Characterizing the Subgingival Microbiome of Pregnant African American Women

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ABSTRACT

Objective: To generate preliminary data about the subgingival microbiome of pregnant African American women to calculate power for a future larger study and to explore associations among the microbiome, periodontal inflammation, and preterm birth.

Design: Comparative descriptive pilot study design.

Setting: Urban area in the southeastern United States.

Participants: Thirty-four African American women in the third trimester of pregnancy.

Methods: Based on visual assessment, participants were placed in two groups: healthy gingiva and gingivitis. Saliva samples were analyzed for interleukin-1β (IL-1β), matrix metalloproteinase–8 (MMP-8), and C-reactive protein (CRP). DNA was extracted from subgingival plaque samples, and amplicons of the fourth hypervariable region were sequenced.

Results: We found no differences in overall microbiome diversity between the healthy gingiva ($n = 22$) and the gingivitis ($n = 12$) groups although significant differences were found among the bacterial taxa present. The gingivitis group had greater levels of salivary IL-1β and MMP-8, whereas CRP was not different between groups. Overall microbiome diversity was positively associated with the CRP level. We found no significant relationships among the subgingival microbiome, periodontal inflammation, and preterm birth.

Conclusion: Gingivitis in pregnancy did not appear to shift the overall composition or diversity of the subgingival microbiome although differences in several bacterial taxa suggest that inflamed gingiva in pregnant women are associated with a disruption in the stability of the subgingival microbiome. A correlation between the abundance of bacteria and CRP also suggests an association between the microbiome and systemic inflammation. These findings provide support for future research about how the oral microbiome and progression of periodontal disease in pregnant women link with adverse pregnancy outcomes.

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Oral health is integral to overall good health and well-being (Petersen, 2009). Pregnant women are particularly susceptible to alterations in oral health because of a variety of factors, including hormonal shifts that contribute to inflammation and decreased immunocompetence (Steinberg, Hilton, Iida, & Samelson, 2013); changes in dietary habits, such as frequent snacking or increased consumption of carbohydrate-rich and decay-promoting foods; stomach acids from nausea and vomiting that contribute to the breakdown of tooth enamel; and decreased likelihood of seeking dental care during pregnancy (Detman, Cottrell, & Denis-Luque, 2010). Furthermore, the assessment and promotion of oral health are often overlooked by prenatal health care providers (George et al., 2012).

In the United States, it is estimated that almost half of all adults older than age 30 years have periodontitis (Eke et al., 2015). Gingivitis, the most common oral disease in pregnancy, has a prevalence of 50% to 70% (Anil et al., 2015). Symptoms of gingivitis in pregnancy typically emerge during the first trimester (Giglio, Lanni, Laskin, & Giglio, 2009) and present as red, swollen gingival margins and bleeding that occurs with the slightest provocation (Han, 2011).

Gingivitis, which represents the milder form of periodontal disease, is an inflammatory condition that affects the soft and hard structures that support the teeth; it is a direct, immune-inflammatory response to the microorganisms that inhabit the biofilm (plaque) at the interface between the surface of the tooth and the gingiva (Lang, Schätzle, & Löe, 2009). Underlying this condition is a complex interaction between these microorganisms and the host defense system. In response to microbial pathogens, host-mediated destruction of periodontal tissue is caused by hyperactivity of leukocytes and the generation of cytokines, eicosanoids, and matrix metal-loproteinases (MMPs; AlMoharib et al., 2014). These biomarkers of the inflammatory process can be found in gingival crevicular fluid and whole saliva (AlRowis et al., 2014). For some, progressive gingivitis leads to periodontitis, which is a polymicrobial inflammatory disease that involves the irreversible destruction of supportive soft tissue and bone and ultimately leads to tooth loss (Lang et al., 2009). The incidence of periodontitis in pregnant women ranges between 5% and 20% (Polyzos et al., 2010).

Periodontal Disease and Preterm Birth

In 1996, Offenbacher et al. reported that women with periodontal disease had a seven-fold increase in the risk of preterm birth. Since then, results from several other studies have shown an association between periodontal disease and preterm birth. For example, after controlling for other known risk factors of preterm birth, moderate to strong associations were found between periodontal disease and/or inflammation and spontaneous preterm birth (Guimarães, Silva-Mato, Miranda Cota, Siqueira, & Costa, 2010; Khader et al., 2009; Macedo et al., 2014; Stadelmann et al., 2014). However, the evidence is not clear (Hunter & Yount, 2011). In critical reviews of the literature, authors cautioned that the association should be considered in light of a high degree of variability in study populations, recruitment, and assessment (Agueda, Echeverría, & Manau, 2008; Ide & Papapanou, 2013). Furthermore, the treatment of periodontal disease during pregnancy has yielded mixed outcomes (Jeffcoat et al., 2011; Macones et al., 2010; Polyzos et al., 2010). These inconsistencies suggest a gap in knowledge with regard to the underlying mechanisms of the observed association between periodontal disease and preterm birth.

