck cancers in HIV+ patients (Anaya-Saavedra et al., 2013).

HIV-associated salivary gland disease is a swelling of the major salivary glands, particularly the parotid gland, and is similar in presentation to Sjogren’s syndrome (Jeffers & Webster-Cyriaque, 2011). Pain and facial disfigurement can also accompany the primary complaint of xerostomia (Jeffers & Webster-Cyriaque, 2011). Several studies have shown an increase in the incidence in patients on HAART (J. S. Greenspan & Greenspan, 2002; L. L. Patton et al., 2013).

1.4.2 HIV Treatment

At the beginning of the AIDS epidemic there was no effective treatment for HIV and the death rate approached 100% (Palmisano & Vella, 2011). In 1987, azidothymidine (AZT) was the first pharmacologic therapy approved by the FDA to treat HIV. AZT did not produce a long-lasting increase in CD4+ cells (Palmisano & Vella, 2011). AZT is a Nucleoside Reverse Transcriptase Inhibitor (NRTI) which are nucleoside analogs that inhibit the viral protein reverse transcriptase (Das & Arnold, 2013). Similar to NRTI’s, Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI) interfere with HIV reverse transcriptase but do so as a non-competitive inhibitor (Das & Arnold, 2013). Subsequent anti-retroviral drugs target other steps in the HIV infection and replication cycle. Integrase inhibitors a class of anti-retroviral drugs that prevent the integration of HIV DNA into the host DNA (Metifiot, Marchand, & Pommier, 2013). A class of antiretroviral drugs known as fusion inhibitors prevent HIV infection of the host cells (Biswas, Tambussi, & Lazzarin, 2007). Protease inhibitors are designed to prevent HIV replication by inhibiting a key protein required for viral replication (Wensing, van Maarseveen, & Nijhuis, 2010). When the protease
inhibitor class of antiretroviral drugs was introduced in 1996, they were combined with other antiretroviral drugs (Palmisano & Vella, 2011). This regimen became known as Highly Active Antiretroviral Therapy or HAART (Palmisano & Vella, 2011). As new classes of anti-retroviral drugs have been developed, further combinations of drug classes and new drugs within classes have been developed and are now standard of care for HIV (World Health Organization, 2013). There are currently 26 antiretroviral medications from six different drug classes and additional new drugs are currently in development (Li & Foisy, 2014).

The goal of HAART is to reduce HIV viral load and increase the amount of CD4+ T-cells. With the widespread use of HAART in the US, significant reduction in the morbidity and mortality associated with HIV/AIDS has been observed (Llibre et al., 2009). Most oral lesions associated with HIV infection such as OHL, OPC, KS, and major aphthous ulcers have gone down in countries with high HAART utilization. In the US, as well as in other developed countries, an increase in the incidence of oral warts and salivary gland disease in the post-HAART era has been reported (D. Greenspan et al., 2001; Navazesh et al., 2003). The biological mechanism explaining the increasing prevalence of the different oral lesions is not well understood. Some groups suggest the oral epithelium is more permeable and disturbed during HIV infection (Challacombe & Naglik, 2006; Hille, Webster-Cyriaque, Palefski, & Raab-Traub, 2002). There is evidence to suggest increased permeability of the oral epithelium involves up regulation of various cell surface receptors including HIV receptors (Challacombe & Naglik, 2006). Many of the studies conducted in the United States on HIV associated oral lesions were conducted before HAART and during previous generations of HAART. More data is needed to determine what the incidence of oral lesions is with the rapidly evolving HAART.
1.4.3 Immune Reconstitution Inflammatory Syndrome

In some cases, when HAART is initiated during a period of immune suppression, a paradoxical worsening of preexisting infections known as immune reconstitution syndrome or immune reconstitution inflammatory syndrome (IRIS) may occur (Meintjes, Scriven, & Marais, 2012). Approximately 25-30% of patients starting a HAART regimen present with worsening clinical presentation of preexisting conditions despite decreasing HIV viral loads and commensurate increase in CD4+ T-cell lab values (French et al., 2000; Narita, Ashkin, Hollender, & Pitchenik, 1998; Tsang & Samaranayake, 2010). “Immune reconstitution is defined as a CD4+ count of > 200 cells/mm³ and an increase of ≥100 cells over baseline any time since starting HAART” (Arici et al., 2001; Tsang & Samaranayake, 2010) IRIS usually takes place within the first 30 days of starting a HAART regimen (Tsang & Samaranayake, 2010). In the oral cavity, IRIS-associated oral lesions can theoretically be the result of any pathogen that can cause an oral lesion. A limited study on the subject has suggested Kaposi’s sarcoma, oral candidiasis, oral warts, salivary gland disease, herpes zoster, and/or hairy leukoplakia are the more common oral lesions seen in IRIS (Tsang & Samaranayake, 2010).

1.4.4 HIV and Psychological Stress

Despite extraordinary advances in the pharmacological treatment of HIV, inconsistency exists in the individual response to treatment. While much of this variability has not been thoroughly explained, some have proposed that psychological stress can play a role in the variability of response to treatment (Chida & Vedhara, 2009). Meta analysis of the pertinent literature has concluded that certain personality types, coping styles, and/or and psychological distress tend to be associated with a diminished HIV-disease prognosis (Chida & Vedhara, 2009). There
are many parameters by which HIV-disease progression can be assessed, including viral load, CD4+ decline, AIDS diagnosis, and AIDS mortality, to name a few. As oral soft tissue lesions are also a measure of HIV disease progression, are they could be associated with worse measures of psychological stress. The results of stress, personality type, and coping styles have not been studied for its possible association with the prevalence of oral lesions in a HIV+ population on HAART or to the oral soft tissue lesions uniquely associated with HIV infection. It should be noted that, independently of this gap of knowledge, an association between psychological stress and certain oral soft tissue lesions has been established by others (Akcali, Huck, Tenenbaum, Davideau, & Buduneli, 2013; Chida & Mao, 2009; C. d. B. Gallo, Mimura, & Sugaya, 2009; Manolache, Seceleanu-Petrescu, & Benea, 2008; Stock et al., 2001). Consequently, while the association between psychological stress and oral soft tissue lesions in HIV investigated in this study is novel, associations between markers of mental and oral health are to an extent well-established (please see section 1.4.9 below).

