Title

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Journal

Journal of clinical periodontology, 46(11)

ISSN

0303-6979

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Publication Date

2019-11-01

DOI

10.1111/jcpe.13179

Peer reviewed

HHS Public Access

Author manuscript

J Clin Periodontol. Author manuscript; available in PMC 2020 November 01.

Published in final edited form as:

J Clin Periodontol. 2019 November ; 46(11): 1072–1082. doi:10.1111/jcpe.13179.

Biomarkers of oral inflammation in perinatally HIV-infected and perinatally HIV exposed, uninfected youth

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Abstract

Aim: To examine oral biomarkers that have been associated with periodontal disease progression in HIV infected adults in perinatally HIV-infected and HIV-exposed but uninfected youth.

Material and Methods: This was a cross-sectional, multi-center substudy of youth participating in the Oral Health Pediatric HIV/AIDS Cohort study. Gingival crevicular fluid repository samples from participants with and without periodontal disease (using Gingival Index and Bleeding on Probing parameters on dental examination) were tested for concentration levels of inflammatory biomarkers. Associations were assessed using Wilcoxon test and Spearman correlation.

Results: For perinatal HIV youth (n=129), the biobiomarkers consistently elevated ($p < 0.05$) in sites with gingival index≥2 and in sites with bleeding on probing were interleukin 1β, 6 and 13, Macrophage Inflammatory Protein-1α, and Metalloproteinase-9. Serum tumor necrosis factor-α and soluble CD14 were positively correlated with a summary count of elevated cytokines. No associations were seen among HIV-uninfected subjects (n=71).

Conclusions: The association of oral biomarkers of inflammation with clinical indicators of periodontal inflammation and systemic immune activation suggest that perinatal HIV-infected youth may be at higher risk for developing significant periodontal disease, associated with tooth

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CONFLICT OF INTEREST: None

loss and HIV progression. More frequent dental care of this group is needed to prevent potential periodontal progression.

Keywords

oral health; perinatal HIV infected youth; periodontal inflammation; cytokines

Introduction

Systemic immune activation is a hallmark of HIV infection and is established as an independent predictor of morbidity and mortality in HIV-infected persons (Liu et al., 1997, Liu et al., 1998, Deeks et al., 2004, Giorgi et al., 1999). The actual cause of this immune activation remains incompletely understood. However, a strong association has been found between translocation of microbial products (particularly lipopolysaccharides) through the gastro-intestinal mucosa, especially among those with sustained failure in CD4 T-cell reconstitution despite long-term anti-retroviral therapy (ART)(Marchetti et al., 2008, Hunt et al., 2011, Vujkovic-Cvijin et al., 2013, Xu et al., 2013). Some have suggested that the gut model can be translated to the oral cavity as an important source of systemic immune activation.

In addition to systemic markers of inflammation, oral inflammatory markers have also been found to be increased in HIV-infected persons. Regardless of HIV disease, the mouth is a known source of inflammation stemming from gingivitis and periodontitis (Ertugrul et al., 2013, Giannobile et al., 2009, Kinney et al., 2014). Numerous inflammatory markers and markers of bone metabolism have been associated with periodontitis, often preceding clinical evidence of attachment loss (Becerik et al., 2011, Giannobile et al., 2009, Ka et al., 2014, Kido et al., 2001, Kinney et al., 2014, Kunimatsu et al., 1993). The multiple bacteria species that have been found to be associated with periodontal inflammation represent a source of potential bacteremia and bacterial translocation in non HIV-infected as well as HIV-infected persons (Eberhard et al., 2013, Noack et al., 2001, Genco et al., 2005, Huang et al., 2009, Huang et al., 2011). Elevated peripheral blood markers of systemic immune activation including interleukin (IL)-6, tumor necrosis factor (TNF)-α, and C-reactive protein have been associated with periodontal inflammation as well as with the presence of known periodontal pathogens (Eberhard et al., 2013, Noack et al., 2001, Genco et al., 2005).

Several studies have demonstrated higher levels of oral inflammatory cytokines, and collagenolytic enzymes from HIV-infected vs. non-infected adults with and without periodontal diseases in the gingival crevicular fluid (GCF) and/or saliva (Alpagot et al., 2003, Alpagot et al., 2008, Alpagot et al., 2006, Falasca et al., 2008, Mellanen et al., 1998, Nishanian et al., 1998). It is entirely plausible that the presence of oral inflammatory cytokines/chemokines even in the absence of visible periodontal inflammation, results in a breakdown of epithelial barriers and a consequent translocation of microbial products from the gingiva. This will, in turn, instigate an HIV-associated systemic immune activation with its associated morbidity.

The Pediatric HIV/AIDS Cohort Study (PHACS) Oral Health Substudy (OHS), a cross-sectional study comprising a comprehensive oral examination performed by trained dentists

in perinatally HIV-infected (PHIV) and perinatally HIV-exposed but uninfected (PHEU) youth, provides the unique opportunity to explore this association (Moscicki et al., 2016). The aim of this study was to examine markers of inflammation and bone metabolism that have been associated with periodontal disease progression from GCF in areas with and without evidence of clinical periodontal inflammation in PHIV and PHEU youth. In addition, we examined the association between biomarkers of oral and systemic inflammation among PHIV youth. We hypothesized that these markers would be markedly elevated in the face of clinical inflammation in HIV-infected persons and in turn this would be associated with markers of systemic immune activation.