> **Lack of knowledge about the subgingival microbiome in pregnancy limits the understanding of periodontal inflammation as a potentially modifiable risk factor for preterm birth.**

Within-Race Study of the Microbiome

African American women are at increased risk for preterm birth and periodontal disease compared with non-Hispanic white women (Martin, Hamilton, Osterman, Driscoll, & Drake, 2018). Socioeconomic disparities related to prenatal oral hygiene practices and dental service use during pregnancy also exist (Boggess et al., 2010; Eke, Dye, Wei, Thornton-Evans, & Genco, 2012) and are major public health concerns. Several structural and behavioral factors may be at work, including limited health care resources (Lee, Milgrom, Huebner, & Conrad, 2010), lack of understanding about the importance of prenatal preventive oral health practices (Marchi, Fisher-Owens, Weintraub, Yu, & Braveman, 2010), and poor adherence to recommendations from dentists and maternal health care providers regarding oral health care (Lee et al., 2010; George et al., 2012). To investigate the high rate of preterm birth in African American women and prevent confounding that may result from comparisons across racial groups, studies that are focused on risk within a racial subgroup are needed.

Link Between Periodontal Disease and Preterm Birth

One theory regarding the underlying link between periodontal disease and preterm birth relates to an immune-inflammatory mechanism. Healthy pregnancy appears to have unique inflammatory phases that correspond with implantation of the conceptus and fetal development (Mor, Aldo, & Alvero, 2017). These inflammatory phases can be disrupted by external stimuli such as infections (Mor et al., 2017) that place the fetus and the pregnancy at risk (Han, 2011). Although vaginal infections that occur during pregnancy are thought to be the most common source of systemic inflammation (Romero, Dey, & Fisher, 2014), periodontal infections that initiate systemic inflammation or infectious agents that actually translocate through the bloodstream to the uterus may likewise increase risk during pregnancy (Madianos, Bobetsis, & Offenbacher, 2013). During the period between successful implantation and the onset of labor, the woman, placenta, and fetus are symbiotic, and the predominant environment is

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shifted toward an anti-inflammatory state (Mor et al., 2017) mediated by anti-inflammatory cytokines such as interleukin (IL)-4, IL-5, IL-10, and IL-13 and granulocyte-macrophage stimulating factor. This shift is thought to be critical to prevent the rejection of the fetal allograft (Challis et al., 2009). Dysregulation of this anti-inflammatory state would be represented by a shift toward the proinflammatory cytokines represented by IL-1, IL-2, IL-6, IL-12, IL-15, IL-18, interferon-γ, and tumor necrosis factor–α (Challis et al., 2009). An elevation in proinflammatory cytokines caused by periodontal disease could then potentially play an important role in this dysregulation of the normal immunologic state during pregnancy (Han, 2011). Supporting this theory are findings from studies that show that pregnant women with periodontal disease have elevated levels of plasma C-reactive protein (CRP), which indicates elevated systemic inflammation (Sharma, Ramesh, & Thomas, 2009). Evidence also suggests that periodontal disease is associated with an increased level of proinflammatory cytokines in the peripheral circulation (Andrukhov et al., 2011).

The Subgingival Microbiome

Despite the suspected connection between periodontal disease and preterm birth, current knowledge about the subgingival bacterial environment most closely related to periodontal disease in pregnant women is incomplete. As a result, our ability to understand its relationship to inflammation and to assess or treat early signs of periodontal infection/inflammation as a potentially modifiable risk factor for preterm birth is limited. Several pathogens are thought to play roles in periodontal infection and inflammation in nonpregnant individuals, including *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia* (Lamont, Koo, & Hajishengallis, 2018), and *Prevotella intermedia* (Lima et al., 2015). Evidence implicates these organisms in periodontal disease in pregnancy but is inconsistent (Adriaens, Alessandri, Spörri, Lang, & Persson, 2009; Carrillo-de-Albornoz, Figuero, Herrera, & Bascones-Martínez, 2010; Novak et al., 2008; Vettore, Leao, Leal, Feres, & Sheiham, 2008). This may be because older methodologies in which only specific periopathogens were targeted were not powerful enough to identify the hundreds of other species of bacteria in the oral cavity (Dewhirst et al., 2010).

More recent research findings about the oral microbiome based on next-generation sequencing in nonpregnant populations suggest that our understanding of infection/inflammation must move from a focus on individual pathogens to the ecological context of a particular microbial community (Abusleme et al., 2013; Duran-Pinedo et al., 2014; Griffen et al., 2012). Also, a state of periodontal inflammation may represent a shift in the overall ecological balance of microbial flora rather than the appearance of individual pathogens. Given this, it is critical to investigate the subgingival microbiome of pregnant women to identify associations among the microbiome, inflammation, and preterm birth. This is particularly the case for African American women who are at increased risk for periodontal disease and preterm birth (Eke et al., 2015; Martin et al., 2018). Therefore, the primary objective of this pilot study was to generate preliminary data about the characterization of the subgingival microbiome of pregnant African American women that could be used to calculate power for a larger study. The secondary objective was to explore associations among the maternal subgingival microbiome, inflammatory indicators of periodontal disease, and preterm birth.

Methods

Design

We used a comparative descriptive design for this pilot study and obtained approval from the institutional review board at Emory University before the commencement of study activities.

Setting and Sample

We enrolled 34 pregnant African American women from two prenatal clinics located in Atlanta, GA, between December 2016 and February 2017. Participants were recruited from an ongoing larger investigation of the associations among a woman's oral, vaginal, and gut microbiomes during pregnancy and preterm birth (Corwin et al., 2017). Inclusion criteria for the larger parent study included self-identification as African American, 18 to 40 years of age, ability to speak and read English, singleton gestation, no chronic medical problems, and no use of prescription medications. The recruitment of women to participate in this pilot study occurred during the third trimester of pregnancy when participants in the parent study were asked if they would also be willing to allow inspection of their gingival tissue and collection of saliva and subgingival microbiome samples. To be included, women had to have a minimum of 20 natural teeth and no professional dental cleaning in the past 3 months.

