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Anti-Periodontopathic Antibody Response in Chronic HIV Infection

Ivo SahBandar[1-3], Joshua Terakubo[1], Robert J Pattison[1,*], Eun-young Park[1,2], Martin K Oishi[4], Matthew M Oishi[5], Cecilia Shikuma[1], Lishomwa Ndhlovu[1-3], and Dominic Chow[1]

[1]Hawaii Center for AIDS, Honolulu, Hawaii, USA

[2]Department of Tropical Medicine, Medical Microbiology and Pharmacology, John A. Burns School of Medicine, University of Hawaii Manoa, Honolulu, Hawaii, USA

[3]Weill Cornell Medicine, New York, USA

[4]Department of Dental Hygiene, University of Hawaii Manoa, Honolulu, Hawaii, USA

[5]Department of Preventive and Community Dentistry, University of Iowa College of Dentistry, Iowa City, Iowa, USA

***Corresponding authors:** Robert J Pattison, Hawaii Center for AIDS, Honolulu, Hawaii, USA, Tel: 414-745-1797; E-mail: rjpattis@hawaii.edu

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Abstract

Background: Human Immunodeficiency Virus (HIV) infection may alter antibody responses against periodontopathic bacteria, increasing the susceptibility for gingival and periodontal disorders in HIV-infected individuals. We describe the oral health of HIV-infected individuals receiving stable Antiretroviral Therapy (ART) and determine their association with serum anti-periodontopathic bacteria antibody level.

Methods: HIV-infected individuals on at least three months of stable ART were enrolled and underwent routine dental and gingival examination including assessment for dental carries along with periodontal, gingival, and plaque measurements. Antibody to periodontal bacteria was determined by using the immunoassay "checkerboard immunoblotting" to assess serum IgG or IgM antibodies to periodontal organisms.

Results: Forty HIV-infected subjects were enrolled: 92.5% males, 72.5% Caucasian, with median CD4 count of 437.6 cells/mL. 75% of our study participants had an undetectable HIV viral load. Ten percent (4/40) of our population were diagnosed with CDC periodontitis scores of 2 and higher while 37.5% (15/40) had gingivitis determined by the gingival score of 2 or higher. Higher anti-Campylobacter rectus, Capnocytophaga ochracea, and Peptostreptococcus micros antibody levels were significantly associated with higher gingivitis scores (all p values<0.05).

Conclusions: A relationship was seen between high gingival score and high levels of antibodies against three periodontopathic bacteria that have been associated with clinical disease. We speculated that HIV-associated oral microbiota imbalance may result in a higher risk of gingival and dental pathology development in this population.

Keywords: HIV; Periodontopathic; Bacteria; Antibody; Oral; Microbiota

Introduction

Human Immunodeficiency Virus (HIV) infection is an important risk factor for gum and dental disorders. Gingival and periodontal inflammation and infection are common among HIV-infected individuals where prevalence rates of 49% gingivitis, 50.6% moderate periodontitis, and 9.5% severe periodontitis have been observed [1,2]. [1,2]. Gingivitis and periodontitis are often insidious and painless and caused by microorganisms colonizing the biofilm of dental plaque. Untreated gingivitis can lead to the development of severe periodontal disease and tooth loss. Gingivitis and periodontitis may provide an avenue for oral pathogens to enter the bloodstream, resulting in systemic infection and inflammation [3,4]. HIV has been reported to impact the host's immune reaction to periodontal pathogenic microorganisms and the development of periodontitis [5]. The role of the periodontitis on inflammation is unclear but postulated to increase the risk of non-AIDS comorbidities such as cardiovascular diseases and insulin resistance. Indeed, there is likely an even higher predisposition for inflammation related morbidities in HIV-infected individuals compared to the general population [6]. We hypothesized the risk of these comorbidities is higher in HIV-infected individuals, in particular with the interplay between systemic immune activation and periodontitis-related systemic infection.