1.4.5 HIV and Perceived Stress

“Stress has been shown to predict more rapid CD4 cell decline and viral load increase, as well as the presentation of clinical symptoms, and progression to AIDS” (Ironson et al., 2014). Ironson et al correlated perceived stress with reduced effectiveness of antiretroviral medications (Ironson et al., 2008). Perceived stress can be measured by the ten-item Perceived Stress Scale (PSS-10) test. The test consists of ten items designed to test “the degree to which persons perceive situations in their life as excessively stressful relative to their ability to cope” (Cohen, Kamarck, & Mermelstein, 1983; Taylor, 2014). Higher levels of perceived stress as
measured by the PSS-10 test was associated with a decline in CD4+ counts (Remor, Penedo, Shen, & Schneiderman, 2007).

1.4.6 HIV and Depressive Symptoms

Much like perceived stress, depressive symptoms have been shown to have a negative impact on HIV disease as measured by various parameters. The Center for Epidemiologic Studies Depression Scale (CES-D) is a 20-item questionnaire used to evaluate depression over the previous seven-day time span (Radloff, 1977). It has been shown to be an effective tool for measuring depression in an HIV+ population (Kalichman, Rompa, & Cage, 2000). The CES-D has been used to show an association between increases in depressive symptoms and decreases in CD4+ T-cell counts and increase in the rate of CD4+ T-cell decline, as well as an increase in AIDS mortality (Anastos et al., 2005; Burack et al., 1993; Ickovics et al., 2001).

1.4.7 HIV and Childhood Trauma

The Childhood Trauma Questionnaire (CTQ) uses a 27-item questionnaire to measure emotional, physical, and sexual abuse, and emotional and physical neglect (Bernstein et al., 2003). There is evidence to suggest that individuals with a greater childhood trauma experience, as measured by the CTQ, have altered immune profiles (Leserman et al., 2005; Woods et al., 2005). In HIV+ patients, childhood trauma has not been shown to be reflected in objective measures of disease, but it has been shown to correlate to increased self-reported, HIV-related symptoms (Kalichman, Sikkema, DiFonzo, Luke, & Austin, 2002). There is no data correlating CTQ scores nor other measures of trauma to oral soft tissue lesions.
1.4.8 Stress Response and HIV

The mechanisms to explain the association between psychological stress response and HIV outcome have not been fully elucidated (Ironson et al., 2014). The mechanisms by which psychological stress interacts with physiology is known as the Hypothalamic-Pituitary-Adrenal (HPA) axis (Zapanti, Terzidis, & Chrousos, 2008). Stress hormones such as cortisol are released in response to activation of the HPA system by psychological stress (Zapanti et al., 2008). Cortisol directly downregulates the immune system (Antoni et al., 2005). It also increases HIV infection of lymphocytes (Ironson et al., 2014) and down-regulates T-cell activation (Patterson et al., 2013). It is thought that overstimulation of the HPA axis by increased levels of psychological stress in HIV+ patients would further accentuate immune suppression. This could explain, in part, the poorer prognosis observed in HIV+ patients with high levels of stress (Kumar, Kumar, Waldrop, Antoni, & Eisdorfer, 2003).

1.4.9 Oral Health and Psychological Stress

The influence of psychological stress on periodontal lesions has been investigated (Akcali et al., 2013), although similar studies in an HIV+ population have not been reported. The etiological agents of periodontal disease are known, but the variability in disease progression between individuals cannot be fully explained by the abundance of the etiologic agent in the mouth. It is thought that host-mediated factors play a significant role in periodontal disease progression (Akcali et al., 2013). The majority of studies that have looked at the association of psychological stress and periodontal disease have found a positive correlation between increased levels of psychological stress and worsening of periodontal disease (Akcali et al., 2013; McCracken, 2009; Peruzzo et al., 2007)
Higher levels of perceived stress have been shown to be a risk factor for recurrent herpes simplex-1 (HSV) lesions (Stock et al., 2001). A meta analysis found that psychological stress was strongly associated with symptomatic oral HSV recurrence (Chida & Mao, 2009). Oral mucosal wound healing was shown to be decelerated in association with higher levels of perceived stress (Marucha, Kiecolt-Glaser, & Favagehi, 1998). Additionally, psychological stress has been implicated in higher incidences of both recurrent stomatitis and disease exacerbation in patients with oral lichen planus (C. d. B. Gallo et al., 2009; Manolache et al., 2008; Soto Araya, Rojas Alcayaga, & Esguep, 2004).

1.4.10 The Use of State of the Art Technology to Study the Oral Microbiome

Microbiome is a term used to refer to all the microorganisms living in a certain environment and their collective genome (Chen & Jiang, 2014). The human oral cavity has one of the most diverse microbiomes in the human body, with over 700 species detected (Aas, Paster, Stokes, Olsen, & Dewhirst, 2005). Many of the species of bacteria in the human mouth have not yet been cultured and culture-independent methods for identification have become increasingly relevant to the studies of the oral microbiome. In fact of the 700 species of bacteria identified in the mouth, only 250 have been isolated and cultured (Paster, Olsen, Aas, & Dewhirst, 2006).