Results from two recent studies conducted in nonpregnant populations (Abusleme et al., 2013; Griffen et al., 2012) indicated that the effect sizes for differences in the Shannon and Chao1 indices (used to measure the richness and evenness of microbial communities) range from large to very large (.82–1.4). Power analysis estimates from G*Power software version 3.1 (Faul, Erdfelder, Buchner, & Lang, 2009) indicated that 40 total participants (20 per group) were required to detect large effect sizes with 80% power. Given the 1-year time constraint of the funding mechanism and the pilot nature of this study, we determined that a study with 30 total participants (15 per group) would produce sufficiently large effect sizes with 70% power for foundational data to support the design of future adequately powered subgingival microbiome studies.

Procedures

Three teeth (#25, 28, and 30) from the right lower quadrant of each participant's mouth were assessed for visual signs of gingival inflammation using the Modified Gingival Index (MGI; Lobene, Weatherford, Ross, Lamm, & Menaker, 1985) by a single trained examiner. The MGI scores for the three teeth were averaged, and participants with mean MGI scores less than or equal to 1 were placed in the healthy group; those with scores greater than 1 were assigned to the gingivitis group. Saliva and subgingival plaque samples were then collected from participants in both groups and were visually assessed using the sterile paper point method (Zhou & Li, 2015). Supragingival plaque was first removed with sterile gauze and each tooth site held dry using cotton rolls while one sterile paper point was inserted into the pocket of each tooth for 20 seconds. The three paper points were pooled and immediately placed in 750 µl MoBio buffer contained in sterile MoBio bead tubes (MoBio Laboratories, Inc, Carlsbad, CA). Saliva and plaque samples were placed on ice and transported for storage at −80°C until ready for analysis.

DNA isolation and 16S ribosomal RNA gene library preparation and sequencing. Specimens were sent to Microbiome Insights (Vancouver, BC, Canada), leaders in the field of microbiome research, for extraction and sequencing. Pooled paper points were homogenized, and DNA was isolated using the MoBio PowerMag Soil DNA Isolation Kit. The highly conserved 16S ribosomal RNA (rRNA) gene, which is widely used to characterize taxonomic diversity in microbial communities, was polymerase chain reaction

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Diseased gingiva appear to result from perturbations within the ecology of normally existing oral bacterial taxa.

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amplified with dual-barcoded primers targeting the fourth hypervariable region according to the protocol outlined by Kozich, Westcott, Baxter, Highlander, and Schloss (2013). Normalized library concentrations of 1 to 2 ng/µl (as per the specifications of the Sequal Prep normalization kit; Thermo Fisher Scientific, Waltham, MA) were used. Amplicons were sequenced with an Illumina MiSeq using the 250–base pair paired-end kit (version 2; Illumina, Inc., San Diego, CA). Sequences were denoised, taxonomically classified using a built-in Ribosomal Database Project Classifier with the Greengenes (version 13_8) as the reference database, and clustered into 97% similarity operational taxonomic units (OTUs) with the Mothur software package version 1.39.5 (Schloss et al., 2009) following the recommended procedure. The default Mothur Needleman alignment was used to join reads. Ambiguous base pairs in joined reads were filtered out, and a maximum length of 275 base pairs was specified.

The potential for contamination was addressed by cosequencing DNA amplified from specimens and from four each of template-free controls and extraction kit reagents processed the same way as the specimens. Two positive controls consisting of cloned SUP05 DNA were also included (number of copies = 2 * 10^6). OTUs were considered putative contaminants and were removed if their mean abundance in controls reached or exceeded 25% of their mean abundance in specimens. An average of 12,382 quality-filtered reads were generated per sample. The resulting data set had 4,248 unique OTUs.

Salivary immune marker assays. Saliva samples were used for immunoassay analysis to determine the concentrations of IL-1β, MMP-8, and CRP. Enzyme-linked immunosorbent assays for IL-1β, CRP (Salimetrics, Carlsbad, CA), and MMP-8 (Fisher Scientific, Pittsburgh, PA) and appropriate, compatible software programs were used to generate the raw data reports. All study samples were assayed in accordance with the manufacturers' guidelines.

Measures

The MGI is based on visual inspection, eliminates the need for probing or pressure, and therefore does not disrupt the subgingival plaque (Lobene

et al., 1985). The scale of the index makes early detection and subtle changes in gingival inflammation detectable (Lobene et al., 1985). This instrument has shown interrater reliability for early visual changes in gingivitis, with an average interrater correlation coefficient of .81 (Lobene et al., 1985). The MGI also has a positive correlation (r = .88, p = .0094) with dental plaque (Sosa-Jurado et al., 2014) and satisfies the criteria for a gingival index specified in the guidelines from the Council on Scientific Affairs (Council on Dental Therapeutics, 1986) and has been generally accepted for use in clinical trials (Ata-Ali et al., 2015; He, Qu, Chang, & Wang, 2018; Kinane et al., 2015).

Clinical and Demographic Data

Additional data were obtained from the parent study data, including self-reported clinical variables (smoking status, illicit drug use, oral infection, and oral self-care by self-report), self-reported demographic variables (age, income, and education) verified by clinical record review, and gestational age at birth by clinical record review. Gestational age at birth was based on the last menstrual period and/or ultrasonography before 14 weeks of gestation in accordance with standard clinical criteria, and preterm birth defined as a birth that occurred before 37 weeks of gestation (American College of Obstetricians and Gynecologists, 2014).

