Oral Inflammation and Systemic Immune Activation Among Children Living with HIV in Kenya

John Sumkai Atiiga

A thesis
submitted in partial fulfillment of the requirements for the degree of
Master of Public Health

University of Washington

2025

Committee:

Jennifer Slyker

Grace John-Stewart

Irene Njuguna

Ana Lucia Seminario

Program Authorized to Offer Degree:

Global Health

University of Washington

Abstract

Oral Inflammation and Systemic Immune Activation Among Children Living with HIV in Kenya

John Sumkai Atiiga

Chair of Supervisory Committee:
Jennifer Slyker
Department of Global Health

Background

Poor oral health can lead to chronic pain, decreased nutritional intake, lower quality of life, and increased risk of systemic inflammation-related diseases. Children living with HIV (CLHIV) have an elevated risk for oral diseases, including gingivitis, which is incompletely resolved with antiretroviral treatment (ART). Oral inflammation may be an important contributor to systemic inflammation in CLHIV, and an attractive target for intervention. The goal of this study was to assess associations between early-life HIV infection and oral inflammation after over 10 years of ART, and to assess whether oral inflammation was associated with systemic inflammation and immune activation.

Methods

This nested study involved two Kenyan cohorts of CLHIV: a "later-treated" cohort enrolled at ages 15 months to 12 years with ART initiated according to clinical stage/CD4 criteria, and an "early-treated" cohort enrolled below 1 year old, with immediate ART initiation. Bleeding on Probing score (BOP score) was calculated during an oral examination to quantify the

percentage of sites with gingivitis. The enzyme-linked immunoassay (ELISA) was used to measure the inflammation markers IL-6 in plasma and soluble (s)-CD14 in saliva. Flow cytometry was used to measure frequencies of activated CD4+, CD8+, and monocyte subsets in blood. Linear regression was used to assess the association between early ART and oral inflammation markers, and the association between oral inflammation and markers of systemic inflammation and immune activation.

Results

A total of 58 early-treated and 27 later-treated CLHIV were evaluated. The median duration on ART at oral assessment/saliva collection was 11 (interquartile range [IQR]10, 13) years. There was a trend for higher median sCD14 levels (p=0.063). BOP scores were also higher in the later-treated but not significantly so (p=0.2).

In analyses, adjusted for age at oral health assessment and sex, pre-ART CD4 percentage <15% (coefficient = 0.145, p = 0.006), higher pre-ART HIV RNA level (coefficient = 0.028, p = 0.012), and initiating ART after 1 year (later-treated) (coefficient = 0.152, p= 0.015), were significantly associated with higher BOP score. None of the variables analyzed was associated with higher sCD14 level.

Higher BOP scores were significantly associated with higher frequencies of activated CD4+ (coefficient = 0.351, p = 0.020), and activated CD8+ (coefficient = 0.579, p = 0.010) T cells, but were not associated with frequencies of intermediate, classical, or non-classical monocytes. sCD14 was not associated with frequencies of activated T cells nor any monocyte subset.

Conclusion

Later ART initiation, pre-ART immunosuppression, and higher pre-ART HIV RNA level were associated with more severe gingivitis after more than a decade of ART in CLHIV, and more severe gingivitis was associated with higher frequencies of activated T cells. Our findings

suggest improving oral health in CLHIV may be a novel approach to addressing residual T cell activation in CLHIV on long-term ART.

ACKNOWLEDGEMENT

This thesis was made possible by the invaluable support and mentorship of my thesis committee chair, Dr. Jennifer Slyker, and committee members Drs. Grace John-Stewart, Irene Njuguna, and Ana Lucia Seminario, who generously gave their time and expertise at every step. Coming from a clinical background with limited research experience, I was fortunate to have Dr. Slyker as an exceptional mentor. She guided me throughout this project with unwavering support and also supported my broader career goals, opening up a range of opportunities along the way. My committee members were incredibly generous with their time and always made themselves available to answer questions and offer guidance. I am deeply grateful.

Although not formally listed as committee members, I would also like to extend my sincere appreciation to Jodi Byers and Dr. Orvalho Augusto, who were instrumental to the success of this project. Special thanks to Drs. Dalton Wamalwa and Arthur Kemoli; the OPH, PAD, and KPS staff; study participants; the CFAR Immunology Core; Dara Lehman, Morgan Litchford, and Whasun Chung. Your contributions in various forms were crucial and are truly appreciated.

To the Department of Global Health at the University of Washington, and my wonderful cohort of Global Health MPH students, you have shown me kindness, encouragement, and community throughout this journey.

Finally, to my family and friends, especially my wife, Jemima, and our children, Anaya and John Jr., your constant support, even from afar, has meant the world to me. I cannot thank you enough.

Table of Contents

INTRODUCTION

Oral health has broad benefits for overall health, including better nutrition, lower risk of several non-communicable diseases (NCDs), and generally better quality of life (1,2). Oral health is neglected in many settings and is an important driver of health inequity. An estimated 26% to 90% of African children experience oral diseases in the form of gingivitis (3,4), which is inflammation of the soft tissues surrounding the tooth structure. This high prevalence is partly due to limited access to preventive oral care and treatment (5). Gingivitis results from suboptimal oral hygiene practices, leading to irritation of the gums by plaque accumulation, but can also be affected by diet, age-related changes in hormones, pregnancy, and chronic comorbidities such as type II diabetes or HIV. Routine professional cleanings can greatly help control the accumulation of plaque but is not accessible by many people. Children living with HIV (CLHIV) are at a higher risk for oral diseases and more severe manifestations of oral diseases, including gingivitis, caries, and opportunistic infections (6,7). Combination antiretroviral therapy (ART) greatly improves oral health outcomes and restores the oral microbiome (8). However, even in the setting of ART, CLHIV have a high risk of gingivitis (9).