Materials and Methods

Study Design and Population

Patients were recruited from the Adolescent Master Protocol (AMP) of PHACS, a prospective cohort study designed to determine the impact of HIV infection and antiretroviral therapy (ART) on PHIV youth and includes a comparison group of PHEU youth. The design of the study and eligibility criteria have been defined in several publications (Moscicki et al., 2016, Alperen et al., 2014, Van Dyke et al., 2011). The OHS protocol (Moscicki et al., 2016, Ryder et al., 2017) was a cross-sectional substudy within AMP with a single visit per subject.

Institutional Review Boards (IRB) at clinical sites and the Harvard T.H. Chan School of Public Health approved the study. Parents/legal guardians provided written informed consent for their child's participation. Youth consented/assented per local IRB guidelines.

Data collection in the PHACS OHS including a full mouth examination and assessment of clinical gingival inflammation, probing depths and clinical attachment loss is detailed elsewhere (Moscicki et al., 2016). Briefly, sites scheduled a comprehensive OHS visit within 3 months of a regularly scheduled AMP annual visit. Dentists performing the examinations had all undergone training and calibration regarding standardized examinations, specimen collection and documentation (Moscicki et al., 2016). Diagnosis of periodontitis was based on the CDC-AAP case definition (Eke et al., 2012). Gingivitis was defined as absence of periodontitis and ≥ 10% bleeding on probing (BOP) of all sites (6 sites per tooth) around all teeth present.

In the interest of standardizing sample collection without biases, it was decided that samples of GCF collected prior to periodontal probing would not target specific periodontal disease sites but be taken from two gingival sites susceptible to inflammatory changes: the mesial buccal sites of the permanent upper right and lower left first molars. Only serum samples drawn within a window from 30 days before to 2 days after the OHS visit were used.

For this substudy, we selected 200 GCF repository samples from the 335 PHIV/PHEU participants in the OHS. We used indicators of overall oral health (i.e. periodontitis by CDC-AAP classification and gingivitis by % sites with BOP) to select the 100 most healthy and 100 least healthy participants to ensure enough variation of the outcome measures at the specific GCF collection sites. None of the 100 healthy participants had evidence of

periodontitis or gingivitis, and 22 of them had minimal % BOP. The least healthy participants included 85 with periodontitis and 15 with gingivitis and high %BOP, with BOP at one or both GCF collection site(s).

In this analysis we looked at 2 measures of periodontal inflammation at each of the two sites: 1) Gingival Index (GI) using a modification of the GI of Löe and Silness (Ryder et al., 2017, Loe and Silness, 1963), where the visual parameters were 0: normal gingiva, 1: mild inflammation/slight change in color/slight edema, 2: moderate inflammation/erythema, edema, and loss of stippling, and 3: severe inflammation/marked erythema and inflammation, tendency toward spontaneous bleeding; and 2) BOP as present or absent.

We also examined two other parameters associated with clinical signs of periodontitis: probing depth ≥5 mm at neither or one/both collection sites, and CAL ≥4 mm at neither, or one/both collection sites. A UNC#12 probe was used to measure probing depths and gingival margin position to the CEJ to calculate CAL. Since the frequency of these two parameters was low as expected in this young population, these two were excluded in the analysis. Details for data collection of periodontal clinical measures, and correlations with other local and systemic clinical and laboratory findings in this study population, are described previously (Moscicki et al., 2016, Ryder et al., 2017).

Inflammatory cytokines and markers of bone metabolism

Prior to gingival probing, the study dentist collected GCF using a standard Periopaper® strip at each site held in place for 10 seconds. Both were promptly placed in a single empty 1.5 ml via l (Engebretson et al., 2004). Samples were immediately placed on ice until transported to the local laboratory within 4 hours of collection and stored at minus 70°C until shipped to the PHACS repository. GCF samples were tested for candidate markers (Kinney et al., 2014, Becerik et al., 2011, Ka et al., 2014, Kido et al., 2001, Kunimatsu et al., 1993) including; i) inflammatory markers: IL-1β, IL-6, IL-8, IL-10, IL-12p70 and IL-13, TNF-α, interferon (IFN)-γ, β2 Microglobulin (B2M), Prostagladin E2 (PGE2), and Macrophage Inflammatory Protein (MIP)-1α, ii) collagenolytic markers: Matrix Metalloproteinase (MMP)-1, MMP-8 and MMP-9, iii) bone resorption and metabolism markers: pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (CTXI), osteoprotegerin (OPG), osteocalcin (OC), and osteopontin (OPN), and iv) mediators of inflammation: soluble TNF receptor (sTNFR) I and sTNFR II. Markers of systemic immune activation included sCD14, sCD163, IL-6, and TNF-α.