Analysis

Clinical and demographic data were compared between groups with the use of independent t tests and chi-square tests as appropriate. Inflammatory markers were non-normally distributed as determined by the Shapiro-Wilk test; thus, the logarithmic function was used to transform the observations, after which independent t tests were used to compare the two groups.

OTUs, which are clusters of organisms grouped by DNA similarity, were aggregated into each taxonomic rank (phylum, class, order, family, genus, and species), and the most abundant were plotted according to relative abundance. The generation of diversity scores that could be used to calculate power for a future larger study was a primary objective of this pilot study. Alpha diversity, a measure of species diversity within a particular ecosystem, was estimated with Chao1 and Shannon indices using the Vegan R package (Oksanen, 2015) on raw OTU abundance tables after filtering out contaminants. The Chao1 index is used to describe richness, or the total number of species

Table 1: Sociodemographic and Oral Health Characteristics of Participants (N = 34)

Characteristic	Frequency, n (%)
Education	
< High school completion	4 (11.8)
≥ High school completion	27 (79.4)
Missing	3 (8.8)
Income	
< 100% federal poverty level	17 (50)
≥ 100% federal poverty level	14 (41.2)
Missing	3 (8.8)
History of mouth/gum infection	
Yes	5 (14.7)
No	26 (76.5)
Missing	3 (8.8)
Brushed teeth in the last 2 days	
Yes	27 (79.4)
No	1 (2.9)
Missing	6 (17.6)
Flossed in the last month	
Yes	11 (32.4)
No	20 (58.8)
Missing	3 (8.8)
Visited the dentist in the last month	
Yes	3 (8.8)
No	28 (82.4)
Missing	3 (8.8)
Smoked cigarettes in the last month	
Yes	2 (5.8)
No	32 (24.1)
Missing	1 (2.9)
Other drug use	
Yes	1 (2.9)
No	33 (97.1)

present in a community (i.e., the greater the Chao1 score, the greater the number of species present [Lemos, Fulthorpe, Triplett, & Roesch, 2011]). The Shannon index is used to describe richness with evenness (Magurran, 2013). Communities numerically dominated by one or a few species exhibit a low Shannon score, whereas communities in which abundance is distributed equally among species will exhibit high evenness. Chao1
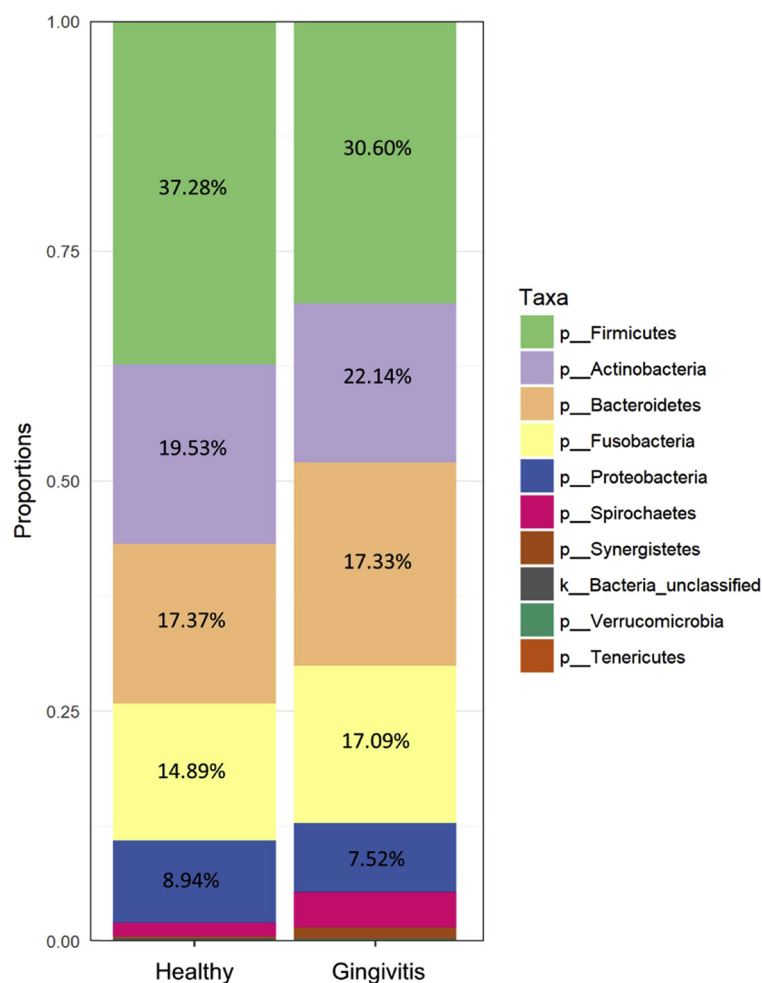
Figure 1. Taxonomic composition of the most abundant phyla in the healthy and gingivitis groups.

calculations were performed with 1,000 permutations at a rarefaction depth of 3,000 reads. The significance of diversity differences was tested with analysis of variance (ANOVA). To estimate beta diversity across samples, OTUs that occurred in fewer than 10% of the samples with a count of less than 3 were excluded, and then Bray-Curtis indices were computed.