Oral inflammation in CLHIV was associated with age and CD4 count in a US cohort (10). In the same study, gingivitis prevalence was 6% in CLHIV under one year old, 55% in one-year-olds, and up to 87% in CLHIV aged two years and older (10). The Kenya National Oral Health Survey in 2015 reported a prevalence of gum disease in over 90% of Kenyan children (11). Factors that may affect the severity of oral inflammation in CLHIV include social determinants of health that affect knowledge of, and access to oral hygiene and dental care access, including maternal age, education, socioeconomic status and gender of child (12,13). Risk factors unique to CLHIV include the age at which ART was initiated and the ART regimen administered (14,15). A US study revealed that an earlier ART initiation (< 6 months after estimated HIV

infection) was associated with lower levels of T-cell activation and viral load during long-term therapy than a late initiation (16). Antiretroviral formulation also affect oral health and adherence; alcohol-based medications (such as lopinavir/ritonavir (LPV/r)) have poor tolerability (which promotes non-adherence) while syrup-based medications (such as nevirapine (NVP)) may be better tolerated but include sweeteners that promote the growth of pathogenic oral microbes (15).

Gingivitis has been associated with increased systemic immune activation in healthy populations (17). Experimental studies have established a causal link between oral inflammation and increased systemic inflammation following the cessation of dental hygiene and upon resumption of oral hygiene practices, systemic inflammation returns to normal (18,19). Additional observational studies in healthy populations have demonstrated an association between oral inflammation and atherosclerotic vascular diseases (ASVDs) (20,21).

Inflammation and immune activation are also central to HIV pathogenesis. HIV infection causes damage to the intestinal mucosa, which leads to the translocation of microbial products from the gut to the systemic circulation, which promotes a state of chronic inflammation (22). Systemic inflammation and immune activation are improved by, but not completely resolved by ART (23). This persistent inflammation is associated with a higher risk of several non-communicable diseases (NCDs), including ASVDs (24) and metabolic syndrome/dyslipidemia in people living with HIV (PLHIV) (25).

Because oral inflammation and HIV infection both promote systemic inflammation and immune activation, optimizing oral health may be a novel strategy to optimize health outcomes and reduce NCD risks in PLHIV. To date, the association between oral and systemic inflammation has not been studied in CLHIV. Understanding these relationships could motivate the incorporation of routine oral health preventive care into HIV care to achieve broad health

benefits. To address this knowledge gap, we assessed associations between early-life HIV infection and oral inflammation after years of ART and evaluated whether oral inflammation was associated with systemic inflammation and immune activation.

METHODS

Study population:

The study utilized existing samples and data from an ongoing longitudinal cohort of Kenyan CLHIV followed since ART initiation. Children were initially enrolled in two studies focused on optimizing care and treatment for CLHIV (26,27). The "later-treated" Pediatric Adherence Diary Study (PAD) (begun in 2004), enrolled children between 15 months and 12 years old who met contemporaneous criteria to begin ART (*moderate (World Health Organization (WHO) clinical stage 2 with CD4 <15%) to severe (WHO clinical stage 3 or 4) HIV-1 disease)*. The "early-treated" Optimizing Pediatric HIV-1 Treatment Study (OPH) study (NCT00428116) (begun in 2007) enrolled children under 12 months old and initiated ART immediately, regardless of the CD4 count or HIV stage. More details about the two cohorts can be found elsewhere (26,28). Some children from both cohorts remain in extended follow-up for studies of HIV reservoirs.

For both cohorts, study visits were conducted at 3 to 6 monthly intervals, with the administration of a standardized questionnaire and blood collection. Clinical data, adherence and ART information, HIV RNA level, and CD4 counts were collected at each visit.

Oral Health Assessments

Children enrolled in the 2 cohorts were eligible for the Children's HIV Oral Manifestations Project (CHOMP) oral health assessments. Caregivers were asked to provide written and oral

consent for participation. Study Protocols were reviewed and approved by the University of Washington and Kenyatta National Hospital Institutional Review Boards (IRBs). The oral examinations were conducted in 2019, during routine study visits and were performed by local Community Oral Health Officers (COHOs) who had been trained in the diagnosis of dental diseases and oral manifestations of HIV, including gingivitis and periodontitis according to a standardized protocol using WHO tools (29). Children with caries or other conditions were referred to the University of Nairobi School of Pediatric Dentistry clinic for treatment.

Saliva collection was done using the passive drool technique (30). The study coordinator held a sterile 5mL tube with gloved hands and asked participants to hold their saliva for 15 seconds and then spit into the tube. This process was repeated until 5mL of saliva was collected or until five minutes had passed, whichever came first. Saliva was stored with no additives at -80 C. Following saliva collection, the COHOs performed oral examinations using dental mirrors under natural light, augmented with headlamps, while patients were lying in medical beds. The clinical extent of gingival inflammation (gingivitis) was measured by using the Bleeding on Probing Score (BOP Score), a physiological marker reflecting the percentage of gingival surfaces that bleed upon gentle probing with a periodontal probe. These were recorded by hand on a diagram of the mouth. A BOP score < 10% defines a healthy gingiva or minimal gingival inflammation, a BOP score between 10% and 30% defines localized gingivitis, and a BOP score > 30% defines generalized gingivitis (31).

ELISA Assays

Soluble CD14 (sCD14), a marker of monocyte activation was measured in pg/ml of saliva using a commercially available kit. The MSD platform (Meso Scale Discovery) was used to measure the inflammatory marker IL-6 in pg/ml of plasma.

Flow Cytometry Assays

Measures of systematic activation included T-cells and monocytes, and assessments were conducted in children from the early-treated cohort 6 years after ART initiation; all the children were below the age of 12 years at the time of these assessments with a median (IQR) age of 6 years. Frequencies of activated CD4+ T cells, CD8+ T cells, and monocytes were measured using high-dimensional flow cytometry. Cells were stained, collected, and analyzed by the Center for AIDS/STD Research (CFAR) Immunology Core. Activated T helper cells were defined as $CD3^{+}CD4^{+}CD38^{bright}HLA\text{-}DR^{+}$; activated CD8+ T cells were defined as $CD3^{+}CD8^{+}CD38^{bright}HLA\text{-}DR^{+}$ and monocyte subsets (HLA-DR+CD11b+) were defined as classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), and non-classical ($CD14^{+/low}CD16^{++}$).