Although the advent of Antiretroviral Therapy (ART) has dramatically reduced mortality of patients with HIV infection, the incidence of oral and periodontal diseases in HIV-infected individuals continues to be elevated [7,8], and oral microbiota has been speculated to be one of the leading causes of systemic inflammation despite HIV virologic suppression [8,5]. Serum immunoglobulin antibodies have been used to classify and analyze periodontal status [9]. Periodontopathic bacteria and the host immune response to the bacteria have also been suggested to play a significant role in the pathogenesis of early onset periodontitis [10]. HIV infections affect

antibody responses against periodontopathic bacteria as the reduced serum immune response may increase the susceptibility for periodontal infection in HIV-infected individuals [11,12]. Indeed, HIV may be creating an environment that leads certain types of periodontopathic bacteria to flourish and increased levels of the circulating antibodies against the periodontopathic bacteria. Additionally, increased periodontopathic bacteria may be responsible for chronic systemic immune activation that may lead to the worsening of the underlined HIV condition altogether [11,12].

Studies reporting the interplay between HIV infection and gingival and periodontal diseases in the ART era are lacking, specifically, studies focusing on the antibody responses against periodontopathic bacteria in HIV-infected individuals on stable ART. We sought to describe the oral health profile and the presence of gingival and periodontal diseases in HIV-infected individuals on stable ART and their association with serum anti-periodontopathic bacteria antibody.

Methods

Study design: This study is cross-sectional, descriptive, observational study, evaluating the oral health of HIV-infected individuals receiving ART and their association with serum anti-periodontopathic bacteria antibody level. Forty HIV-infected adults >18 years of age with ELISA confirmed HIV infection on stable ART for at least three months. Individuals were recruited through the University of Hawaii ambulatory based HIV clinic. This study was approved by the University of Hawaii Committee on Human Studies and informed consents were obtained from all participants in our population. Pregnant females were excluded from the study as the oral microbiome during pregnancy is largely unknown. Clinical data was collected, oral examinations were conducted and analyzed, venous blood was collected, and sera was separated and preserved in aliquots in -80°C until they were used for the downstream assays.

Oral Examination: The oral clinical examination consisted of the following procedures: tooth status, dental caries, and oral and blood specimen collection and storage. Tooth status was recorded for all teeth in the mouth. Each tooth was classified as either sound, decayed, filled, missing, crown, decayed root fragment, sound root fragment, or implant. Teeth were also identified as permanent or primary. Oral examinations, periodontal measures and samples were obtained by a single licensed and certified dentist.

Periodontal measures: Periodontal clinical parameters were measured on all teeth in the mouth using a manual periodontal probe. Clinical parameters included; Plaque Scores (PL) for four sites on each tooth was assigned using a 0 to 3 scale. Absence of plaque or stain=0, deposits covering less than one-third of the clinical crown surface=1, less than two-thirds=2, more than two-thirds=3. Gingival Index Score (GI) is a 0 to 3 scale were each facial aspect was scored as normal=0, mild inflammation=1, moderate inflammation=2, or severe inflammation=3. Bleeding Upon Probing (BoP) was recorded as presence or absence at each probed site Each quadrant was recorded after that quadrant was probed. Probing Pocket Depth (PD) was measured on all teeth at six sites per tooth (i.e.mesiofacial, facial, distofacial, mesiolingual, lingual, and distolingual) from the free gingival margin to the base of the sulcus and was recorded in whole millimeters. Cemento-enamel Junction (CEJ) measures (Recession) were assessed on all teeth at six sites per tooth (i.e., same sites as PD) in whole millimeters from the CEJ to the gingival margin. If a reading fell between a two-millimeter marking on the probe, the lesser of the two readings was recorded. If the gingival margin was coronal to the CEJ, recession was recorded as a positive value. If a reading fell between a two-millimeter marking on the probe, the lesser of the two readings was recorded. If the gingival margin was coronal to the CEJ, recession was recorded as a positive value. If the gingival margin was apical to the CEJ, recession was recorded as a negative value. CEJ measurements were taken at the same spot as the recorded sulcus depth (deepest point at each site). Finally, Clinical Attachment Level (CAL) was calculated as PD minus CEJ and will reflect the linear distance from the CEJ to the base of the sulcus at each site. CAL was calculated during data analysis.