We visualized beta diversity, which describes differences across samples, using nonmetric multidimensional ordination. Variation in community structure was assessed with permutational multivariate ANOVA with the gingivitis group as the main fixed factor and using 4,999 permutations for significance testing. We used general linear models using the DESeq2 package (Love, Huber, & Anders, 2014) to determine the statistical significance of differences in the abundance of individual OTUs between groups with p adjustment

using the Benjamini–Hochberg procedure (Love et al., 2014). We investigated associations among the alpha diversity scores, transformed levels of biochemical indicators of periodontal disease (IL-1β, MMP-8, and CRP), and gestational age at birth (in weeks) using Pearson product–moment correlation coefficients.

Results

The mean age of participants was 26.03 ± 5.12 years. Other sociodemographic and oral health characteristics of the participants can be found in Table 1. Of the 34 participants enrolled, 22 had healthy gingiva, and 12 had visual signs of gingivitis as indicated by mean MGI scores greater than 1. We found no significant differences between groups in age, income level, education level, smoking, other drug use, reported mouth/gum infection, or oral self-care habits.

OTUs Higher in Healthy Groups

OTUs Higher in Gingivitis Groups
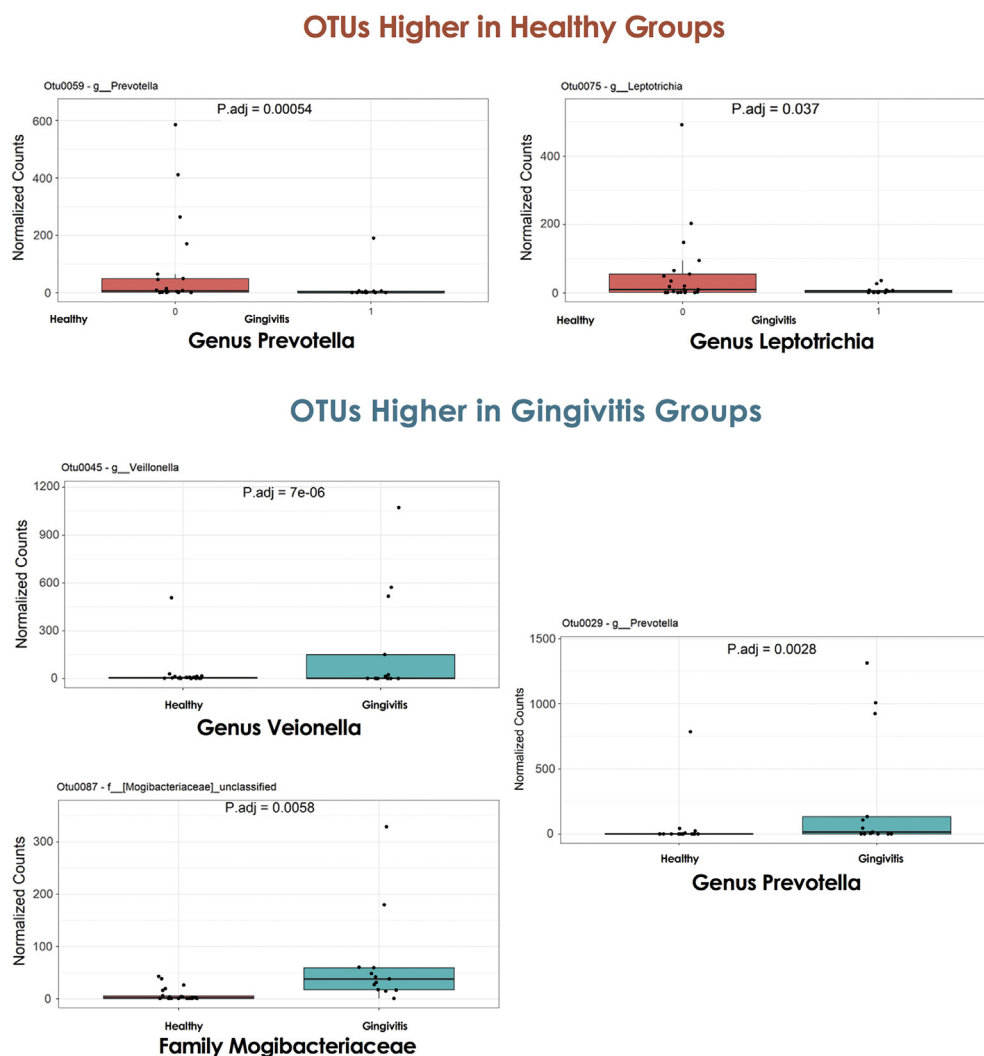
Figure 2. Individual operational taxonomic unit (OTU) differences between healthy and gingivitis groups.

Characterization of the Oral Microbiome in Periodontal Health and Disease

As illustrated in Figure 1, comparison of the two groups at the level of the phylum revealed a similar overall microbiome composition between groups. However, significant differences existed in several OTUs that were classified at the genus and family levels. OTUs that belonged to the genera *Prevotella* and *Leptotrichia* were more prevalent in healthy participants, whereas OTUs that belonged to the family *Mogibacteriaceae* and genera *Veionella* and *Prevotella* were more prevalent in participants in the gingivitis group ($p < .01$; see Figure 2).