Statistical Analysis

All analyses were performed using the R 4.3.4 statistical package and considered alpha=0.05, using 2-sided tests. Drawing from previous research (12,13) and hypothesized relationships based on biologic knowledge, we developed directed acyclic graphs (DAGs) to *a priori* propose potential confounders and mediators. Given the known effects of age, puberty, and sex on oral inflammation, we adjusted for age at oral health assessment (above and below 12 years) and sex.

Descriptive statistics were used to summarize the demographic and clinical characteristics of the study population and their caregivers across the two cohorts. Categorical variables were reported as counts and percentages, while continuous variables were summarized as medians with interquartile ranges (IQR). Inferential analyses were conducted using Fisher's exact test, Pearson's chi-squared test, and Wilcoxon rank-sum test to assess differences in distributions between the two cohorts.

We determined medians and interquartile ranges of biochemical and physiologic markers of oral inflammation (sCD14 and BOP Score) among subjects. A Wilcoxon rank sum test was done to determine whether the difference in the distribution of sCD14 and BOP scores between the two cohorts was significant. A Pearson correlation test was also done to measure the correlation between sCD14 levels and BOP scores.

We assessed correlates of oral inflammation, including immunosuppression, WHO clinical stage (Stage III/IV vs. Stage I/II), HIV RNA levels, initial ART regimen (non-nucleotide reverse transcriptase inhibitor (NNRTI)-based vs. protease inhibitor (PI-based)-based), later- versus early-treated cohort (i.e., OPH vs. PAD), age at ART initiation, age at oral health assessment. We hypothesized that pathogen exposure (disease, viremia, inflammation), medications, and immunosuppression at this critical period of tooth development and establishment of the oral microbiome have the potential to impact long-term oral health. We used linear regression to assess the association between exposures and the outcomes of sCD14 and BOP score. The model adjusted for potential confounders related to oral hygiene practices, including age at oral health assessment and sex.

We used linear regression to measure the association between oral inflammation and markers of systemic inflammation and immune activation, adjusting for sex and age at oral health assessment.

RESULTS

Cohort characteristics (Pre-ART)

A total of 83 CLHIV formed our study sample. Of these, 56 were early-treated, receiving ART before 12 months, and 27 were later-treated children who initiated ART between the ages of 15 months and 12 years. Given the enrollment criteria for the two different studies, the early-treated cohort were younger, had earlier ART initiation, better immunologic status, and were younger when their oral health and immunologic assessments were conducted (p-value < 0.001 for each, Table 1). Infant sex and caregiver level of education were similar between the early and later-treated children. More early-treated children had their mother as a primary caregiver at enrollment (98% vs 70%, p-value < 0.001). At enrollment, the early-treated children had significantly higher absolute CD4 counts (median 1326 versus 522 cells/mm^3, p-value < 0.001) and CD4% (median 19% versus 7%, p-value < 0.001). Baseline viral load was similar between cohorts (6.10 versus 6.05 $\log_{10}$ HIV RNA copies/ml P-value = 0.4).

Oral Inflammation

Children were assessed for markers of oral inflammation (sCD14 and BOP score) at a median age of 10 years of age for the early-treated and 17 years of age for the late-treated. Oral inflammation (sCD14 and BOP) levels were high in both early- and later-treated groups. There was a trend for higher median sCD14 levels (p-value = 0.063) in later-treated than the early-treated groups. BOP levels were also higher in the later-treated group but not significantly so (p-value = 0.2) (Table 2; Figures 4, 5). There was no significant correlation between salivary sCD14 levels and BOP score (Pearson Coeff= 0.012; p-value = 0.912).

Early Life Predictors of Oral Inflammation

In unadjusted analyses, older age at oral health assessment and older age at ART initiation were significantly associated with higher salivary sCD14 levels, but none remained significant after adjustment (p-value > 0.05).

Pre-ART severe immunosuppression (CD4% <15%), HIV RNA level, cohort and age at oral health assessment were significantly associated with higher BOP scores in unadjusted analysis. In analyses adjusting for age and sex, children with severe immunosuppression at ART initiation had a ~15% higher mean BOP score compared to children with better immune status (Coeff=0.145; 95% CI: 0.042, 0.248; p-value = 0.006) and each 1-$\log_{10}$ increase in $\log_{10}$ HIV RNA level predicted a 2.8% increase in BOP score (Coefficient=0.028, 95% CI: 0.006, 0.051; p-value = 0.012). In unadjusted analyses, there was also a trend for higher BOP scores in the later-treated cohort, which became significant after adjusting for confounders (adjusted Coeff = 0.152 (95% CI: 0.029, 0.275; p-value = 0.015). Older age at oral health assessment was also associated with higher BOP scores (adjusted Coeff=0.033 (95% CI: 0.004, 0.061; p-value = 0.022)**.** The remaining variables, including WHO clinical stage, initial ART regimen, and age at ART initiation, were not significantly associated (p-value > 0.05) with either marker of gingival inflammation.

Oral Inflammation and Systemic Inflammation/Immune Activation

We did not find significant associations between plasma IL-6 and oral inflammation measured by either sCD14 or BOP score (Table 4i), similarly monocyte subset frequencies were not associated with oral inflammation (Table 4i, Table 4ii). However, higher percentages of activated CD4+ T cells and activated CD8+ T cells were associated with higher BOP scores (Table 4iii), with coefficients of 0.351 (95% CI: 0.057, 0.644; p-value = 0.020) and 0.579 (95% CI: 0.140, 1.020; p-value = 0.010), respectively.