Measurement of serum antibody to periodontal bacteria: Serum were analyzed to determine levels of periodontal pathogen-specific antibodies to a number of periodontal organisms including the following: *Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Bacteroides forsythus, Treponema denticola, Fusobacterium nucleatum, Actinobacilus Actinomycetemcomitans, Campylobacter rectus, Eikenella corrodens, Selenomonas noxia, Peptostreptococcus micros, Eubacterium nodatum, Veillonella parvoula*, *Capnocytophaga ochracea, Streptococcus intermedius, Streptococcus oralis, Streptococcus sanguis*, *Actinomyces naeslundii*. Protein A from *Staphylococcus aureus* (Roche Diagnostics, Indianapolis IN) was used as an immunoglobulin binding substrate. We utilized the immunoassay "checkerboard immunoblotting" to assess serum IgG or IgM antibodies to the listed periodontal organisms: first, Protein A and the different bacterial suspensions were loaded onto nitrocellulose membranes (Hybond EC L, Amersham Arlington Heights IL) using a Miniblotter and incubated overnight at 4°C. The membranes were then removed from the Miniblotter and non-specific binding sites were blocked twice with 5% dehydrated skim milk (Disco Laboratories, Detroit MI) in TBST buffer [10.1% Tween 20 (Sigma) in Tris saline buffer (20 mM), 137 mM NaCl, (pH 7.6)], followed by the treatment with 3% (v/v) H_2O_2 in 100% methanol and washing with 500 ml of TBST. Diluted purified IgG or IgM (Sigma Immunochemicals, St Louis MO)-as standards-and serum samples were then loaded into the Miniblotter and incubated at room temperature for 1 hr. After incubation, the diluted serum samples and the immunoglobulin standards were aspirated, and the membrane was washed with 500 ml of TBST.

Detection and quantitation of signals: Fab fragment of goat anti-human IgG (or IgM) peroxidase-conjugated antibody (Roche Diagnostics, Indianapolis, IN) was used as the second antibody. The membrane was incubated with diluted antibody at room temperature, for 1 hour and then washed. Detection was accomplished by using the ECL Western blotting detection reagents (ECLWestern, Amersham Life Sciences) and the Lumi-Imager™ workstation (Boehringer-Mannheim) which captures chemiluminescence. The antibody concentrations of the unknown samples (patient sera) were then estimated using the standard curve obtained from the purified human IgG or IgM preparations, using the LumiAnalyst™ software. The sensitivity of the assay was optimized for the detection of approximately 10 ng/ml immunoglobulin.

Analysis: Gingival and periodontal scores were assessed as categorical variables. Gingival and periodontal scores were divided into the following groups: score of 0, score of 1-2 and score >3. Continuous variables were presented as medians along with their interquartile range (Q1, Q3) and analyzed by Kruskal-Wallis one way analysis of variance test. A two-sided probability of $p<0.05$ was used to determine statistical significance. All statistical analyses were performed using the JMP statistical program (SAS Institute Inc. Cary, NC).

Results

Among the 40 subjects, 92.5% were males with a median age of

55 years old (Q1, Q3: 47.22, 61.97). Clinical and demographic study characteristics are displayed in table 1. The ethnicity groups of our subjects were 72.5% White, 15% Hispanic, and 12.5% Asian. The CD4 count of our population was a median of 437.6 cells/mm^3 (265, 640). Subjects received the following antiretroviral regimen of NRTIs, NNRTIs, and PIs: 62.5%, 45%, and 42.5% respectively. Comorbidities found among study subjects include type 2 Diabetes (10%) and Hypertension (25%) (Table 1). There were no statistically significant associations between gingivitis and periodontitis between any of these sociodemographic variables.