Beyond taxonomic description of the subgingival microbiome, our primary objective was to generate diversity scores that would be used to calculate power for a future larger study. We found no significant differences in the measures of alpha diversity between groups in the Chao1 or Shannon index, meaning that the two groups were similar in terms of the abundance and evenness of OTUs within each sample ($p > .05$; see Figure 3). Similarly, a visualization of beta diversity using nonmetric multidimensional ordination and permutational multivariate ANOVA using distance matrices confirmed that the groups were similar ($Pr[>F] = 0.0977$; see Figure 4).

Associations Among Oral Microbiome, Biochemical Indicators of Periodontal Disease, and Preterm Birth

Our results indicated that participants in the gingivitis group had significantly greater levels of
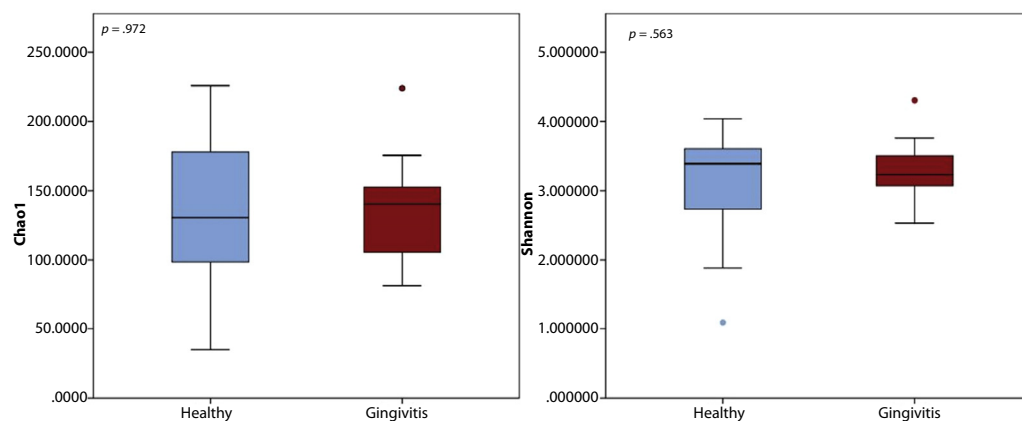
Figure 3. No significant difference in Chao1 and Shannon alpha diversity between groups indicates the two groups were similar in terms of the abundance and evenness of operational taxonomic units within each sample.

salivary IL-1β and MMP-8; salivary CRP levels did not significantly differ (see Figure 5). We found moderate to strong positive correlations between all three salivary inflammatory markers (IL-1β, MMP-8, and CRP; see Table 2). A weak positive association was found between alpha diversity (Chao1) and CRP ($r = .374$, $p = .029$; see

Figure 6). We found no relationship between gestational age at birth and any of the inflammatory markers or alpha diversity.

Discussion

Despite the prevalence of gingivitis in pregnancy and its observed associations with preterm birth
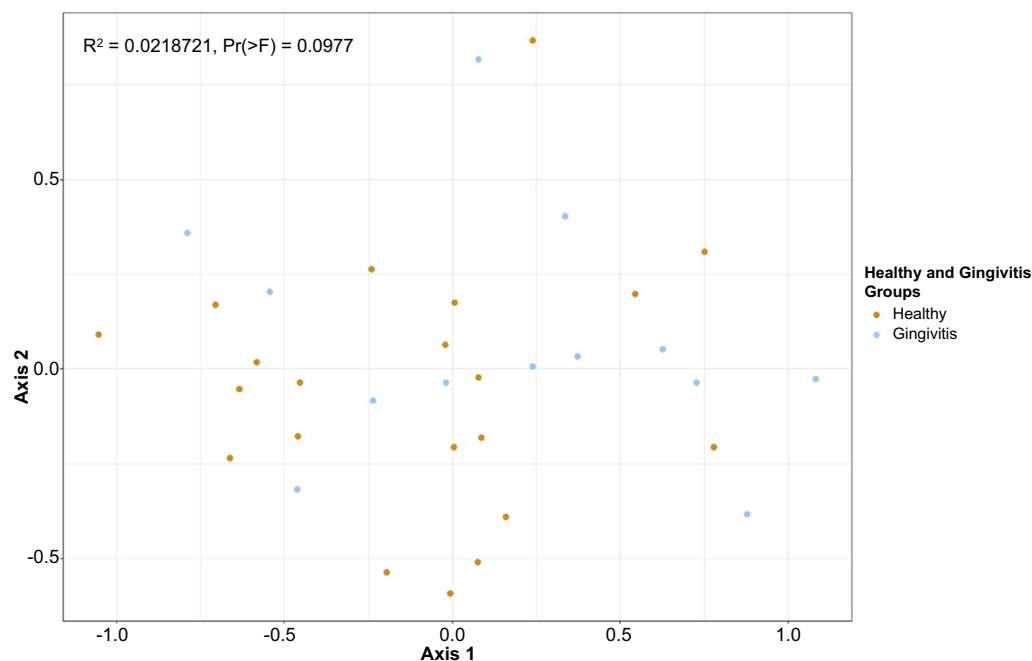
Figure 4. This nonmetric multidimensional ordination plot reflects the beta diversity between healthy and gingivitis groups. Each point represents one microbiome sample. The orange dots reflect healthy group samples, and the blue dots represent gingivitis group samples. Points that cluster together reflect similarity. The lack of clustering indicates that the healthy and gingivitis communities are not very different from one another. We confirmed this statistically with the R^2 value, which showed that the percentage of variation between groups was not significant.