Sample preparation

Microcentrifuge tubes containing periostrips with GCF were kept at −70ºC until testing. For the elution of GCF from the strips, 400 μL of phosphate-buffered saline (1xPBS) containing 0.05% Tween-20 buffer was added to each tube, and samples were kept at +4°C overnight on a shaking platform. Next day, each sample was vortexed for 30 sec and centrifuged for 5 minutes at 6000xg before processing for ELISA or Luminex analysis as described below. For assessment of intra- and inter-plate variability plasma and serum samples of a healthy person were assayed in duplicates on each Luminex and ELISA plate. Dilution buffer defined by each protocol was set as a blank.

Enzyme-linked immunosorbent assays (ELISA)

ELISA was used for the quantitative detection of B2M (Abcam, Cambridge, MA, USA), PGE2 (R&D Systems, Minneapolis, MN, USA), and CTXI (Cloud-Clone Corp, Houston, TX, USA) from GCF samples, and for sCD14 (R&D Systems, Minneapolis, MN, USA) and sCD163 (R&D Systems, Minneapolis, MN, USA) detection from serum samples. B2M was assayed at 1:40, PGE2 at 1:20, CTXI at 1:2 dilution, sCD163 as neat (undiluted) and sCD14 at 1:200, following manufacturer's instructions, respectively. Data were analyzed using SoftMax Pro version 5 ELISA software (Molecular Devices, Sunnyvale, CA). The lowest value detected or extrapolated by the proprietary software was used as the minimum detectable value. Concentrations lower than extrapolated values calculated were considered out of range.

Luminex-based multiplex assays

Commercially available Luminex-based immunoassays were used for detection of inflammatory cytokines IL-1β, IL-6, IL-8, IL-10, IL-12p70, IL-13, IFN-γ, TNF-α and MIP-1α (Millipore, Temecula, California, USA); MMP-1, MMP-8, MMP-9 (R&D Systems, Minneapolis, MN, USA); sTNFR I and sTNFR II (EMT Millipore, Temecula, California, USA); and OPG, OC and OPN (Millipore, Temecula, California, USA) in GCF. The 96-well plates with GCF samples were run on Luminex 200 (Bio-Rad, Hercules, CA, USA). For all biomarkers samples were run neat (undiluted), except for MMPs which were run at 1:10 dilution (1:20 for out of range samples at the higher end). Regression curves (5-parameter logistic) were fit, and unknown concentrations in pg/ml were determined using Bio-Plex Manager Software version 6.1.

For detection of IL-6 and TNF-α in serum, serum samples were processed on a MagPix instrument using xPonent software (Luminex Corp., Austin, TX, USA). Regression curves (5-parameter logistic) were fit, and unknown concentrations in pg/ml were determined using MiraiBio MasterPlex QT software (Hitachi). The lowest value detected or extrapolated by the proprietary software was used as the minimum detectable value. Concentrations lower than extrapolated values calculated were considered out of range.

Statistical analysis

Periodontal outcomes as clinical evidence of periodontal inflammation included: GI of 2 or greater at neither, or one/both collection sites (GI2), and BOP at neither, or one/both collection sites. Demographic characteristics and periodontal outcomes were described by HIV status. PHIV and PHEU participants were compared by a Wilcoxon test for the continuous measures, and by a Fisher's Exact test for discrete measures.

OC and OPN were undetectable in over 90% of samples --likely due to the absence of marked clinically detectable bone resorption in our youth-- and were excluded from analysis. To study the association between inflammation cytokines with clinical outcomes by HIV infection status, the remaining 18 cytokine concentration levels were examined comparing participants with clinical evidence at neither versus one/both collection site for GI2, and BOP within PHIV and PHEU participants separately. Too few participants had both sites involved to use as a separate group and when we compared the 3 groups (neither, one,

both), similar cytokines were found associated with statistical significance as when we compare neither to one/both combined. Cytokine concentration levels were plotted for each of these groups, and the groups were compared by Wilcoxon test. The level of inflammation in GCF was also summarized across cytokines by a composite variable. For each cytokine, each participant was classified as falling into the top quartile (concentration >Q3 from the pooled PHIV and PHEU samples) or falling into the bottom three quartiles. The count of the number of cytokines in the top quartile was determined for each participant (theoretical range: 0 to 18) and was used as a surrogate of the intensity of inflammation. The distribution of this count was compared between PHIV and PHEU participants using a Wilcoxon test. Within PHIV or PHEU participants, the count was also compared between the neither versus one/both periodontal outcome groups by Wilcoxon test.

The association between oral inflammation and systemic immune activation was assessed by determining the Spearman correlation coefficient ρ between each of the 18 oral cytokine concentrations, the count of elevated cytokines, and % sites with BOP of the whole mouth with each serum cytokine. These correlations were further adjusted by viral load or CD4 count at time close to oral examination using Spearman partial correlation. Serum IL-6 was undetectable in over 60% of samples demonstrating the overall good HIV health in our PHIV and was excluded from this analysis.

Results

Demographics and laboratory studies of the 200 selected subjects are described in Table 1. The distributions are consistent with what has been observed in the overall AMP cohort (Alperen et al., 2014, Tassiopoulos et al., 2013). PHIV are older and less likely to be Hispanic. There was no significant difference between PHIV and PHEU for number of sites with GI ≥2 and BOP, or full mouth average of BOP and plague index (Table 2).