The original technologies used to quantify and identify bacteria involved culture of the bacteria. The fact that many of the bacteria in the human body and specifically the oral cavity, are not amenable to culture in a laboratory severely limits the utility of culture-based techniques for investigating the microbiome. Consequently, nucleic acid-based techniques have replaced culture-based techniques for the oral cavity. The first techniques available to identify bacteria using nucleic acid-based techniques were probes that could identify species-specific sequences for individual species of bacteria. These technology advances have made it possible to identify bacteria that are not culturable.
of bacterial species in a culture-independent technique was the microarray system. With the advent of Human Microbiome Project sponsored by the NIH, microbial microarrays have been replaced by DNA sequencing technology, particularly high-throughput pyrosequencing of the bacterial 16S rRNA gene. With each paradigm shift in bacterial identification technology, the resolution with which the microbiome can be studied has risen exponentially. Greater than 800 reference strains can now be detected in a single sample using 16S pyrosequencing.

The mouth harbors several habitats for distinct microbiomes. The buccal, gingival and hard palate mucosa harbor a similar microbiome (Ligtenberg & Almståhl, 2015). The biodiversity of anatomical sites is described and measured in several different ways. The number of species in a given site is described as species richness but this does not take the abundances of each species relative to one another into account (Zhou et al., 2013). Evenness describes how close the numbers of each species in a given habitat are to one another; oral sites tend to have the highest evenness compared to another human anatomic sites (Zhou et al., 2013). Simpson diversity is used to describe biodiversity, taking to account both richness and abundance (Simpson, 1949). Shannon diversity takes both species richness and evenness into account (Zhou et al., 2013).

1.4.11 HIV and the Oral Microbiome

Disturbance in the oral microbiome has also been suggested as a possible contributor to oral and systemic disease in HIV+ patients on HAART. Navazesh et al found that potentially pathogenic oral bacteria were increased in HIV+ patients on HAART including Peptostreptococcus micros, Campylobacter species, Eubacterium species, and Tannerella forsythia (Navazesh et al., 2005). They also noted that...
measured by CD4+ cell counts and HIV viral load) (Navazesh et al., 2005). The diversity of the oral flora this study examined was limited by traditional bacterial culture techniques to only 23 bacterial species (Navazesh et al., 2005). Nonetheless, this study suggested that “HAART is an independent and significant risk factor for the occurrence of certain bacterial pathogens” in the oral cavity of HIV+ patients (Navazesh et al., 2005). Current generation DNA sequencing technology offers great potential to expand on the Navazesh et al study for a more complete picture of the oral microbiome and its relationship to HIV diseases and its associated co-morbidities.

1.4.12 Microbiome and Stress

The microbiome and behavior is most extensively studied in the gut microbiome and is known as the gut-brain axis. A number of animal studies have shown that the gut microbiome not only influences behavior but also is also required for normal function of the central nervous system (Clarke et al., 2013; Diaz Heijtz et al., 2011; Neufeld, Kang, Bienenstock, & Foster, 2011). The relationship between the gut microbiome and the CNS is a bidirectional relationship. Studies in neonatal rats show that experimental stress “leads to long term changes in the diversity and composition of gut microbiota”, see Figure 1 (Foster & McVey Neufeld, 2013; Garcia-Rodenas et al., 2006). Demonstrating the two-way relationship, intestinal microbiota have been shown experimentally to be necessary for some of the stress-induced changes in the immune response (Allen et al., 2012).
Figure 1 A bidirectional interaction between the gut microbiome and the gut-brain axis.

A bidirectional interaction between the gut microbiome and the gut-brain axis determines normal homeostasis and is proposed to influence the risk of disease. Alterations in the gastrointestinal (GI), central nervous system (CNS), autonomic nervous system (ANS) and immune system can lead to changes in mucosal barrier function, inflammation, stress reactivity, and behavior which in turn can influence the balance between health and disease. Figure adapted from Foster and Neufield 2013.

1.4.13 Microbiome and Oral Soft Tissue Lesions

Variations in the oral microbiome have been proposed as a method of testing for several diseases of the mouth such as dental caries, (Yang et al., 2012), periodontal disease (Sakamoto, Umeda, Ishikawa, & Benno, 2000), halitosis (Riggio et al., 2008) and oral squamous cell carcinoma (Pushalkar et al., 2011). Diseases of more distant anatomical sites such as pancreatic cancer (Farrell et al., 2012), diabetes (Casarin et al., 2013), atherosclerosis (Koren et al., 2011), inflammatory
bowel disease (Docktor et al., 2012), and miscarriage, low birth weight, and pre-term birth (Mitchell-Lewis, Engebretson, Chen, Lamster, & Papapanou, 2001; Offenbacher et al., 2006) have also been reported to have a detectable effect on the composition of the oral flora.

Changes in oral flora have not been extensively investigated for their association to oral soft tissue lesions, particularly for some of the lesions and presentations most commonly diagnosed in HIV. Hijazi et al suggested that changes in the oral microbiome play a role in the initiation of recurrent apthous stomatitis (RAS) but did not provide data supporting causality (Hijazi et al., 2014). Hijazi et al found statistically significant differences in species diversity including increased levels of *Streptococcaceae* in healthy controls and increased levels of *Bacteroides* in patients with a history of RAS. These differences were observed even if those patients did not have active RAS lesions at the time sampling was performed (Hijazi et al., 2014). Kraneveld et al have looked at the role oral candida load has on the oral bacterial microbiome in edentulous patients with dentures. They reported that the diversity of the bacterial microbiome decreased as the candida load increased (Kraneveld et al., 2012).
2 MATERIALS AND METHODS

2.1 Clinical Exam

In collaboration with the UIC Department of Psychiatry, 29 HIV+ individuals currently adhering to a HAART regimen were given an oral soft tissue examination and completed the following mood, trauma, and stress questionnaires: Childhood Trauma Questionnaire (CTQ), Perceived Stress Scale (PSS), Schedule of Life Events, PTSD Checklist-Civilian version (PCL-C), Center for Epidemiologic Studies Depression Scale (CESD-10).