DISCUSSION

We hypothesized that early-life immunosuppression and high HIV RNA levels at ART initiation would be associated with oral inflammatory disease in CLHIV who were assessed in adolescence/young adulthood, and that oral inflammation would be associated with systemic inflammation and immune activation. To our knowledge, this is the first longitudinal study evaluating the relationship between characteristics during HIV in childhood and later oral inflammation in adolescence and the first study evaluating the association between oral and systemic inflammation and immune activation in youth living with HIV (YLHIV) followed up from early childhood. We found that immunologic/virologic status at the time of ART initiation in childhood were associated with later gingivitis in YLHIV. Pre-ART CD4 percentage <15% and higher HIV RNA levels were significantly associated with higher BOP scores. Among YLHIV, gingivitis was also associated with concurrently higher CD4+ and CD8+ T cell activation despite viral suppression after many years of successful ART. Together, these data suggest the importance of incorporating oral health into HIV treatment for CLHIV.

As expected, older age at oral health assessment was significantly associated with increased oral inflammation (32). Prior research in adults without HIV have found an association between gingivitis and age (33–35). Similarly, sCD14 levels among children in this study were positively associated with age. These age-related differences could be attributed to several factors in our cohort, including lack of routine dental care, cumulative exposure to dietary and microbial stimuli over time, and hormonal changes associated with puberty (36,37).

Children in the later-treated cohort had higher sCD14 and BOP levels compared to children who initiated ART empirically after HIV diagnosis. We also found that CLHIV with advanced immunosuppression pre-ART and higher HIV RNA levels were associated with higher BOP scores years later in adolescence and young adulthood. Pre-ART CD4 < 15%, high pre-ART

HIV RNA levels, and later treatment were significantly associated with having more sites of gingival inflammation. This finding is especially notable given the many years of successful ART and optimized clinical experience of being in a longitudinal research study. Delayed ART initiation and more advanced HIV disease could contribute to long-term gingival health through several inter-related mechanisms, including microbiome disruptions and the emergence of pathogenic oral species (P. gingivalis, Fusobacterium, and other harmful bacteria), and mucosal membrane damage (22).

Despite observing that pre-treatment immunosuppression and viral load predicted gingivitis as assessed by BOP, these factors did not predict sCD14 levels in YLHIV. The lack of association was surprising to us, given prior research describing associations between HIV disease status and systemic sCD14 levels. It is notable that we did not find a correlation between sCD14 and BOP scores; this lack of correlation could be due to a dilution effect of measuring sCD14 in bulk saliva rather than in gingival crevicular fluid of the inflamed areas. It is also likely our small sample size may have limited the statistical power to detect smaller effect sizes. Future studies with larger sample sizes may provide stronger evidence on the relationship between these early HIV factors and long-term oral morbidities.

We did not find an association between BOP score or salivary sCD14 levels and IL-6 levels in blood. However, we did find that high BOP scores were associated with significantly higher levels of activated CD4+ and CD8+ T-cells in blood. This finding is consistent with previous experimental studies that have established a causal link between increased oral inflammation following the cessation of dental hygiene and heightened systemic immune activation/inflammation as measured by IL-6 and C-reactive protein(CRP) (18,19). Higher T cell activation persists despite ART and predicts an increased risk of all-cause morbidity and

mortality in both YLHIV and adults. Our results suggest that oral inflammation could contribute to systemic cellular activation and influence HIV progression through this pathway.

Our study has several strengths, including the longitudinal clinical assessment of cohorts of children with well-managed HIV infection over nearly 2 decades, and the availability of robust clinical and laboratory data. To our knowledge this is the first study evaluating BOP and sCD14 levels with well-established cohorts of CLHIV. However, certain limitations should be considered. Since this select cohort received comprehensive, high-quality HIV care from early childhood, our findings may not be generalizable to broader populations or other settings of CLHIV with less access to HIV care. Additionally, the small sample size may have limited our ability to detect small effect sizes between covariates and outcomes. Furthermore, we did not have data on oral hygiene practices within these cohorts. Given that oral hygiene is a key predictor of both early HIV-related health experiences and oral inflammation, the lack of data on this represents a potential source of confounding that we were unable to control for in our analyses. Finally, these cohorts were accrued at a much earlier stage of the HIV pandemic, and may not be generalizable to current HIV regimens. It is unclear whether modern integrase-inhibitor-based regimens might mitigate some of the effects of HIV on the oral mucosa.

In summary, we found that advanced HIV progression at the time of ART initiation may have relevance for the progression of oral diseases more than a decade later, and that gingival inflammation was associated with concurrent T cell activation among YLHIV who perinatally acquired HIV. This work highlights the need for preventative care and oral health screening for CLHIV and YLHIV, as timely detection and management of oral inflammation can help prevent further deterioration and associated cardiovascular and metabolic complications associated with immune activation. In health systems, such as Kenya, in which universal health

care covers children aged five years and younger, integrating oral health into routine pediatric medical care could be a cost-effective and efficient approach to enhance the quality of life and reduce HIV-associated immune activation in children and adolescents with HIV infection.

REFERENCES

1. Mbawalla HS, Masalu JR, Åstrøm AN. Socio-demographic and behavioural correlates of oral hygiene status and oral health related quality of life, the Limpopo - Arusha school health project (LASH): A cross-sectional study. BMC Pediatr. 2010 Nov 30;10:87.

2. Sheiham A. Oral health, general health and quality of life. Bull World Health Organ. 2005 Sep;83(9):644.

3. Poni NA, Ribas-Pérez D, Flores-Fraile J, Hernández-Franch PV, Rodríguez-Menacho D, Castaño-Séiquer A. Descriptive Study of Oral Health in an Indigenous Child Population of Baka Pygmies in Cameroon. Dent J. 2023 Oct 12;11(10):237.

4. Azodo CC, Agbor AM. Gingival health and oral hygiene practices of schoolchildren in the North West Region of Cameroon. BMC Res Notes. 2015 Aug 29;8(1):385.

5. Shomuyiwa DO, Bridge G. Oral health of adolescents in West Africa: prioritizing its social determinants. Glob Health Res Policy. 2023 Jul 19;8:28.