Inflammatory biomarkers and periodontal parameters

The median cytokine concentration and interquartile range (IQR) are displayed based on the number of GCF collection sites with clinical evidence of inflammation (neither vs one/both), i.e. GI ≥ 2 (Figure 1) and BOP (Figure 2) for PHIV. For ease of presenting the associations between cytokines and the outcomes, the figures show the difference on the logarithm scale relative to the median of the “neither” group. The concentration was statistically significantly higher in sites with GI ≥ 2 for ILs-1β, −6, −10, and −13, MIP-1α, MMP-8 and −9, CTXI, OPG, and sTNFR-1. The concentration was statistically significantly higher in sites with BOP for ILs-1β, −6, and 13, MIP-1α, MMP-9, and sTNFR-II. Inflammation markers that were consistently elevated in those with GI ≥ 2 and BOP were ILs-1β, −6 and −13, MIP-1α, and MMP-9. None of the inflammatory biomarkers showed significantly different concentrations for any of the periodontal parameters among the PHEU (Supplemental Figures 1and 2 in the Supplement). Supplemental Figures 3-18 in the Supplement show box plots and individual concentration values for each of the inflammatory markers for PHIV and PHEU.

Summary counts of cytokines in the top quartile were similar for PHIV and PHEU youth (median (Q1,Q3) =3 (1,6) vs 3 (1,7), respectively). Among the PHIV, summary counts were

strongly correlated with GI ≥ 2 [median 3 (interquartile range 1,5) vs 5.5 (2,9) for no GI ≧2 vs one or both GI ≧2, respectively; p=0.006] and BOP [median 2 (1,5) vs 5 (2,8) for no BOP vs BOP, respectively; p=0.004]. Among PHEU, no statistical significance was found for GI ≥ 2 among PHEU [3 (1,6.5) vs 4.5 (2,7), respectively; p=0.40] and BOP [3 (0,6) vs 5 (1,6.5), respectively, p = 0.20]

Correlation between serum and GCF inflammatory markers and periodontal inflammation.

Median and mean serum cytokines levels are shown in supplemental Table. Although serum cytokines were not significantly associated with individual GCF cytokines, a positive association was observed with the summary counts of elevated GCF cytokines. Among PHIV, serum TNF-α ($\rho = 0.19$, p-value = 0.03) and sCD14 ($\rho = 0.20$, p-value = 0.02) were positively correlated with the summary counts. These correlations remained similar after adjusting for viral load or CD4 count. Among PHEU, serum TNF-α was associated with the GCF summary counts with marginal statistical significance ($\rho = 0.23$, p-value = 0.06), but sCD14 was not associated ($\rho = 0.11$, p-value = 0.35). No associations were found for serum sCD163 in either cohort. Neither BOP or GI>2 were significantly associated systemic markers. In addition, we examined the association between whole mouth inflammation (defined by %BOP and average plaque index of the entire mouth) and systemic immune markers and found no significant positive correlations. This remained true when adjusting for CD4 count and viral load (data not shown).

Discussion

Several inflammatory, collagenolytic and bone metabolism markers were elevated in the PHIV with evidence of clinical periodontal inflammation. In contrast, no associations were seen in the PHEU group despite the average level of these clinical measures of inflammation being similar. These differences in the association of clinical and laboratory markers between the PHIV and PHEU groups are striking since these markers are thought to be more sensitive indicators of inflammation than gross visual inspection and are also considered to predict progression to more severe periodontal disease (Moutsopoulos and Madianos, 2006).The strong association with number of cytokines found elevated in those with inflammation vs. those without underscores the likelihood that there is significant underlying inflammation.

Gingivitis is a local reaction to pathogens which is modulated by environmental and genetic factors (da Silva et al., 2017). GCF is a transudate that is critical for epithelial health concentrated with macromolecules derived from local tissue and serum (Ebersole, 2003). In response to pathogens, this transudate becomes an inflammatory exudate with contributions from both local tissue and serum comprised of polymorphonuclear and mononuclear cells such as T-cells, B-cells, plasma cells and macrophages. These cells produce a large repertoire of inflammatory and immune mediators. There is good evidence that antibodies found are also locally as well as systemically produced contributing to the inflammatory response (Ebersole, 2003). Early stages of gingival inflammation are due to inflammatory responses to the microbial by-products of plaque bacteria which have crossed the gingival barrier (Wang, 2015, Socransky et al., 1998). This early response in the tissue is

predominated by T-cell infiltration reflective of the elevated pro-inflammatory cytokines (such as ILs-1β and −6) as observed in our study. B-cell infiltrates are more indicative of the later stages of periodontal disease and antibodies are directed toward bacterial product or outer bacterial membranes (Ranney, 1991). IL-6, which is thought to regulate B-cell expansion in oral tissue, has been linked as a sensitive marker of progression and tissue destruction (Manolagas, 1995). IL-6 also drives osteoclast activity and bone resorption. It has been suggested that these biomarkers can be used to predict which patients will have progressive disease (Tomas et al., 2017). Unfortunately, more advanced periodontal disease is common in adults with HIV (Ryder et al., 2012, Heron and Elahi, 2017). Since little is known regarding PHIV youth and biomarkers of disease progression, follow-up of this cohort will be essential and is currently underway.