From the oral cavity examination, 37.5% of our subjects had gingival disease and 50% had gingival plaque (Table 1). Furthermore, only 4 patients (10%) had Center for Disease Control and Prevention (CDC) periodontitis scores of 2 or higher, while 27.5% had a score of 1 and the remaining 62.5% had a score of 0. Patients missing teeth include 67.5% of our population other than their wisdom teeth. 25% have oral lesions defined as suspicious lesions or conditions that should be referred to a dentist. All patients bled upon probing of the gingiva and had gingival recessions (Table 1).

There was a higher diversity of antibodies against periodontopathic bacteria in subjects with increasing gingivitis scores (Table 2). There was a higher antibody response of *Campylobacter rectus, Capnocytophaga ochracea, and Peptostreptococcus micros* in subjects with higher gingival scores (Figure 1). The antibody responses of *Campylobacter rectus, Capnocytophaga ochracea,* and *Peptostreptococcus micros* were significantly higher in the highest gingival score group compared to the group with the lowest gingival score (all p values<0.05). There was no significant correlation between antibody levels of periodontopathic bacteria with higher periodontal score. Nor were there differences between the viral load, CD4 count, or CD4/CD8 and the gingival and periodontal scores.

Table 1: Clinical characteristics.

Characteristics (n=40)	N (%)
Age median (yrs.)	55.235 (Min=26.82 Max=72.62)
Gender Male	37(92.5%)
CD4 count (cell/mm^3)	437.6 (Min=4 Max=879)
Gingival score	0: 5 (12.5%) 1: 20 (50%) 2: 11 (27.5%) 3: 4 (10%)
Plaque Score	0: 25 (62.5%) 1: 11 (27.5%) 2: 2 (5%) 3: 2 (5%)
Comorbidities	
Hypertension	10 (25%)
Diabetes (Type II)	4 (10%)
Ethnicity	White 29 (72.5%) Hispanic 6 (15%) Asian 5 (12.5%)
Antiviral Therapy	
NRTIs	25 (62.5%)
NNRTIs	18 (45%)
PIs	17 (42.5%)
Viral Load	
Undetectable (<50 Copies/mL)	30 (75%)
At least one missing tooth (expect wisdom teeth)	27 (67.5%)
With any oral lesion	10 (25%)
Pocket depth of at least 4 mm	27 (67.5%)
With gingival recession	40 (100%)
With bleeding or probing	40 (100%)
Alcohol Usage	30 (90%)
Tobacco usage	18 (45%)
Illicit drug use	23 (57.5%)

NRTIs: Nucleoside Reverse Transcriptase Inhibitors, NNRTIs: Non-Nucleoside Reverse Transcriptase Inhibitors, PIs: Protease Inhibitors

Discussion

This cross-sectional study of the oral health profile showed that gingivitis is common among HIV-infected individuals on stable ART. The rate of gingivitis in our population is higher than what has been reported in HIV patients early on or without ART [13]. Conversely, the rates of periodontitis were lower than reported previously [14]. This shift from periodontitis to less severe gingivitis may reflect the effects of ART on the oral microflora and immune response. Host cellular and humoral immunology response may play an important role in periodontal disease progression [2,7,8,15]. Previous studies reported the HIV-associated immune disruption marked by low CD4 count was associated with the development of severe periodontitis [16]. Most of our study participants were on stable ART and virally suppressed, suggesting ART played an important role in reducing the development of severe periodontal disease in HIV-infected individuals. This result is in accordance with study by Nobre ÁVV, et al. [17] in Canada that showed the reduction in clinical severity of chronic periodontitis in HIV-infected individuals with long-term use of ART. The shift from periodontitis to gingivitis may indicate the need of a more aggressive management and earlier oral health management.