6. Seminario AL, Kemoli A, Benki-Nugent S, Chebet D, Gomez L, Wamalwa D, et al. Caregivers' report of HIV-associated oral manifestations among HIV-unexposed, exposed, and infected Kenyan children. Int J Paediatr Dent. 2021 Nov;31(6):708–15.

7. Cassone A, Cauda R. Candida and candidiasis in HIV-infected patients: where commensalism, opportunistic behavior and frank pathogenicity lose their borders. AIDS Lond Engl. 2012 Jul 31;26(12):1457–72.

8. Narayanan A, Kieri O, Vesterbacka J, Manoharan L, Chen P, Ghorbani M, et al. Exploring the interplay between antiretroviral therapy and the gut-oral microbiome axis in people living with HIV. Sci Rep. 2024 Aug 1;14:17820.

9. Lam PPY, Zhou N, Wong HM, Yiu CKY. Oral Health Status of Children and Adolescents Living with HIV Undergoing Antiretroviral Therapy: A Systematic Review and Meta-Analysis. Int J Environ Res Public Health. 2022 Oct 8;19(19):12864.

10. Ramos-Gomez FJ, Petru A, Hilton JF, Canchola AJ, Wara D, Greenspan JS. Oral manifestations and dental status in paediatric HIV infection. Int J Paediatr Dent. 2000 Mar;10(1):3–11.

11. Kenya National Oral Health Policy 2022-2030 | WHO | Regional Office for Africa [Internet]. [cited 2025 Mar 30]. Available from: https://www.afro.who.int/countries/kenya/publication/kenya-national-oral-health-policy-2022-2030

12. Štefanová E, Baška T, Holubčíková J, Timková S, Tatarková M, Sovičová M, et al. Selected Behavioural Factors Affecting Oral Health in Schoolchildren: Results from the Health Behaviour in School-Aged Children (HBSC) Slovak Study. Int J Environ Res Public Health. 2020 Oct;17(20):7516.

13. Castilho ARF de, Mialhe FL, Barbosa T de S, Puppin-Rontani RM. Influence of family environment on children's oral health: a systematic review. J Pediatr (Rio J). 2013 Mar 1;89(2):116–23.

14. McLigeyo A, Wekesa P, Owuor K, Mwangi J, Isavwa L, Mutisya I. Factors Associated with Treatment Outcomes Among Children and Adolescents Living with HIV Receiving Antiretroviral Therapy in Central Kenya. AIDS Res Hum Retroviruses. 2022 Jun 1;38(6):480–90.

15. Lockman S, Hughes M, Sawe F, Zheng Y, McIntyre J, Chipato T, et al. Nevirapine-Versus Lopinavir/Ritonavir-Based Initial Therapy for HIV-1 Infection among Women in Africa: A Randomized Trial. PLoS Med. 2012 Jun 12;9(6):e1001236.

16. Jain V, Hartogensis W, Bacchetti P, Hunt PW, Hatano H, Sinclair E, et al. Antiretroviral Therapy Initiated Within 6 Months of HIV Infection Is Associated With Lower T-Cell Activation and Smaller HIV Reservoir Size. J Infect Dis. 2013 Oct 15;208(8):1202–11.

17. Hasturk H, Kantarci A, Van Dyke TE. Oral Inflammatory Diseases and Systemic Inflammation: Role of the Macrophage. Front Immunol. 2012 May 16;3:118.

18. Eberhard J, Grote K, Luchtefeld M, Heuer W, Schuett H, Divchev D, et al. Experimental Gingivitis Induces Systemic Inflammatory Markers in Young Healthy Individuals: A Single-Subject Interventional Study. PLoS ONE [Internet]. 2013 [cited 2024 Jul 22];8(2). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3567060/

19. Peric M, Marhl U, Gennai S, Marruganti C, Graziani F. Treatment of gingivitis is associated with reduction of systemic inflammation and improvement of oral health-related quality of life: A randomized clinical trial. J Clin Periodontol. 2022 Jun 1;49.

20. Lockhart PB, Bolger AF, Papapanou PN, Osinbowale O, Trevisan M, Levison ME, et al. Periodontal Disease and Atherosclerotic Vascular Disease: Does the Evidence Support an Independent Association? Circulation. 2012 May 22;125(20):2520–44.

21. Tonetti MS, Van Dyke TE, working group 1 of the joint EFP/AAP workshop. Periodontitis and atherosclerotic cardiovascular disease: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. J Periodontol. 2013 Apr;84(4 Suppl):S24-29.

22. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006 Dec;12(12):1365–71.

23. Marchetti G, Tincati C, Silvestri G. Microbial Translocation in the Pathogenesis of HIV Infection and AIDS. Clin Microbiol Rev. 2013 Jan;26(1):2–18.

24. Kaplan RC, Hanna DB, Kizer JR. Recent Insights into Cardiovascular Disease (CVD) Risk Among HIV-Infected Adults. Curr HIV/AIDS Rep. 2016 Feb;13(1):44–52.

25. Ali MK, Magee MJ, Dave JA, Ofotokun I, Tungsiripat M, Jones TK, et al. HIV and Metabolic, Body, and Bone Disorders: What We Know From Low- and Middle-Income Countries. JAIDS J Acquir Immune Defic Syndr. 2014 Sep 1;67:S27.

26. Wamalwa D, Benki-Nugent S, Langat A, Tapia K, Ngugi E, Moraa H, et al. Treatment interruption after 2-year antiretroviral treatment (ART) initiated during acute/early HIV in infancy: a randomized trial. AIDS Lond Engl. 2016 Sep 24;30(15):2303–13.

27. Wagner A, Slyker J, Langat A, Inwani I, Adhiambo J, Benki-Nugent S, et al. High mortality in HIV-infected children diagnosed in hospital underscores need for faster diagnostic turnaround time in prevention of mother-to-child transmission of HIV (PMTCT) programs. BMC Pediatr. 2015 Feb 15;15:10.