Dr. Herve Sroussi and Dr. Robert Schwartz at the University of Illinois at Chicago performed the oral clinical examinations. Participants were given a head and neck examination and the presence of lymphadenopathy, salivary gland enlargement, saliva production, the presence and location of the following oral lesions: angular chelitis, candidiasis (pseudomembranous and erythematous), leukoplakia, hairy leukoplakia, herpes labialis, herpetic lesion intraoral, aphthous ulcers (major or minor), denture stomatitis, denture ulcer, other ulcer, oral papilloma/wart, Kaposi's sarcoma and abscess.

Sterile cotton swabs were used to swab the base of the tongue and tonsillar pillars for microbial analysis. Three milliliters of unstimulated saliva were collected in 3ml of sterile phosphate buffered saline (PBS). All samples were stored at -20°C until analysis.

Based upon clinical observation and palpation, lymphadenopathy was defined as an enlargement of the pre- or postauricular, submandibular, submental, occipital,
posterior cervical, supraclavicular, or anterior cervical lymph nodes. Consistency and
tenderness were noted. Size of enlarged nodes was recorded as >1cm or <1cm.
Salivary gland enlargement was defined as a unilateral or bilateral enlargement of the
parotid glands. Saliva production was determined by the ability to express saliva from
either Stensen duct. Angular chelitis was defined as fissures or linear ulcers at the
commisures of the mouth. Pseudomembranous candidiasis was defined as the
presence of creamy white or yellow plaques anywhere in the mouth on an erythematous
or normal appearing mucosa that could be removed by scraping with cotton swab.
Erythematous candidiasis was defined as an erythematous area of mucosa, with a flat
or fissured surface, at any location in the mouth. Hairy leukoplakia was defined as a
corrugated, vertically oriented, white plaque that does not rub off. Herpetic lesions were
defined as ulcers or vesicles on keratinized mucosa or vermillion border of the lips and
adjacent facial skin with crusting. Aphthous ulceration was defined as “single or multiple
recurrent, well-circumscribed, painful ulcer(s) on non-keratinized tissue, measuring 0.2
to 0.5 cm with border, from which no etiologic agent can be identified” (J. S. Greenspan,
Barr, Sciubba, & Winkler, 1992). Major aphthous ulceration is similar to minor with the
distinction being size greater 0.5cm. Oral papilloma was identified as papillary
outgrowths of the oral mucosa.

2.2 Psychological Testing

Psychological testing was performed at the UIC Clinical Research Center (CRC)
by clinical staff under the supervision of a clinical psychologist. Stress was measured by
the 10 question Perceived Stress Questionnaire (PSS-10). Scores were split at the
median (22) for statistical comparison to microbiome data. Mood was assessed by the
20-item Center for Epidemiologic Studies Depression Scale (CES-D). The CES-D is used to evaluate depression over the previous 7-day time span. Scores greater than 16 on the CES-D were considered to have depression for the purpose of microbiome data analysis. The Childhood Trauma Questionnaire (CTQ) measured sexual abuse and emotional neglect. The CTQ has subset tests to measure emotional, physical, and sexual abuse, and emotional and physical neglect. Severe scores on the subset tests were used for microbiome data analysis. The CTQ score was assessed as follows as described by Bernstein et al (Bernstein et al., 2003):

Total score:
25-31 no trauma
41-51 low-moderate
56-68 moderate to severe
73-125 severe to extreme

Subset tests in the CTQ:
5 none
6-7 low
8-12 moderate
>12 severe

2.3 Lab Values

Ten milliliters of blood was collected for CD4+ T-cell and viral load analysis. Viral load was assayed using Roche® COBAS AmpliPrep®/COBAS TaqMan® system. CD4+ T-cells were assayed using single platform method and multiparameter flow cytometry.
2.4 Microbiome Analysis

Microbiome analysis was based on previously published techniques by Caporaso et al in 2012 (Caporaso et al., 2012). PCR was used to amplify the bacterial genomic DNA using primers targeting conserved regions of bacterial 16S rRNA genes. The amplicons were then labeled using custom primers to incorporate sample-specific barcodes. The labeled amplicons were then mixed and sequenced on a next-generation sequencer. The sequences were separated into groups of sequences with the same barcode (each sample). Quality control was performed on each pool of sequences, and poor quality data was removed.

Retaining sample origin information, the data were combined. Groups of sequences with high similarity (>97%) were created from all samples. These groups, called Operational Taxonomic Units (OTUs), can be thought of as "species". The number of sequences from each sample in each OTU group was noted. One representative sequence from each OTU was further annotated for taxonomy, and this taxonomy was applied to all sequences in the OTU. The taxonomic information was compiled into tables with sample by taxon information. For each taxon, the number of sequences from each sample was reported. The table, a biological observation matrix (BIOM), was the basis of downstream statistical analyses. The BIOM was used to calculate diversity indices for each sample and to perform comparisons of all the samples within the study using the ordination technique of principal coordinate analysis.
2.5 Statistical Analysis

Independent t-tests were conducted to examine group differences (oral lesions yes vs. no) in mood, trauma, and stress. Given the small sample sizes, Cohen’s d effect sizes (small effect = 0.2; medium effect = 0.5; large effect = 0.8) were also calculated. Statistical significance was set at p<0.05.