Clinical periodontal inflammation is defined often by several parameters. In this study we used two measures of inflammation BOP and GI. We noted that 5 of the biomarkers were associated with both BOP and GI giving us confidence that these markers were not associated by chance. The association with MMP-9 was of special interest since it functions to degrade types 1–3 collagens. MMP-9 is produced by gingival keratinocytes in response to pathogens and also has been identified as a marker of disease progression (Sorsa et al., 2006, Leppilahti et al., 2014). Somewhat surprising was the observed elevation of IL-13 in those with GI ≥ 2 since it is considered a Th2 type cytokine which dampens inflammation. We hypothesized that this observation may be a normal response to counteract potentially destructive inflammation (Souza et al., 2013).

Concerning was the association between the systemic immune activation markers of HIV, specifically sCD14 and TNF-α, and periodontal inflammation defined by multiple elevated cytokines. The use of summary counts offers an overall picture of the intensity and diversity of inflammation (Scott et al., 2013, Hwang et al., 2015, Arnold et al., 2016). Although there is no consensus on “biomarkers” of systemic immune activation, elevation of sCD14 has been shown to be a relatively sensitive marker for predicting HIV progression(Williams et al., 2016). Other markers, such as TNF-α have more controversial roles (de Medeiros et al., 2016, Vaidya et al., 2014). The association for sCD14 among PHIV but not PHEU underscore the potential risk of HIV progression. In contrast, serum TNF-α appeared to be a more general marker of systemic inflammation since it was elevated in both PHIV and PHEU with GCF elevated cytokines. The lack of association between visual inflammation and systemic immune activation markers underscore the limitations in visual inspection. Another interpretation is that clinical periodontal inflammation is not necessarily a risk for HIV progression unless there is a corresponding measurable intense inflammatory response.

Limitations of our analysis include the cross-sectional nature of this study and using only selected sites for GCF sampling which is not reflective of overall periodontal health. Another limitation was the age difference between PHIV and PHEU. The frequency of gingivitis is observed to increase with age, however there was no difference in proportion with gingivitis between the two cohorts in our previous publication (Moscicki et al., 2016). The proportion with visible disease of the two cohorts was also similar in our selected samples. Another limitation was the use of GI which is considered more subjective than

other parameters such as BOP, probing depth and CAL (Ainamo and Bay, 1975, Wei and Lang, 1981).

Of note, a new classification system for periodontal diseases for educational and clinical purposes was recently introduced. It involves four stages of disease severity (I-IV) and three grades of rate of progression (A-C). In addition to probing depths and clinical attachment loss, this system uses radiographs, and also may include furcation involvement, masticatory factors and past dental records to determine current and predict future rates of progression (Papapanou et al., 2018, Tonetti et al., 2018). At the time this cross-sectional multicenter study took place, this classification system had not been published, and this latter clinical information was not available. As a follow-up of these participants is currently underway, a comparison between biomarker profiles at baseline and follow-up, and determining correlations between these biomarkers and changes in clinical measures, may add new insights into the application of this new classification system in clinical research.

In conclusion, periodontal inflammation in PHIV youth is associated with elevation of local biomarkers that have been shown in some studies to predict progression to severe periodontal disease that can lead to tooth loss. This same association was not observed in the PHEU suggesting PHIV youth may have a greater potential for progression. Elevation of these local biomarkers was also associated with serum markers of systemic immune activation. Detection of these biomarkers may be used to identify individuals who would benefit from more intensive hygiene and more frequent examinations to prevent progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS:

We thank the children and families for their participation in PHACS, and the individuals and institutions involved in the conduct of PHACS. The study was supported by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development with co-funding from the National Institute on Drug Abuse, the National Institute of Allergy and Infectious Diseases, the Office of AIDS Research, the National Institute of Mental Health, the National Institute of Neurological Disorders and Stroke, the National Institute on Deafness and Other Communication Disorders, the National Institute of Dental and Craniofacial Research, and the National Institute on Alcohol Abuse and Alcoholism, through cooperative agreements with the Harvard T.H. Chan School of Public Health (HD052102) (Principal Investigator: George Seage; Project Director: Julie Alperen) and the Tulane University School of Medicine (HD052104) (Principal Investigator: Russell Van Dyke; Co-Principal Investigator: Ellen Chadwick; Project Director: Patrick Davis). Data management services were provided by Frontier Science and Technology Research Foundation (PI: Suzanne Siminski), and regulatory services and logistical support were provided by Westat, Inc (PI: Julie Davidson).

Note: The conclusions and opinions expressed in this article are those of the authors and do not necessarily reflect those of the National Institutes of Health or U.S. Department of Health and Human Services

The following institutions, clinical site investigators, and staff participated in conducting the Pediatric HIV/AIDS Cohort Study Adolescent Master Protocol in 2012, in alphabetical order: **Baylor College of Medicine**: Ester Yang*, William Shearer, Mary Paul, Norma Cooper, Lynette Harris; **Bronx Lebanon Hospital Center**: Karen Kemp-Posterman*, Selene Wun*, Murli Purswani, Mahboobullah Baig, Anna Cintron; **Children's Diagnostic and Treatment Center**: Jennifer Zeni*, Ana Puga, Sandra Navarro, Doyle Patton, Deyana Leon; Children's Hospital, Boston: Man Wai Ng*, Sandra Burchett, Nancy Karthas, Betsy Kammerer; **Ann and Robert H. Lurie Children's Hospital of Chicago:** Ray Jurado*, Johnny Kuttab*, Ashlee Vorachek*,Ram Yogev, Margaret Ann Sanders, Kathleen Malee, Scott Hunter; **Jacobi Medical Center**: Victor Badner*, Ronald Garreett*, Andrew Wiznia, Marlene Burey, Molly Nozyce; St. **Christopher's Hospital for Children**: Susan Chialastri*, Janet Chen, Latreca