28. Pankau MD, Wamalwa D, Benki-Nugent S, Tapia K, Ngugi E, Langat A, et al. Decay of HIV DNA in the Reservoir and the Impact of Short Treatment Interruption in Kenyan Infants. Open Forum Infect Dis. 2018 Jan;5(1):ofx268.

29. Oral health surveys: basic methods - 5th edition [Internet]. [cited 2025 Mar 16]. Available from: https://www.who.int/publications/i/item/9789241548649

30. SalivaBio's Passive Drool Saliva Collection Method [Internet]. Salimetrics. [cited 2025 Mar 24]. Available from: https://salimetrics.com/collection-method/passive-drool-saliva-collection-device/

31. Deng K, Pelekos G, Jin L, Tonetti MS. Gingival bleeding on brushing as a sentinel sign of gingival inflammation: A diagnostic accuracy trial for the discrimination of periodontal health and disease. J Clin Periodontol. 2021 Dec;48(12):1537–48.

32. Michaud DS, Fu Z, Shi J, Chung M. Periodontal Disease, Tooth Loss, and Cancer Risk. Epidemiol Rev. 2017 Jan;39(1):49–58.

33. Isaza-Guzmán DM, Aristizábal-Cardona D, Martínez-Pabón MC, Velásquez-Echeverri H, Tobón-Arroyave SI. Estimation of sCD14 levels in saliva obtained from patients with various periodontal conditions. Oral Dis. 2008 Jul;14(5):450–6.

34. Changes in salivary microbial sensing proteins CD14 and TLR2 with aging - ProQuest [Internet]. [cited 2025 Mar 16]. Available from: https://www.proquest.com/openview/99eb626927c506f4dbb09ab70def47c6/1?pq-origsite=gscholar&cbl=33690

35. AlQallaf H, Hamada Y, Blanchard S, Shin D, Gregory R, Srinivasan M. Differential profiles of soluble and cellular toll like receptor (TLR)-2 and 4 in chronic periodontitis. PLoS ONE. 2018 Dec 20;13(12):e0200231.

36. Chaitra TR, Manuja N, Sinha AA, Kulkarni AU. Hormonal effect on gingiva: pubertal gingivitis. BMJ Case Rep. 2012 Jul 27;2012:bcr2012006193.

37. Glowacka J, Opydo-Szymaczek J, Mehr K, Jarzabek-Bielecka G, Glowacki J. Factors affecting puberty gingivitis in Polish girls with adolescent idiopathic scoliosis. Ginekol Pol. 2020;91(3):103–10.

APPENDIX

TABLES

Table1: Demographic Characteristics and Early Life HIV-related Factors of the Optimizing Pediatric HIV-1 Treatment Study (OPH) and the Pediatric Adherence Study (PAD) cohorts of Children Living with HIV

Characteristic	N	Overall Median (IQR) or n (%) N = 83[1]	Early-treated (OPH) Median (IQR) or n (%) N = 56[1]	Later-treated (PAD) Median (IQR) or n (%) N = 27[1]	p-value[2]
Age at enrollment into research cohort (months)	83	9 (4, 33)	4 (3, 10)	42 (27, 58)	<0.001
Age at ART Initiation (months)	83	7 (4, 27)	4 (4, 7)	42 (27, 58)	<0.001
Female	83	35 (45.8%)	25 (44.6%)	13 (48.1%)	0.8
Primary caregiver (Biological mother)	83	74 (89.2%)	55 (98.2%)	19 (70.4%)	<0.001
Caregiver level of Education (Beyond Primary)	81	39 (48.1%)	25 (45.5%)	14 (53.8%)	0.5
CD4 count cells/mm3	83	1070 (522, 1572)	1326 (735, 1757)	522 (102, 982)	<0.001
CD4 percentage	83	16 (9, 23)	19 (14, 25)	7 (4, 14)	<0.001
HIV RNA level at Enrollment (log10)	83	6.07 (4.76, 6.72)	6.10 (4.71, 6.87)	6.05 (4.76, 6.53)	0.4
Age at Oral Health and IL6, sCD14 Assessment (years)	83	11.00 (10.00, 15.00)	10.00 (10.00, 11.00)	17.00 (15.00, 19.00)	<0.001
12 years or below at oral Health and IL6, sCD14 Assessment	83	53 (63.9%)	53 (94.6%)	0 (0.0%)	
12 years or below at T cell and monocyte assessment	51	51 (100%)	53 (100%)	0 (0.0%)	

[1] n (%)

[2] Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

*Notes: The eligibility criteria for OPH was children under 12 months, and the eligibility criteria for PAD was children between 15 months to 12 years.

*Notes: Children in the OPH Cohort were treated empirically, and those in the PAD cohort were treated according to the clinical/CD4 count criteria at the time.

*Note: A total of 12 children in the OPHD cohort were enrolled from the ARA and INK cohorts at above 12 months of age. However, all of them had ART initiation below 12 months of age.

Table 2: Oral inflammation markers

Characteristic	N	Overall Median (IQR) N = 83	Early-treated (OPH) Median (IQR) N = 56	Later-treated (PAD) Median (IQR) N = 27	p-value[1]
sCD14 level (pg/ml)	81	18,955 (12,830, 26,097)	16,983 (11,715, 25,994)	20,661 (14,627, 29,791)	0.063
Bleeding on Probing (BOP) Score[2]	82	0.36 (0.23, 0.56)	0.35 (0.20, 0.54)	0.43 (0.28, 0.64)	0.2

[1] Wilcoxon rank sum test

[2]BOP Score = Proportion of bleeding sites upon gentle probing over the total number of sites probed around gums.