> **These results provide support for further research about the microbiome as an explanatory mechanism that links periodontal disease and adverse pregnancy outcomes.**

and other adverse conditions, little is known about the subgingival bacterial environment of pregnant women. To develop nursing assessment and interventions that can mitigate this risk, it is critical to understand the microbiome of pregnancy gingivitis and its relationship with local inflammation and adverse birth outcomes. Therefore, the aim of this study was to characterize the subgingival microbiome of a small group of pregnant African American women to generate preliminary data that would help us calculate power for a larger study. Additionally, we aimed to explore associations between the maternal subgingival microbiome, inflammatory indicators of periodontal disease, and preterm birth.

The top six phyla represented in the subgingival space of both participant groups were *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Fusobactera*, *Proteobacteria*, and *Spirochaetes*. In a previous study of nonpregnant populations, researchers used 16S rRNA sequencing and reported that up to 96% of the bacteria in our mouths belong to one of these six phyla (Dewhirst et al., 2010). Broadly speaking, pregnancy or gingivitis during pregnancy does not appear to shift the taxonomic composition of the subgingival space.

Despite this overall similarity, differences in individual OTUs existed between groups (see Figure 2). The five OTUs that differentiated participants with healthy gingiva from those with signs of gingivitis were all characterized as taxa that are commonly found in the oral cavity and contain species that are known periodontal pathogens or

opportunistic pathogens. For example, the genus *Prevotella* is the largest genus in the phylum *Bacteroidetes* (Dewhirst et al., 2010) and contains species such as *Prevotella intermedia* that are known periodontal pathogens (Cobb et al., 2017). *Leptotrichia* and *Veionella* contain species that are typically found in the oral cavity and are known to act as opportunistic pathogens (Eribe & Olsen, 2017) or to stimulate the growth of other pathogenic organisms (Eribe & Olsen, 2017; Knapp et al., 2017). Although our ability to draw species-level conclusions was limited by our choice to sequence the fourth hypervariable region of the 16S rRNA gene, our results confirmed findings from contemporary microbiome studies that indicated that diseased gingiva in pregnant women appeared to result from perturbations within the ecology of normally existing oral flora rather than singular pathogens (Costalonga & Herzberg, 2014).

These perturbations can lead to shifts in the diversity of microbial communities that affect the balance of commensal organisms to pathogenic organisms and are associated with periodontal disease in nonpregnant populations (Cobb et al., 2017). Microbial community diversity can be described in two ways. Alpha diversity describes how many OTUs are present in a community and how evenly they are distributed; beta diversity describes the diversity between two different environments. Chao1 and Shannon are two common indices used to describe diversity within a bacterial community, as previously described. Based on findings in nonpregnant populations, alpha diversity should be greater in individuals with periodontal disease (Abusleme et al., 2013; Griffen et al., 2012) because gingival bleeding creates a nutrient source, and deepened periodontal pockets create anaerobic niches. Both provide ideal conditions for the proliferation of
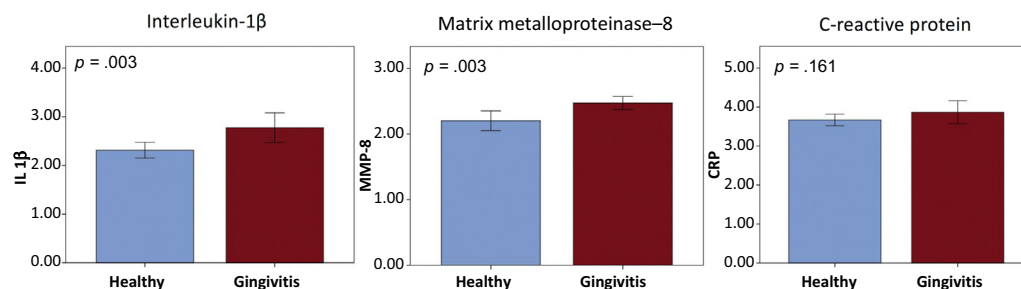
Figure 5. Salivary interleukin-1β (IL-1β) levels and matrix metalloproteinase–8 (MMP-8) levels were significantly greater in the gingivitis group, whereas salivary C-reactive protein (CRP) levels did not significantly differ between groups.

Table 2: Correlations Between Three Inflammatory Markers ($N = 34$)

| Inflammatory Marker	1	2	3
1. Interleukin-1β	—	.743[**]	.603[**]
2. Matrix metalloproteinase–8	.743[**]	—	.409[*]
3. C-reactive protein	.603[**]	.409[*]	—

$*p < .05$. $**p < .01$.

more organisms and a successively more diverse bacterial community. Despite the differences in specific OTUs, we found no significant difference in alpha or beta diversity between the two participant groups, which suggests that these individual OTU differences were not large enough to affect overall community diversity. This is likely attributable to the limited range of disease in this cohort because the gingival status of most participants indicated only mild inflammation.

Another potential consideration is the race of the participants. Recently, researchers have begun to elucidate substantial divergences in the microbiome among individuals from different races and ethnicities (Gupta, Paul, & Dutta, 2017). For example, Mason and colleagues (2013) found that 33 of 77 genera in the oral cavity significantly differed in abundance between non-Hispanic Black, non-Hispanic White, Chinese, and Hispanic individuals in the United States and that it was possible to identify an individual's ethnicity from subgingival microbial signatures. Although the microbiome profile of the African American women in our sample does not clearly distinguish itself as unique, subtle variations in taxonomic abundance may have affected the diversity scores and contributed to the lack of difference between groups.