Mann-Whitney U tests were used to examine differences in the microbiome of the group with oral soft tissue lesions and the group without oral soft tissue lesions. Statistical significance was set at p<0.07 for the microbiome analysis. The Mann Whitney U test was also used to examine differences between species richness, evenness, Shannon Index, and Simpson Index and high levels of psychological stress. SPSS version 22.0 (Chicago, IL) was used for data analysis.
3 RESULTS

The primary aim of this study was to investigate a possible association between oral lesions and psychological stress in people living with the human immunodeficiency virus (HIV). Informed consent, approved by UIC IRB protocol 2012-0466, was obtained from all subjects enrolled in the study. Twenty-nine HIV-infected subjects had an oral soft tissue examination and completed mood, trauma, and stress questionnaires. The subjects had a mean age of 35.3 years (22.13-45.91 years); 65% were male (n=19) and 93% African American (n=27).

Based on the oral examination, 10 (34%) out of 29 HIV-infected subjects presented with oral soft tissue lesions. Among the 10 individuals with oral lesions, a total of 13 lesions were diagnosed with the most common lesions being oral candidiasis (n=3, 23%) and oral warts (n=3, 23%). Socio-demographic factors, health behaviors, and clinical characteristics known to be associated with an increased risk of having an oral soft tissue lesion were compared in subjects with or without an oral soft tissue lesion. These data are summarized in table 1. Of the measured variables, none were statistically significant when comparing the two groups (see table 1).
### TABLE I
DEMOGRAPHIC AND CLINICAL CHARACTERISTICS FOR HIV-INFECTED INDIVIDUALS WITH AND WITHOUT ORAL LESIONS

<table>
<thead>
<tr>
<th>Variables</th>
<th>Oral Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (n=19)</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
</tr>
<tr>
<td><strong>Socio-demographic factors</strong></td>
<td></td>
</tr>
<tr>
<td>Age, M (SD)</td>
<td>34.4 (8.7)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
</tr>
<tr>
<td>&lt;high school</td>
<td>8 (42)</td>
</tr>
<tr>
<td>High school</td>
<td>5 (26)</td>
</tr>
<tr>
<td>&gt; High school</td>
<td>6 (32)</td>
</tr>
<tr>
<td>Male</td>
<td>13 (68)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>18 (95)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Other</td>
<td>-</td>
</tr>
<tr>
<td><strong>Risky health behaviors</strong></td>
<td></td>
</tr>
<tr>
<td>Number alcoholic drinks/week</td>
<td>1.23 (1.64)</td>
</tr>
<tr>
<td>Current use^1</td>
<td></td>
</tr>
<tr>
<td>tobacco</td>
<td>9 (47)</td>
</tr>
<tr>
<td>marijuana</td>
<td>11 (58)</td>
</tr>
<tr>
<td>cocaine</td>
<td>1 (5)</td>
</tr>
<tr>
<td>heroine</td>
<td>3 (16)</td>
</tr>
<tr>
<td>methamphetamines</td>
<td>1 (5)</td>
</tr>
<tr>
<td><strong>Clinical characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>CD4 Count (cells/µl)</td>
<td></td>
</tr>
<tr>
<td>&gt; 500</td>
<td>9 (50)</td>
</tr>
<tr>
<td>≥ 200 and ≤ 500</td>
<td>6 (33)</td>
</tr>
<tr>
<td>&lt; 200</td>
<td>2 (17)</td>
</tr>
<tr>
<td>Viral Load (HIV RNA (cp/ml))</td>
<td></td>
</tr>
<tr>
<td>Undetectable</td>
<td>7 (37)</td>
</tr>
<tr>
<td>&lt; 10,000</td>
<td>8 (42)</td>
</tr>
<tr>
<td>≥ 10,000</td>
<td>4 (21)</td>
</tr>
<tr>
<td>HAART use</td>
<td>19 (100)</td>
</tr>
</tbody>
</table>

^1“Current” use in the past month; Undetectable ≤ 48 copies/ml
3.1 Associations Between Oral Lesions and Depressive Symptoms, Trauma, and Stress

Independent t-tests were conducted to examine group differences in mood, trauma, and stress comparing subjects with and without an oral soft tissue lesion. Compared to individuals without oral soft tissue lesions, individuals with oral soft tissue lesions reported greater depressive symptoms and emotional neglect, $t (27)=-2.18$, $p=0.04$, Cohen’s $d=-0.85$, and $t (27)=-2.60$, $p=0.01$, Cohen’s $d=-1.01$, respectively, see table 2. Although not statistically different, a similar pattern was also noted for sexual abuse and perceived stress, $t (27)=-1.82$, $p=0.08$, Cohen’s $d=-0.71$ and $t (27)=1.47$, $p=0.15$, Cohen’s $d=-0.57$, respectively. The summary of these results is presented in table II.
# TABLE II