Ivey, Maria Garcia Bulkley, Mitzie Grant; **San Juan Hospital/Department of Pediatrics**: Ramon Gonzalez*, Midnela Acevedo-Flores, Heida Rios, Vivian Olivera; **Tulane University Health Sciences Center**: Janice Townsend*, Margarita Silio, Medea Jones, Patricia Sirois; **University of Colorado Denver Health Sciences Center**: Roopa Gandhi*, Elizabeth McFarland, Emily Barr, Robin McEvoy; **University of Medicine and Dentistry of New Jersey**: Kenneth Markowitz*, Arry Dieudonne, Linda Bettica, Susan Adubato. *Denotes participating dentist.

FUNDING:

The Pediatric HIV/AIDS Cohort Study (PHACS) was supported by the *Eunice Kennedy Shriver* National Institute Of Child Health & Human Development (NICHD) with co-funding from the National Institute Of Dental & Craniofacial Research (NIDCR), the National Institute Of Allergy And Infectious Diseases (NIAID), the National Institute Of Neurological Disorders And Stroke (NINDS), the National Institute On Deafness And Other Communication Disorders (NIDCD), Office of AIDS Research (OAR), the National Institute Of Mental Health (NIMH), the National Institute On Drug Abuse (NIDA), and the National Institute On Alcohol Abuse And Alcoholism (NIAAA), through cooperative agreements with the Harvard T.H. Chan School of Public Health (HD052102) and the Tulane University School of Medicine (HD052104). Supported in part by a research grant from Investigator Initiated Studies Program of Merck Sharp & Dohme Corp.

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Clinical Relevance

Scientific Rationale for Study: Adults with HIV are at risk for significant periodontal disease which can lead to tooth loss as well as HIV progression. Little is known about periodontal inflammation in perinatally HIV-infected youth (PHIV).

Principal findings: Among PHIV youth, we found associations between clinical signs of periodontal inflammation and oral inflammation biomarkers that are associated with progression of periodontal disease in studies among adults. Elevation of these oral biomarkers was associated with systemic immune activation, a marker of HIV progression.

Practical implications: Detection of biomarkers of periodontal inflammation in PHIV youth suggest close dental follow-up using clinical and radiographic testing is needed in these youth.

