Abstract 213

CHANGES IN THE ORAL MICROBIOME WITH ART INITIATION

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BACKGROUND

- The oral microbiome may be different in HIV-infected versus uninfected subjects and is likely influenced by ART.
- Significant oral pathology is associated with advanced HIV disease
  - Common opportunistic oral infections
    - Candidiasis
    - Oral hairy leukoplakia
    - Kaposi's sarcoma
    - HSV
  - Additional oral pathologies
    - HIV salivary disease
    - HPV-associated oral lesions
    - Periodontal disease
    - Xerostomia and ulcerations
- Prior studies have demonstrated shifts in the microbiome
  - 16S sequencing demonstrated *H parainfluenzae* associated with HIV+ and *Streptococcus mitis* with HIV-[1]
  - 16S array demonstrated increase in several species after ART[2]
  - ITS based sequencing of the mycobiome identified *Pichia* as an antagonist of opportunistic fungi in HIV+[3]
- The oral microbiome has been primarily studied to understand periodontal disease.
  - Periodontal disease is associated with other pathology more common in HIV-infected individuals [4-6]
    - Cardiovascular disease, metabolic syndrome and diabetes, and osteoporosis
    - Overgrowth of pathogenic bacteria in periodontitis causes endotoxemia with resultant inflammation.
- The oral microbiome appears to be stable over time and to antibiotic perturbation.
  - The human microbiome project demonstrated that saliva is the most stable site in the oral cavity
  - Other studies have demonstrated both short and long term stability of the oral microbiome

To date, studies of oral microbiome in HIV are limited by small sample size, variations in technique, and their mostly cross-sectional nature. Because of this, longitudinal studies of the changes in the microbiome associated with changes in health in an individual (such as initiation of ART) are likely to result in more reproducible results and may establish sequences of events that cross sectional studies comparing HIV-infected individuals on and off ART to unrelated, uninfected controls cannot. For this study, we compared saliva samples from HIV-infected individuals before and after 24 weeks of a single ART therapy. As individuals are their own controls, and a single ART regimen eliminates effects of differing ART class, we sought to decrease variability to identify the effect of 24 weeks of ART on the oral microbiome.

METHODS

Study cohort: Saliva samples were collected from participants co-enrolled in both A5272 and A5280. A5280 was a randomized-placebo controlled study evaluating the effect of Vitamin D versus placebo on bone mineral density loss after the initiation of EVF/FTC/TDF. A5272 was a prospective, observational study of HIV-1 infected ART-naïve adults (≥18 years old) initiating ART in a controlled clinical trial. It was designed to evaluate oral pathology over the course of early ART. Saliva specimens, CD4+ T-cell count and plasma HIV-1 RNA were collected at several time points. Saliva samples (70 samples from 35 participants) were obtained from participants who had paired samples from entry and week 24, complete data sets, had received EVF/FTC/TDF, and were in the placebo arm of A5280. All participants provided written informed consent. The protocol was approved by the institutional review boards of all sites.

Bacterial 16S rRNA Analysis: Total nucleic acid extracted from 200 ul of saliva. Polymerase chain reaction was performed and amplicons were pooled and sequenced on the Illumina MiSeq platform (Washington University Center for Genome Sciences; 2 × 250 standard run). Data were analyzed using Quantitative Insights into Microbial Ecology (QIIME, version 1.9.1).

Statistical Analysis: Descriptive measures were used to summarize the data. Continuous variables were summarized using median and IQR; categorical variables were summarized using frequency and percent (%). Spearman's rank correlations were used to examine bivariate associations between study variables. Fisher's exact and chi-square tests were used to compare categorical variables between the study groups. Mann-Whitney test and Kruskal Wallis test with Dunn's post hoc analyses (p values in figures) were used for comparing continuous variables. No correction for multiple comparisons was performed unless otherwise stated. Statistical analyses and graphing were performed in R and Prism version 6.05 for Windows (GraphPad Software, http://www.graphpad.com). All p values were two-sided and $p < 0.05$ was considered significant.

RESULTS

Table 1: Baseline demographics and HIV characteristics of study participants

Characteristics		Number (%)
Sex	Men	31 (88.6)
	Women	4 (11.4)
Race	White	16 (45.7)
	Black	11 (31.4)
	Hispanic	7 (20)
	Other	1 (2.9)
IVDU	Never	33 (94.3)
	Previously	2 (5.7)
Age at entry		36.2 (19-61)
CD4+ at entry		353.5 (7-804)
CD4+ <200		5 (14.2)
Baseline HIV VL		118,465 (1091-1,614,781)
VL>100,000		9 (25.7)
Baseline BMI		25.66 (17.4-37.45)