ASSOCIATIONS OF ORAL LESIONS AND DEPRESSIVE SYMPTOMS, TRAUMA, AND STRESS IN INDIVIDUALS WITH HIV

<table>
<thead>
<tr>
<th>Variables</th>
<th>No (n=19)</th>
<th>Yes (n=10)</th>
<th>p-value</th>
<th>Cohen’s d (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depressive symptoms (CES-D)</td>
<td>7.52 (4.26)</td>
<td>12.10 (7.08)</td>
<td>0.04</td>
<td>-0.85 (-1.65 to 0.05)</td>
</tr>
<tr>
<td>Perceived stress (PSS-10)</td>
<td>20.53 (3.83)</td>
<td>23.30 (6.34)</td>
<td>0.15</td>
<td>-0.57 (-1.35 to 0.20)</td>
</tr>
<tr>
<td>PTSD (PCL-C≥44)</td>
<td>25.10 (8.27)</td>
<td>27.40 (9.30)</td>
<td>0.50</td>
<td>-0.26 (-1.03 to 0.50)</td>
</tr>
<tr>
<td>Childhood Trauma (CTQ)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emotional abuse</td>
<td>6.95 (3.99)</td>
<td>8.80 (4.66)</td>
<td>0.27</td>
<td>-0.44 (-1.21 to 0.34)</td>
</tr>
<tr>
<td>Physical abuse</td>
<td>9.00 (5.14)</td>
<td>10.00 (4.92)</td>
<td>0.63</td>
<td>-0.19 (-0.96 to 0.58)</td>
</tr>
<tr>
<td>Sexual abuse</td>
<td>6.89 (3.94)</td>
<td>10.70 (7.42)</td>
<td>0.08</td>
<td>-0.71 (-1.50 to 0.08)</td>
</tr>
<tr>
<td>Emotional neglect</td>
<td>9.05 (3.89)</td>
<td>13.00 (3.89)</td>
<td>0.01</td>
<td>-1.01 (-1.82 to -0.20)</td>
</tr>
<tr>
<td>Physical neglect</td>
<td>8.05 (4.30)</td>
<td>9.00 (3.30)</td>
<td>0.55</td>
<td>-0.24 (-1.00 to 0.53)</td>
</tr>
<tr>
<td>Life events checklist</td>
<td>7.00 (5.32)</td>
<td>8.50 (4.38)</td>
<td>0.45</td>
<td>-0.30 (-1.07 to 0.47)</td>
</tr>
</tbody>
</table>

Note. CES-D= Center for Epidemiologic Studies Depression Scale; PSS 10=Perceived Stress Scale; PCL-C=PTSD Checklist-Civilian Version; CTQ=Childhood Trauma Questionnaire. CI=confidence interval.
3.2 Microbiome Analysis

The microbiome data was evaluated to test a hypothesized association between the bacterial diversity of the microbiome in subjects with or without oral soft tissue lesions. Bacterial richness was reported on both Shannon and Simpson indices. The data indicate there were no statistically significant differences found between overall diversity of the oral bacterial microbiome at the family level when subjects with oral soft tissue lesions were compared to subjects without oral soft tissue lesions (see Table III). There were also no significant differences found between the species evenness or overall richness in groups with and without soft tissue oral lesions, (see Table III). A marginal difference in community using analysis of similarity was observed (ANOSIM). This analysis revealed a difference but that dissimilarity did not reach statistical significance (p-value <0.07). This may partly be due to the imbalance between the number of samples from patients with and without lesions (see Figure 2).

Comparison of the psychological outcomes to the non-parametric microbiome data required sorting the patients with high scores on the various psychological assessments into a group and those with low scores into another group. The median score on the CES-D, PSS, and PCL-C tests were used to split the scores into high and low groups. No statistically significant differences were found between the oral microbiomes of subjects scoring above or below the median score on the CES-D, PSS, or PCL-C tests (see table 3). When subjects with high or extreme levels of the psychological outcomes on the CTQ tests were split based on the established “extreme” scores (Bernstein et al., 2003), the
number of subjects in each group, based on lesion presence, became too small for statistical analysis (see Figure 3).

### TABLE III

**P-VALUE CALCULATIONS**

<table>
<thead>
<tr>
<th>Species</th>
<th>Species Richness</th>
<th>Species Evenness</th>
<th>Shannon Index</th>
<th>Simpson Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Lesion</td>
<td>0.603</td>
<td>0.217</td>
<td>0.201</td>
<td>0.466</td>
</tr>
<tr>
<td>CESD</td>
<td>0.789</td>
<td>0.158</td>
<td>0.364</td>
<td>0.227</td>
</tr>
<tr>
<td>PSS</td>
<td>0.770</td>
<td>0.830</td>
<td>1.000</td>
<td>0.740</td>
</tr>
<tr>
<td>PCLC</td>
<td>0.336</td>
<td>0.570</td>
<td>0.799</td>
<td>0.625</td>
</tr>
</tbody>
</table>
Figure 2 Non metric, multi-dimensional scaling (NMDS) plot of oral microbiome

Each triangle represents a single microbial community associated with a single patient, and the more closely located two triangles are, the more similar the microbial communities are (according to Bray-Curtis metric). Samples are color-coded by presence of lesions in the patient. A marginal difference in community using analysis of similarity was observed (ANOSIM), but this was not significant (p-value <0.07). This may partly be due to the imbalance between the number of samples from patients with and without lesions.
Figure 3 The number of subjects with and without oral lesions and high levels of psychological stress

The numbers of subjects with high levels of psychological stress as measured by the CTQ test was insufficient for statistical analysis. A history of depression was classified as a score on the CES-D ≥ 16. Emotional, physical, and sexual abuse as well as emotional and physical neglect were considered severe with scores on the respective CTQ tests were greater than 12.
4 DISCUSSION

4.1 Interpretation of Results

This study was designed with a primary aim to investigate if an association exists between oral soft tissue lesions and psychological stress in HIV+ subjects. A secondary aim was designed to test whether the oral microbiome mediated the postulated association between oral soft tissue diseases and psychological stress. Based on the oral examination, 10 out of 29 HIV-infected individuals presented with oral soft tissue lesions. Compared to individuals without oral soft tissue lesions, individuals with oral soft tissue lesions were found to have a statistically significant (p>0.05) increase in depressive symptoms and emotional neglect. No significant differences in the oral bacterial microbiome could be detected when comparing subjects with and without oral lesions. The oral microbiome did not differ either when comparing subjects with higher and lower levels of psychological stress.