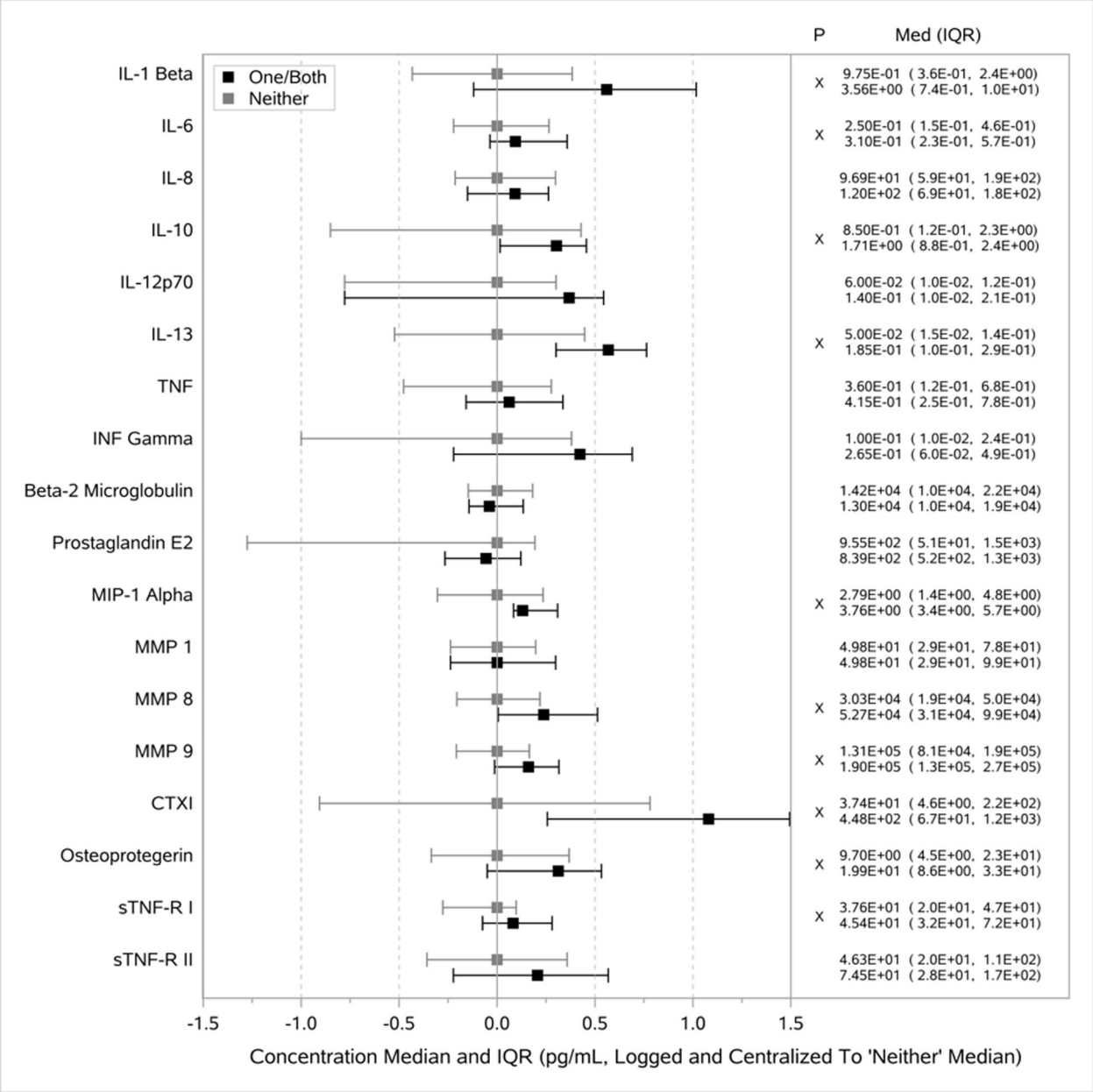

Figure 1. Gingival index ≥ 2

The concentration (pg/mL) of each cytokine among PHIV participants, measured in the pooled sample from the two gingival crevicular fluid collection sites, is displayed on the right. Concentrations are shown separately for participants with a Gingival Index (**GI**) greater than or equal to 2 at neither collection site, and at one/both collection sites; an 'X' in the P column indicates a p-value <0.05 when the two groups were compared by a Wilcoxon test. In the left panel, the median and interquartile range have been logged and centralized to the 'neither' median; the plotted values therefore represent the difference on the log scale relative to the median of the 'neither' group. IL, interleukin; TNF, tumor necrosis factor;

INF, interferon; MIP, Macrophage Inflammatory Protein; MMP, matrix metalloproteinase; pyridinoline cross-linked carboxyterminal telopeptide of type I collagen CTXI.