IL-1β and MMP-8 are known salivary biomarkers of periodontal disease (AlMoharib et al., 2014). Because periodontal disease represents an inflammatory process accompanied by the destruction of connective tissue, circulating molecules such as IL-1β and MMP-8 are integral to these processes and can be detected in the saliva (Sexton et al., 2011). IL-1β was shown to function in concert with other cytokines to regulate the localized inflammatory response in the periodontium (AlRowis et al., 2014). MMP-8 is a leading enzyme in the destruction of tissue in individuals with periodontal disease (AlRowis et al., 2014). As expected, participants in the gingivitis group had significantly greater salivary levels of IL-1β and MMP-8 than participants with healthy gingiva.

In our study, CRP was not different between groups. CRP is produced by the liver and is a systemic marker released during the acute phase of an inflammatory response (AlMoharib et al.,

r = .374, p = .029
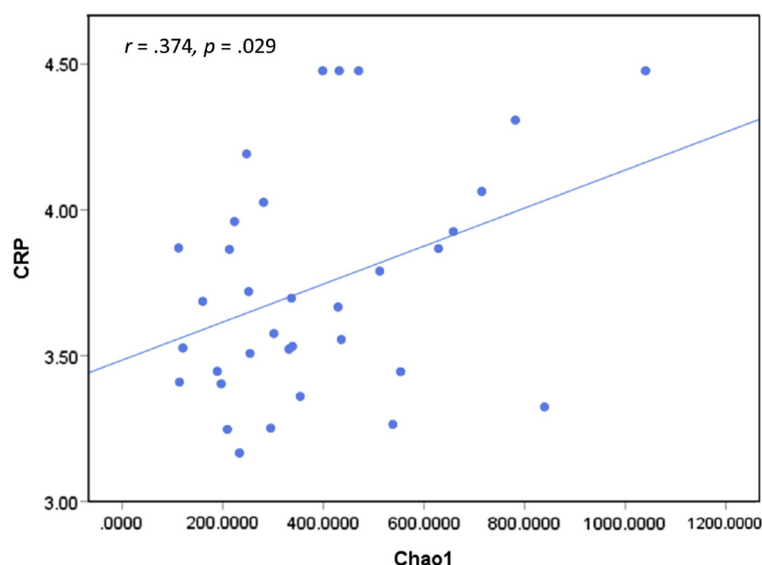
Figure 6. A weak, positive correlation between Chao1 (a measure of alpha diversity) and CRP suggests that increased richness or abundance of microorganisms in the subgingival space triggers an acute-phase response and elevates CRP levels.

2014). CRP reaches the saliva via gingival crevicular fluid or salivary glands (AlMoharib et al., 2014) and is elevated in the saliva of individuals with chronic or progressive periodontal disease (Miller et al., 2010; Shojaee et al., 2013). Plasma CRP has also been shown to be elevated in pregnant women with periodontal disease (Sharma et al., 2009). However, in our study, it is conceivable that gingivitis, a milder form of periodontal disease exhibited by most participants in the gingivitis group, may not have triggered enough of an acute-phase response to elevate systemic levels of CRP (Jain, Gautam, & Naseem, 2011).

Despite this, our analysis provides one clue to suggest an association between the subgingival microbiome and systemic inflammation. The correlation between CRP and Chao1 diversity suggests that increased richness or abundance of microorganisms in the subgingival space triggers an acute-phase response and elevates CRP levels. This important finding suggests an association between the subgingival microbiome in pregnancy and systemic inflammation, which may ultimately affect pregnancy health. Although these results are preliminary, they strongly indicate the need for further investigation of the microbiome as an explanatory mechanism that links periodontal disease in pregnancy with adverse birth outcomes, such as preterm birth.

Our study findings do not have a direct application to nursing practice or to the improvement of maternal–newborn health outcomes; however, the results of our study lay the groundwork for future, more in-depth research investigating the maternal oral microbiome and its relationship to maternal–newborn health. Future microbiome research studies are important for researchers who seek to understand the biobehavioral underpinnings of maternal–newborn health and for clinicians who directly assess, educate, and care for pregnant women. A deeper understanding of the function and diversity of microbes in the mouth will inform both preconception and prenatal education regarding the importance of oral hygiene behaviors and oral health care. Future research may also be instrumental in the development of new tools and diagnostics that shape prenatal oral health assessment, education, and intervention guidelines to promote a healthy mouth and good birth outcomes.

Limitations

Limitations of this study include its cross-sectional design and limited range of periodontal disease

severity; longitudinal investigation of the oral microbiome in pregnancy with a larger cohort with a more diverse range of disease is needed. Furthermore, bias may have been introduced because the investigator performed the visual assessment, the group assignment, and the collection of specimens. Despite these limitations, the results of this pilot study provide new information about the subgingival microbiome of pregnant women with healthy and diseased gingiva.

Conclusion

Because of the effects of hormonal changes in pregnant women, the likelihood of a hyperreactive inflammatory response in pregnancy is heightened (Steinberg et al., 2013). Gingivitis in pregnancy is an early form of periodontal disease that has been associated with preterm birth. Our findings provide an initial description of the microbial environment of the subgingival space of African American women during pregnancy and the relationships among the subgingival microbiome, inflammatory markers of periodontal disease, and gestational age at birth. Information gained from this study provides preliminary support for future research to investigate the progression of periodontal disease in pregnant women and helps explain how this disease might be linked with adverse pregnancy outcomes.

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