This study is the first to show an association between oral soft tissue lesions and psychological stress in an HIV+ population. The relationship between oral soft tissue lesions and psychological stress is very complex. The effect of oral health, and more specifically oral soft tissue lesions, on quality of life is recognized as having an important role on the mental health of patients (Mumcu et al., 2007). The interaction between oral and mental health has been explained, in part, due to the adverse effects oral disease has on nutrition, speech, self-esteem, appearance, and social interaction have on the patient’s quality of life.
(Chiappelli & Cajulis, 2004; Liberali et al., 2013). In light of the study cited above it is plausible that the oral soft tissue lesions observed in this study had a direct, negative effect on the mental health of the subjects.

The relationship between psychological stress and oral health is likely to be bidirectional. Stress may lead to defective immunity and impact the oral flora. Accordingly, psychological stress may affect the presence of oral soft tissue lesions. Independently of the success of HAART in controlling viral replication and preserving immune functions, high levels of psychological stress have been shown to have significant adverse effects on CD4 counts, viral load, AIDS diagnosis and AIDS morbidity (Chida & Vedhara, 2009; Ironson et al., 2005). Accordingly, the association between oral soft tissue lesions and psychological stress found in this study could potentially be mediated through the known effect of psychological stress on the course of HIV-related disease. Lesions of the mouth are considered some of the earliest signs of immune suppression as well as markers for disease progression or treatment failure in both treated and non-treated HIV infection (Coogan et al., 2005; Nokta, 2008; Sroussi & Epstein, 2007). Consequently, oral soft tissue lesions could be an early sign of declining immunity, resulting from the deleterious effects of psychological stress on HIV disease.

The mechanism by which psychological stress interacts with physiology is known as the Hypothalamic-Pituitary-Adrenal (HPA) axis (Zapanti et al., 2008). Stress hormones, such as cortisol, are released in response to activation of the HPA system by psychological stress (Zapanti et al., 2008). Cortisol directly down
regulates the immune system (Antoni et al., 2005) and also increases HIV infection of lymphocytes (Ironson et al., 2014) and T-cell activation (Patterson et al., 2013). It is thought that overstimulation of the HPA axis by increased levels of psychological stress in HIV+ patients would further accentuate immune suppression and could explain, in part, the poorer prognosis observed in individuals with high levels of stress and HIV (Kumar et al., 2003).

4.2 Microbiome and Oral Soft Tissue Lesions

The causative microbial agents of the oral soft tissue lesions found in this study are diverse, consisting of bacteria, viruses, and fungi. While only the bacterial microbiome was investigated in this study, recently published studies provide evidence that imbalances in the mucosal bacterial microbiome are associated with mucosal inflammatory conditions whose etiology was previously attributed to a single infectious agent (Petersen & Round, 2014). While data presented in this thesis did not confirm a disturbance in the oral microbiome of patients with oral lesions, the number of subjects with oral soft tissue lesions was small. More stringent inclusion criteria for study subjects to minimize known factors which disturb the oral microbiome, such as oral hygiene and diet, has the potential to reduce some of the variability inherent to microbiome data (Hijazi et al., 2014).

This study was the result of an interdisciplinary collaboration between oral and mental health researchers. The data presented in this thesis suggest that cooperation in the clinical practice of dentistry and psychology is likely to be beneficial to patient care. Consideration for patients’ oral health has the potential
to improve mental health and conversely, considering a patient’s mental health has the potential to improve their oral health.

4.3 Limitations

Given the dearth of published data on the association between oral soft tissue lesions and mental health in HIV, the current study was designed as a preliminary investigation into the subject. The limitations of the present study include a small sample size; only 10 of the 29 subjects given an oral exam were found to have an oral soft tissue lesion. While other studies report a similar oral soft tissue lesion incidence in an HIV+ population in similar urban settings (Tami-Maury et al., 2011), the overall number of subjects limited the power of this study. Both perceived stress and sexual abuse had a medium effect on oral lesion presence according to the Cohen’s D test, indicating that a larger sample size might confer statistical significance.

While speculations were made in the discussion above as to potential mechanisms linking oral lesions, psychological stress and the oral microbiome, this study is a correlative study that, by design, can only support association and not causality.

The subject sex ratio was skewed; more men enrolled in the study and outnumbered the female subjects by a factor of 2:1. This gender ratio is however representative of the population living with HIV in the United States. Two different clinicians who were not calibrated to diagnose oral soft tissue lesions conducted the oral examinations. While both clinicians have ample experience with such
examination, the diagnosis of the putative oral soft tissue lesions by non-calibrated examiners is an additional recognized weakness of our study.

Depression has a profound effect on the course of HIV, negatively effecting CD4 count and viral load (Carrico et al., 2011; Ironson et al., 2005) as well as AIDS mortality and onset (Antelman et al., 2007; Ickovics et al., 2001). The study presented here excluded subjects with severe depression and thus one of the more powerful indicators of HIV prognosis. It is possible that subjects with severe depression may better demonstrate a correlation between oral soft tissue lesions and psychological disease.

The microbiome study was unable to show statistically significant differences between either subjects with oral soft tissue lesions or high levels of psychological stress. The high level of microbial diversity in the oral cavity necessitates large sample sizes in order to compare the microbiome of groups. The small number of subjects with oral lesions limited the ability to definitively conclude there are no differences in the oral microbiome between the two groups. Differences between the study group may exist in the fungal or viral microbiomes but this was not investigated in the present study.