We utilized immune checkerboard assay to assess the serum antibody response to periodontal microbiota [18]. From the serum anti-periodontopathic bacterial antibody level analysis, we found higher diversity of antibodies against periodontopathic bacteria in subjects with increasing gingival scores. The difference in diversity among subjects with different gingival scores suggests that some pathogenic bacteria become more prevalent as the gingival disease progresses [8,17]. We found significant correlation between higher gingival score with high level of anti-*Campylobacter rectus, Peptostreptococcus micros,* and *Capnocytophaga ochracea,* antibody. *Campylobacter rectus* is a pathogen often implicated in chronic periodontitis [9,12]. The presence of antibody against these bacteria and its correlation with the gingival score may suggest that there was an increased abundance of this bacterium that leads to the development of gingivitis in these subjects. While *Capnocytophaga ochracea* is less often found as the pathogen in periodontitis, this gram-negative anaerobe is often found in the oral cavity of humans and contributes to the formation of dental plaque [8,9]. The correlation between increased antibody level of this bacterium and high gingival score may imply that the antibodies to *Capnocytophaga ochracea* may be an early sign of periodontal disease that may necessitate more aggressive oral care to prevent the development of a more severe periodontal disease. *Peptostreptococcus micros* is also recognized pathogen in adult periodontitis. The presence of high level of antibody against this bacterium and its correlation with high gingival score suggest this bacterium as one of the potential pathogens that may cause periodontal disorders in our study [10,16,19].

Several limitations exist in our study: this is a cross sectional study

Table 2: Levels of IgG antbodies against periodontopathic bacteria.

Bacteria Spp.	IgG level (%)			P
	Gingival Score 0	Gingival Score 1	Gingival Score ≥2	
Aggregatibacter actinomycetemcomitans	0.02	0.11	0.87	.11
Actinomyces viscosus	0.69	0.15	0.16	.14
Bacillus fusiformis	0.15	0.56	0.29	.63
Capnocytophaga ochracea*	**0.05**	**0.16**	**0.79**	**.04**
Campylobacter rectus*	**0.04**	**0.27**	**0.69**	**.04**
Enterococcus casseliflava	0.30	0.27	0.43	.47
Fusobacterium Nucleatum	0.11	0.70	0.19	.23
Helicobater pylori	0.01	0.01	0.98	.05
Porphyromonas gingivalis	0.34	0.35	0.31	.76
Peptoniphilus indolicus	0.09	0.23	0.68	.34
Peptostreptococcus micros*	**0**	**0.13**	**0.87**	**.02**
Prevotella nigrescens	0.32	0.37	0.31	.94
Streptococcus infantis	0	0.27	0.73	.05
Selenomonas noxia	0.43	0.21	0.36	.23
Streptococcus Oralis	0	0.32	0.68	.08
Streptococcus salivarius	0.64	0.32	0.04	.37
Treponema denticola	0.39	0.35	0.26	.68
Variovorax paradoxus	0.37	0.51	0.12	.8

GS: Gingival Score
p value was calculated using Kruskal-Wallis Test to determine group differences

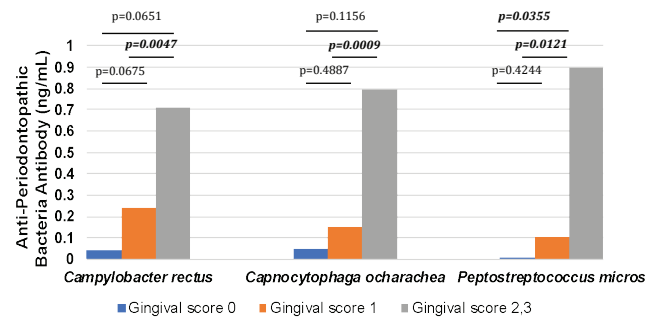

Figure 1: Pathogen antibody level. Graphs showing the different level of antibody against three pathogenic bacteria *Campylobacter rectus*, *Capnocytophaga ochracea*, and *Peptostreptococcus* micros among study participants with different gingival score. The gingival score is divided into 0, 1, and >2.

without a HIV negative control group, with limited number of study participants. Also, this study was based purely on bacteria antibody levels and may not reflect the true bacteria flora microenvironment. Despite these limitations, our results suggest the presence of antibody response to periodontopathic bacteria in HIV-infected individuals on ART may limit the evolution of gingivitis to periodontitis.

A relationship was seen between high gingivitis score and high levels of antibodies against three periodontopathic bacteria that have been associated with clinical disease. We speculated that HIV-associated oral microbiota imbalance may result in a higher risk of gingival and dental pathology development in this population.

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