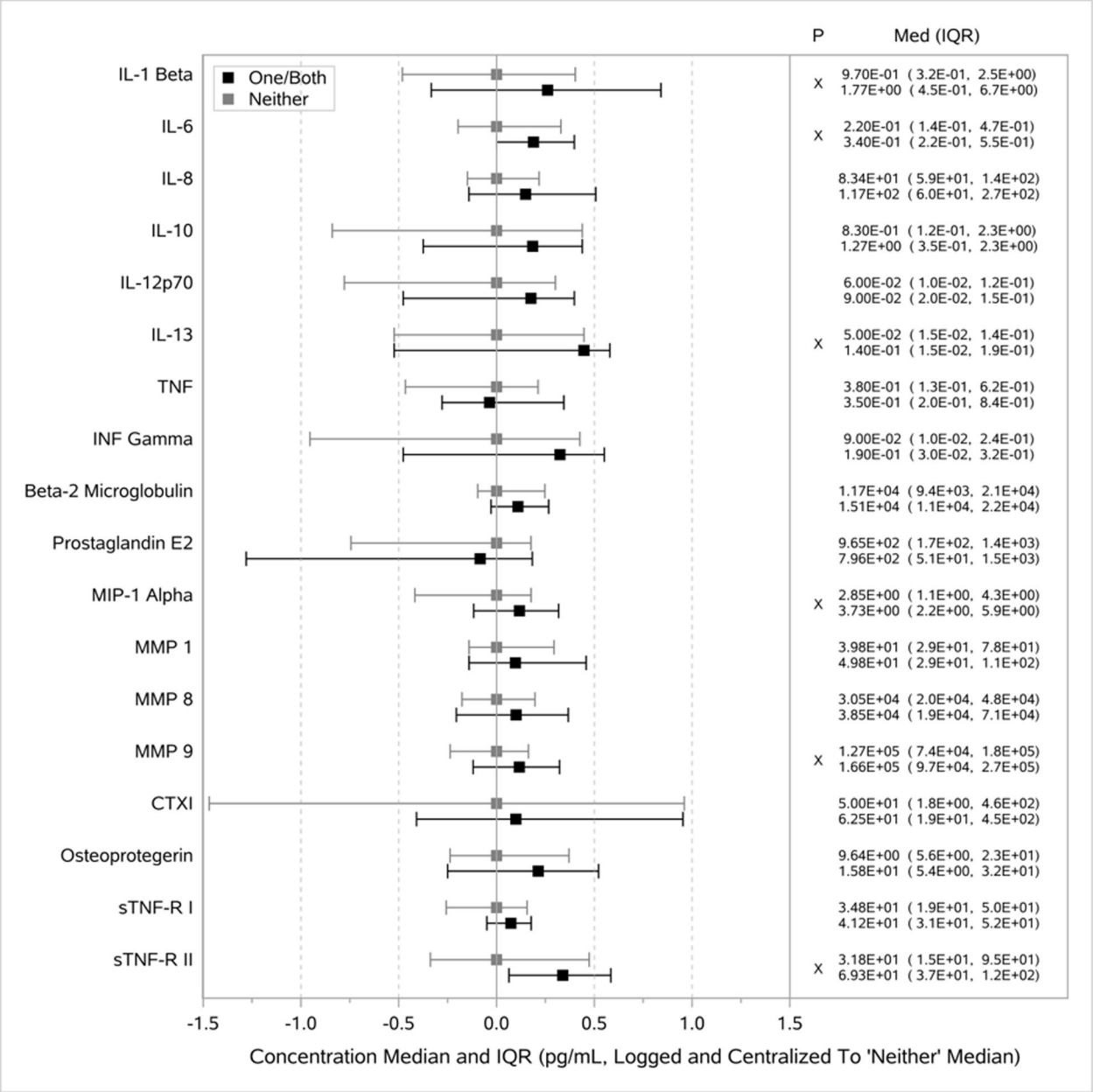

Figure 2: Bleeding on Probing

The concentration (pg/mL) of each cytokine among PHIV participants, measured in the pooled sample from the two gingival crevicular fluid collection sites, is displayed on the right. Concentrations are shown separately for participants with bleeding on probing (**BOP**) at neither collection site, and at one/both collection sites; an 'X' in the P column indicates a p-value <0.05 when the two groups were compared by a Wilcoxon test. In the left panel, the median and interquartile range have been logged and centralized to the 'neither' median; the plotted values therefore represent the difference on the log scale relative to the median of the 'neither' group. . IL, interleukin; TNF, tumor necrosis factor; INF, interferon; MIP,

Macrophage Inflammatory Protein; MMP, matrix metalloproteinase; pyridinoline cross-linked carboxyterminal telopeptide of type I collagen CTXI.

Table 1:

Demographic characteristics among perinatally HIV-infected (PHIV) and perinatally HIV-exposed but uninfected (PHEU) youth from whom we collected gingival crevicular fluid cytokine measures, by HIV infection status.

		Cohort			
		PHIV (N=129)	**PHEU (N=71)**	**Total (N=200)**	**P-Value**
Age at Visit, years, mean (SD)		16.94 (2.69)	14.75 (2.48)	16.17 (2.81)	<.001 *
Age Category, years	10 to <14	22 (17%)	29 (41%)	51 (26%)	<.001 **
No. (%)	14 to <17	39 (30%)	30 (42%)	69 (35%)	
	17 to <19	34 (26%)	8 (11%)	42 (21%)	
	19+	34 (26%)	4 (6%)	38 (19%)	
Male: No. (%)		65 (50%)	37 (52%)	102 (51%)	0.883 **
Black: No. (%)		90 (70%)	40 (56%)	130 (65%)	0.064 **
Hispanic: No. (%)		37 (29%)	37 (52%)	74 (37%)	0.001 **
Maximum Tanner Stage	1	1 (1%)	2 (3%)	3 (2%)	0.013 **
No. (%)	2	3 (2%)	8 (11%)	11 (6%)	
	3	10 (8%)	7 (10%)	17 (9%)	
	4	24 (19%)	18 (25%)	42 (21%)	
	5	91 (71%)	36 (51%)	127 (64%)	
Caregiver Income <$20,001, No. (%)		50 (40%)	45 (63%)	95 (49%)	0.003 **
	Missing	5	0	5	

* Wilcoxon Test

** Fisher's Exact Test

SD, standard deviation

Table 2:

Periodontal outcomes by HIV infection status.

		PHIV (N=129)	PHEU (N=71)	Total (N=200)	P-Value
Periodontal Measure[b] at GCF Collection Sites[a]					
GI ≥2	Neither	98 (77%)	52 (74%)	150 (76%)	0.73 *
No. (%)	One/Both	30 (23%)	18 (26%)	48 (24%)	
	Missing	1	1	2	
BOP	Neither	69 (54%)	42 (60%)	111 (56%)	0.46 *
No. (%)	One/Both	59 (46%)	28 (40%)	87 (44%)	
	Missing	1	1	2	
Periodontal Measure of Entire Mouth					
% BOP	Median (Q1,Q3)	15% (10%,26%)	16% (8%,26)	16% (9%,26%)	0.68 **
Average PI	Median (Q1,Q3)	0.93 (0.52,1.24)	0.88 (0.60,1.27)	0.90 (0.54,1.26)	0.80 **

*Fisher's Exact Test,

**Wilcoxon Rank-Sum Test

GI, gingival index, BOP, bleeding on probing, PI, plague index

[a]Gingival crevicular fluid (GCF) was collected from the mesial buccal aspect of the first permanent upper right and lower left molars. If the first permanent molar was not presented or only partially erupted, then the sample was collected from the mesial site from the first erupted tooth mesial to the first permanent molar site.

[b]Neither denotes that neither of the GCF samples were obtained at sites that had GI ≥2 or BOP. One/Both denotes that one or both GCF samples were obtained at sites that had GI ≥2 or BOP. Missing denotes that BOP and GI information were missing in these two specific sampling sites.