The ability to generalize the findings from the current study to a non-HIV population is limited. HIV+ subjects have a significantly higher prevalence of oral soft tissue lesions and suffer from a baseline immune deficiency independently of their mental health status. The main finding of this study that oral soft tissue
lesions are associated with markers of psychological stress may therefore not be true in a HIV- population.

4.4 Future Directions

The interplay between mental health and oral health is an area of research that is just beginning to be explored. With the samples collected in this study, characterization of both the viral and fungal components of the microbiome are planned to determine if associations with oral lesions, microbiome and mental health exist. The current research protocol will also be used to enlarge the sample size to increase the statistical robustness of the findings.

The next step would be to determine if there is directionality to the association between psychological stress and oral mucosal lesions. To investigate directionality, two interventional control studies could be designed to test if stress reduction affects the incidence of oral mucosal lesions and if treatment of oral mucosal lesions is associated with a reduction in psychosocial stress. An HIV+ population was used in this study because they present with a much higher rate of lesions of the oral mucosa. The ability to generalize these findings to a larger, HIV-negative population is also an important step that requires investigation. Future research will ultimately encourage oral health providers to further consider the role of their patient’s mental health status in treating conditions of the oral cavity. The converse is also true for mental health professionals when treating their patients’ psychological conditions.
5 CONCLUSIONS

- The null hypothesis of the primary aim was rejected. There is a statistically significant association between oral soft tissue lesions and psychological stress.
- The null hypothesis of the second aim failed to be rejected. There is no statistically significant difference between the microbiome of subjects with oral lesions and subjects without oral lesions. Additionally, there is no statistically significant difference between the microbiome of subjects with high levels of psychological stress and subjects without high levels of psychological stress.


lymphocyte counts weakens with time. *J Acquir Immune Defic Syndr*, 42(4), 516-518. doi: 10.1097/01.qai.0000223018.09192.6c


APPENDIX

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 470)
203 Administrative Office Building
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Approval Notice
Continuing Review

June 5, 2013

Leah Rubin, PhD
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RE: Protocol # 2012-0466
“Sex Differences in Cognitive Response to a Hydrocortisone”

Dear Dr. Rubin:

Your Continuing Review was reviewed and approved by the Convened review process on June 5, 2013. You may now continue your research.

Please note the following information about your approved research protocol:

Protocol Approval Period: June 5, 2013 - June 5, 2014
Approved Subject Enrollment #: 93 (10 enrolled to date)
AdditionalDeterminationsforResearchInvolvingMinors: These determinations have not been made for this study since it has not been approved for enrollment of minors.
Performance Sites: UIC, Cook County CORE Center
Sponsor: BIRCH, Building Interdisciplinary Research Careers in Women's Health, Campus Research Board, National Institutes of Health
PAF#: Not Applicable, Not Applicable, 2012-03189
Grant/Contract No: Not Applicable, Not Applicable, 1K01MH098798-01
Grant/Contract Title: Not Applicable, Not Applicable, Mentored Research
Scientist Development Award
Research Protocol(s):

a) Sex Differences in Cognitive Response to a Hydrocortisone Challenge in HIV, Version 5, 1/10/2013

Recruitment Material(s):

a) Telephone Screening Script, Version 2, 7/18/12
b) Internet Recruitment: Craigslist Recruitment Advertisement/ UIC Announcement, Version #2, 07/03/2012
c) CORE Center Screening Script, Version #3, 8/1/12
d) Flyer “Are you HIV+?”, (Group), with SCORE contact info, Version 2.0, 12/14/2012

References: Need for Publication
http://www.chicagohumanresearch.com/  \( \text{PAX: 211-412-3030} \)
Informed Consent/HIPAA Authorization(s):

a) Alteration of Informed Consent for Telephone Script
b) Waiver of Signed Consent Document for the Telephone Script [45 CFR 46.117(c)(2)]
c) Sex Differences in Cognitive Response to a Hydrocortisone Challenge in HIV, Version 6, 1/10/2013

Please note the Review History of this submission:

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<th>Submission Type</th>
<th>Review Process</th>
<th>Review Date</th>
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<td>Continuing Review</td>
<td>Convened</td>
<td>06/05/2013</td>
<td>Approved</td>
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Please remember to:

→ Use your research protocol number (2012-0466) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the enclosure,

"UIC Investigator Responsibilities, Protection of Human Research Subjects"
(http://bigger.uic.edu/depts/over/research/protocolreview/irb/policies/0924.pdf)

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 996-0865. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Tricia Hermanek, BS
IRB Coordinator, IRB # 1
Office for the Protection of Research Subjects

Enclosure(s):
1. Informed Consent Document(s):
   a) Sex Differences in Cognitive Response to a Hydrocortisone Challenge in HIV, Version 6, 1/10/2013

2. Recruiting Material(s):
   a) Telephone Screening Script, Version 2, 7/18/12
   b) Internet Recruitment: Craigslist Recruitment Advertisement/ UIC Announcement, Version #2, 07/03/2012
   c) CORE Center Screening Script, Version #3, 8/1/12
   d) Flyer "Are you HIV+?", (Group), with SCORE contact info, Version 2.0, 12/14/2012
   e) Flyer "Are you HIV+?", (Group), without SCORE contact info, Version 1.0, 12/14/2012
   f) Flyer "Are you HIV+?", (Male), with SCORE contact info, Version 2.0, 12/14/2012
   g) Flyer "Are you HIV+?", (Male), without SCORE contact info, Version 1.0, 12/14/2012
   h) Flyer "Are you HIV+?", (Female), without SCORE contact info, Version 1.0, 12/14/2012
   i) Flyer "Are you HIV+?", (Female), with SCORE contact info, Version 2.0, 12/14/2012
   j) Research Study Information Sheet, Version #1, 12/12/2012

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