Table 3. Correlation Between early HIV experience and oral Inflammation biomarkers measured >10 years later

Correlates	Log_{10} CD14 pg/ml		BOP score	
	Unadjusted (Coefficient, 95% CI); P-value	**Adjusted (Coefficient, 95% CI); P-value (age at oral health and sex)**	**Unadjusted (Coefficient, 95% CI); P-value**	**Adjusted (Coefficient, 95% CI); P-value**
Pre-ART CD4 <15% (immunosuppression)	0.038 (-0.063, 0.141); P = 0.451	0.009 (-0.145, 0.120); P =0.829	0.122 (0.028, 0.216); P = 0.011	0.145 (0.042, 0.248); P = 0.006
cWHO stage III/IV[1] Ref: stage I/II	0.007 (-0.096, 0.111); P = 0.887	-0.030 (-0.147, 0.087); P = 0.611	0.081 (-0.013, 0.177); P = 0.093	0.074 (-0.032, 0.181); P = 0.169
HIV RNA level (log10 copies/ml)	-0.003 (-0.025, 0.019); P = 0.79	0.001 (-0.020, 0.023); P = 0.878	0.0257 (0.002, 0.048); P = 0.027	0.028 (0.006, 0.051); P = 0.012
NNRTI-based (NVP or EFV-based) starting ART regimen Ref: "PI-based"	0.003 (-0.109, 0.116); P = 0.946	-0.050 (-0.173, 0.071); P = 0.411	-0.034 (-0.143, 0.073); P = 0.526	-0.067 (-0.185, 0.050); P = 0.259
Late-treated vs. Early-treated	0.11(0.009, 0.223); P = 0.033	0.064 (-0.204, 0.332); P = 0.636	0.073 (-0.027, 0.174); P =0.153	0.152 (0.029, 0.275); P = 0.015
Age at ART initiation (months)	0.0029 (0.0006, 0.0050); P = 0.012	0.002 (-0.001, 0.005); P = 0.200	0.0019 (-0.0004, 0.0043); P = 0.109	0.002 (-0.001, 0.006); P = 0.164
Age at oral health assessment (years)	0.015 (0.001, 0.029); P = 0.032	0.004 (-0.026, 0.036); P = 0.768	0.0136 (-0.0009, 0.0281); P = 0.066	0.033(0.004, 0.061); P = 0.022

*Notes: Adjustments done for Gender and Age at Oral Health Assessment

[1]cWHO stage is a hierarchical system of clinically classifying HIV infection into four stages.

Table 4. i: Correlation between oral inflammation biomarkers and systemic immune activation biomarkers (Activated IL-6 and Intermediate Monocytes)

Correlates	Log_{10} Plasma IL-6		Activated monocytes (Log_{10} IM_Parent)[2]	
	Unadjusted (Coefficient, 95% CI); P-value	Adjusted (Coefficient, 95% CI); P-value	Unadjusted (Coefficient, 95% CI); P-value	Adjusted (Coefficient, 95% CI); P-value
Log_{10} CD14 pg/ml	-0.233 (-0.562, 0.094); P = 0.160	-0.244 (-0.612, 0.122); P = 0.188	-0.204 (-0.565, 0.157); P = 0.261	-0.233 (-0.576, 0.109); 0.176
BOP score[1]	-0.192 (-0.635, 0.250); P = 0.388	-0.160 (-0.607, 0.286); P = 0.476	0.178 (-0.172, 0.530); P = 0.312	0.155 (-0.169, 0.480); 0.340

*Notes: Adjustments done for Gender and Age at Oral Health Assessment

[1]BOP Score = Proportion of bleeding sites upon gentle probing over the total number of sites probed around gums.

[2]IM_Parent = Intermediate monocytes

Table 4. ii: Correlation Between Oral Inflammation Biomarkers and Systemic Immune Activation Biomarkers (Activated Monocytes – Classical and Non-Classical)

Correlates	Activated monocytes (Log_{10} CM)[2]		Activated monocytes (Log_{10} NCM)[3]	
	Crude (Coefficient, 95% CI); P-value	Adjusted (Coefficient, 95% CI); P-value	Crude (Coefficient, 95% CI); P-value	Adjusted (Coefficient, 95% CI); P-value
Log_{10} CD14 pg/ml	-0.030 (-0.088, 0.027); P = 0.298	-0.027 (-0.084, 0.030); P = 0.345	0.138 (-0.197, 0.473); P = 0.411	0.114 (-0.201, 0.431); P = 0.468
BOP score[1]	-0.013 (-0.084, 0.057); P = 0.705	-0.016 (-0.086, 0.052); P = 0.631	0.287 (-0.058, 0.634); P = 0.101	0.251 (-0.056, 0.560) P = 0.107

*Notes: Adjustments done for Gender and Age at Oral Health Assessment

[1]BOP Score = Proportion of bleeding sites upon gentle probing over the total number of sites probed around gums.

[2]CM_Parent = Classical monocytes

[3]NCM = Non-Classical monocytes

Table 4. iii: Correlation Between Oral Inflammation Biomarkers and Systemic Immune Activation Biomarkers (Activated CD4 and CD8 T-cells)

Correlates	Log_{10} CD4_CD38HLADR		Log_{10} CD8_CD38HLADR	
	Crude (Coefficient, 95% CI); P-value	Adjusted (Coefficient, 95% CI); P-value	Crude (Coefficient, 95% CI); P-value	Adjusted (Coefficient, 95% CI); P-value
Log_{10} sCD14 pg/ml	-0.049 (-0.357, 0.258); P = 0.749	-0.044 (-0.361, 0.271); P = 0.778	0.032 (-0.469, 0.533); P = 0.898	0.048 (-0.470, 0.567); P = 0.851
BOP score[1]	0.347 (0.071, 0.624); P = 0.014	0.351 (0.057, 0.644); P = 0.020	0.010 (0.139, 0.987); P = 0.010	0.579 (0.140, 1.020); P= 0.010

*Notes: Adjustments done for Gender and Age at Oral Health Assessment

[1]BOP Score = Proportion of bleeding sites upon gentle probing over the total number of sites probed around gums.