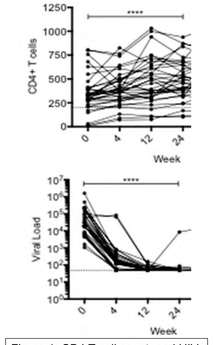


Figure 1: CD4 T cell count and HIV viral load over 24 week study period

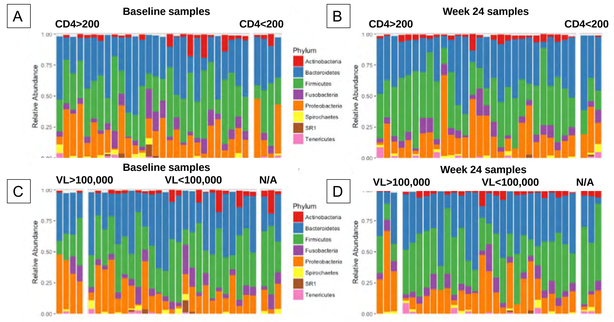


Figure 2: Major taxa (phyla>1%) in each sample. Dominant phyla are shown and demonstrate significant interpersonal variation but overall similar relative abundance in dominant phyla between baseline (A and C) and week 24 (B and D). Samples are broken out for low CD4<200 (A and B) or high viral load >100,000 (C and D.)

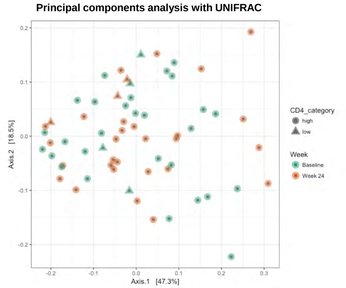


Figure 3: All samples unconstrained ordination analysis (PCoA using the weighted UNIFRAC distance). Colored by time point, CD4 low samples (< 200) are indicated with triangles. Some clustering of low CD4 is seen to the upper left quadrant, but the statistics do not support that the centroids between the two groups (CD4 low vs. CD4 high) are different (permanova p-value 0.128).

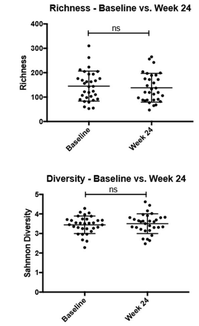
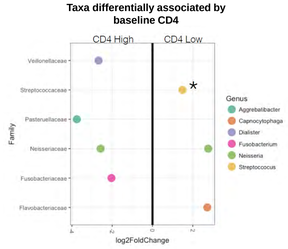


Figure 4: No significant change in richness (number of species) or diversity (relative abundance of species) is seen between baseline and week 24 samples.



* Differential Abundance in baseline and Week 24 samples with high Viral Load (VL category)

Figure 5: Differentially abundant taxa seen between baseline samples with CD4 counts > or <200 cells/ml. A Streptococcus spp is seen which is differentially abundant between low and high viral loads at both baseline and week 24. No taxa are differentially expressed between all samples at baseline or week 24 or between CD4 low and high samples at week 24.

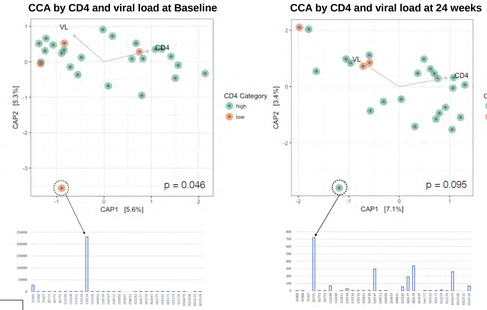


Figure 6: Constrained correspondence analysis (CCA)[7] by CD4 and viral load (actual quantities, not categories). Groups of patients can be distinguished at baseline based on their oral microbiome (p = 0.046). The arrows indicate the variable driving the grouping. For example, the upper left quadrant is high VL and low CD4, upper right is low VL high CD4. This grouping becomes non-significant by week 24.

CONCLUSIONS

Results: Study participants were predominantly men (88.6%); 45.7% were white, 31.4% were African American, and 20% were Hispanic, with a mean age at entry of 36.2. The median CD4 count was 326 (7-804), although 5 participants had entry CD4 counts below 200. The median viral load was 30,136, with 25.7% of participants with baseline viral load >100,000. All but one participant was suppressed by 24 wks of ART. No significant changes were seen in alpha or beta diversity in the oral microbiome between baseline and wk 24 of ART. The dominant taxa seen in saliva were the same at 0 and wk 24. However, participants with baseline CD4<200 and high viral load >100,000 exhibited differences in dominant taxa, demonstrating more *Capnocytophaga*, *Streptococcus sanguis*, and *Neisseria mucosa* in low CD4 samples and more *Neisseria flava*, *Aggregatibacter segnis*, *Fusobacterium nucleatum*, and *Dialister pneumosintes* in samples from participants with CD4>200 at baseline. The clinical importance of this shift is unclear, although *Capnocytophaga* has been associated with periodontal disease, which has been seen in HIV+ participants with advanced immune deficiency. These shifts are partly reversed by 24 wks of ART, and no distinguishing taxa are identified between samples with baseline high or low CD4. Constrained correspondence analysis demonstrates that there are statistically significant differences in the microbiota of samples with low CD4 and high viral load.
Conclusions: We demonstrated no overall significant shift in the salivary microbiome after 24 wks of ART. However, advanced HIV disease is associated with differences in the microbiome, which may be contributing to inflammation and periodontal disease in this population.

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