FIGURES

Figure 1: Flowchart of Timeline, Clinical and Laboratory Assessments

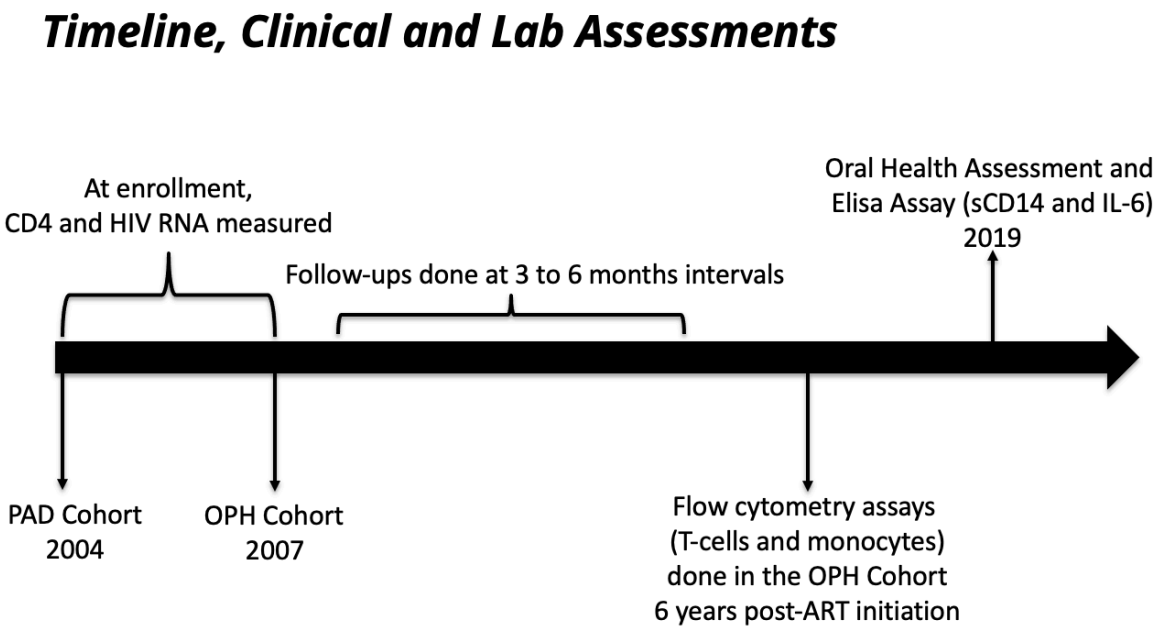

Figure 2: Boxplot of sCD14 level Distribution by Cohort

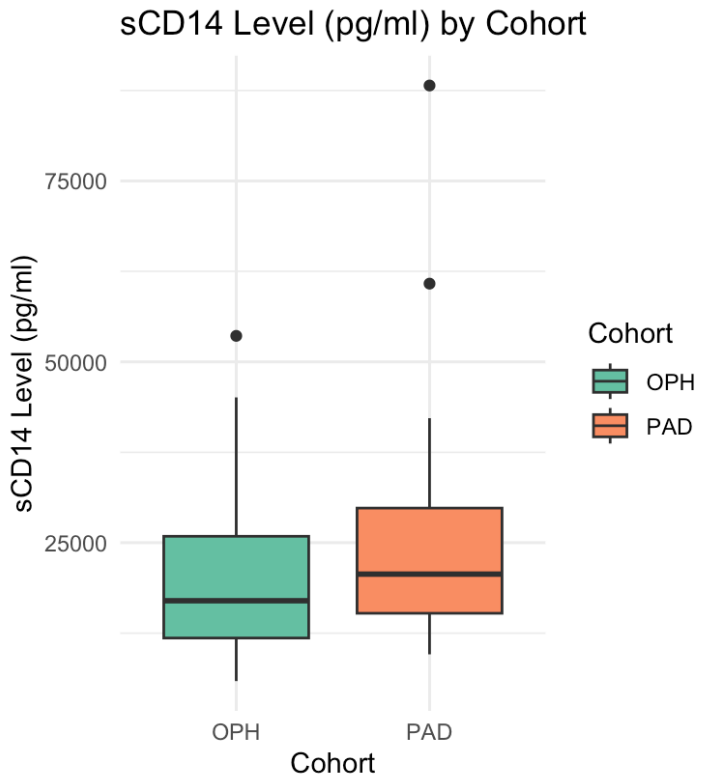

Figure 3: Boxplot of BOP score Distribution by Cohort

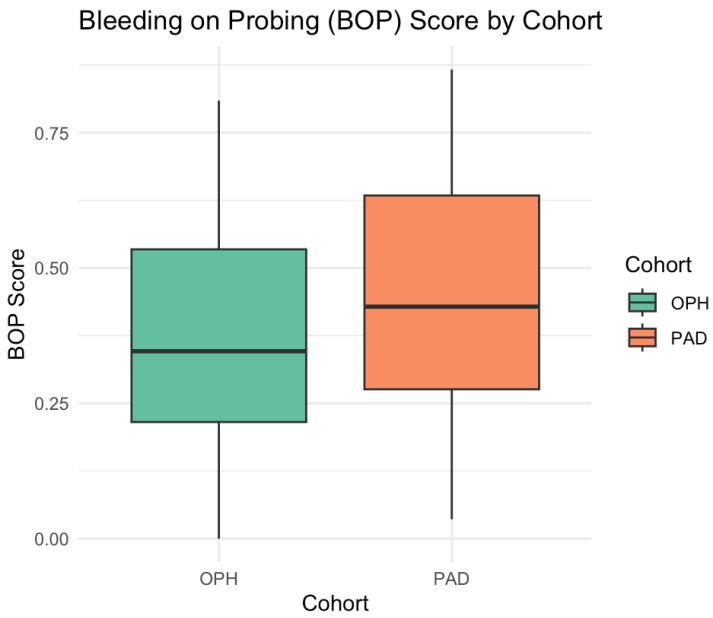

Figure 4: Forest plot of Early HIV Experience Correlates of Oral Inflammation