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Intensive Non-surgical Periodontal Treatment Positively Affects the Microbiome of Chronic Kidney Disease Patients

by

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Oral and Craniofacial Sciences

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

ACKNOWLEDGEMENTS:

I would like to acknowledge the support and encouragement of my Master's Thesis Committee, Drs. Ryder, Xenoudi, and Kiurtsidis throughout the three year process of completing this project. I would also like to thank my co-resident, Dr. Iman Mohammadi, DMD, for being my partner in collecting and analyzing data for this project. Thank you to my other co-residents and Dr. Kapila for their advice and suggestions regarding the presentation of the results of this project. Last but not least, a big thank you to our research coordinator, Faviola Garcia, for her help in scheduling and managing patients and the data for this project.

Intensive Non-surgical Periodontal Treatment Positively Affects the Microbiome of Chronic Kidney Disease Patients

Stacey Anne Lee, DDS

ABSTRACT

Chronic kidney disease (CKD) is characterized by a progressive decline in kidney function. Evidence demonstrating an association between CKD and periodontitis has been increasing. The aim of this study is to investigate the changes in the microbial profile of CKD patients after non-surgical treatment combined with locally delivered minocycline. 42 patients were divided into a periodontal intensive treatment group and a rescue treatment group. In the periodontal intervention group, patients were treated with scaling and root planing with locally delivered minocycline at sites with pocket depths of 5 mm or more. The control group was treated with scaling and root planing with minocycline administration at the conclusion of the study. Subgingival plaque samples were analyzed at the species-level and the percent frequencies of target taxa found in each sample was further analyzed statistically. When divided into the red complex, orange complex, and newly identified pathogens group, the intensive treatment group showed statistically significant decreases in the levels of all three of these groups. In the control group, the orange complex showed a slight, but non-significant decrease in levels while the red complex and group of newly identified pathogens showed a non-significant increase. At the species level, the intensive treatment group showed statistically significant reductions in a greater number of species than the control group. An intensive periodontal treatment consisting of locally delivered minocycline combined with SRP has a significant benefit in decreasing the oral microbial load of this cohort of dentally underserved CKD patients.

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INTRODUCTION

Chronic kidney disease (CKD) has become an increasing global health issue with significant consequences. Death from CKD rose by 31.7% (1.2 million deaths) worldwide from 2005 to 2015 (1). Chronic kidney disease (CKD) refers to a progressive loss of kidney function over time that may lead to kidney failure if left untreated. Kidney function is commonly assessed by calculating a patient's glomerular filtration rate (GFR) from measurements of serum creatinine. GFR refers to the rate at which a kidney filters waste. According to the National Kidney Foundation's Kidney Disease and Outcomes Quality Initiative, there are five stages of CKD based on the patient's calculated GFR: stage 1 (GFR $\geq$ 90), stage 2 (GFR 89-60), stage 3 (GFR 59-30), stage 4 (GFR 29-15), and stage 5 (GFR < 15). Complications of CKD include cardiovascular disease and mortality, anemia, hyperlipidemia, and metabolic bone disease (2). Worldwide, diabetes and hypertension have been found to be the most common risk factors for the development of CKD (3). Development of CKD due to diabetes caused a significant 39.5% increase in deaths between 2005-2015 (1). Evidence has shown that the association among diabetes, hypertension, and CKD is linked to systemic inflammation and an increase in inflammatory biomarkers such as C-reactive protein and interleukin-6.

Associations between systemic conditions and periodontal disease have been widely explored in the literature. Some of these relationships have been more extensively researched than others and there are still many other associations that are becoming more thoroughly investigated. Recent evidence to support a connection between chronic kidney disease and periodontal disease has also been increasing. Both conditions have similar risk factors, including cardiovascular disease and diabetes. The common factor among these conditions is chronic inflammation and elevated levels of C-reactive protein, a marker for inflammation in blood (4,5).

Multiple studies have explored the association between CKD and periodontitis. Data from NHANES III (1988-1994) has shown that after adjusting for confounders such as, diabetes, smoking, and cardiovascular disease, the 10 year all-cause mortality rate for patients with CKD increased from 32% to 41% if periodontitis is also present (6). Furthermore, adults with periodontal disease and edentulism were twice as likely to have chronic kidney disease than those without periodontal disease after eliminating other risk factors such as age, race/ethnicity, smoking status, and hypertension (4).

For decades, the etiology of periodontitis has been debated. The two theories most contested are the non-specific plaque hypothesis and the specific plaque hypothesis. The non-specific plaque hypothesis proposes that the accumulation of bacteria near the gingival margin of teeth leads to inflammation and consequently, periodontal destruction. As the plaque-bacterial biofilm increased, the production of toxic byproducts from the bacteria overwhelm the host's defenses and cause periodontal destruction (7). However, this hypothesis was questioned because not all cases of gingivitis progressed to periodontitis and sampling of periodontitis sites revealed specific groups of bacteria. On the other hand, the specific plaque hypothesis proposed that the bacterial composition of plaque varies in pathogenic potential. Specific bacteria were identified that were most associated with an increase in pocket depth. In 1998, Socransky et al. identified specific clusters of bacteria. The "red complex" bacteria—*Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*—increased in number and prevalence with increasing pocket depth and number of sites with bleeding on probing. Those of other complexes are also related to pocket depth, but the relationship to other clinical parameters are not as strong (8). Subgingival plaque samples in patients with periodontitis were found to harbor a higher

proportion of "red" and "orange complex" species, but supragingival plaque harbored more "green" and "purple complex" species (9).

A change in plaque microbial composition has been found to affect the health of the periodontium and vice versa. Socransky and Haffajee proposed a model in which bacterial species increased in prevalence as the environment shifts from health to disease. Initially, the tissues are colonized by members of the yellow, green, and purple complexes, as well as by the *Actinomyces* species. When gingivitis becomes established, there is an increased prevalence of species of the orange, then red complexes. With increased disease severity, there is a corresponding change in microbial profile, especially an increase in the red complex species and in some of the orange complex species (10). Multiple studies have suggested that there are species other than those in the complexes that are implicated in periodontal disease (11). A recent systematic review identified an additional 17 periodontal disease-associated species, listed in Table 1. These species were found in statistically significantly higher levels and/or prevalence in subjects with periodontal disease than in subjects in periodontal health (12).

Table 1. Newly identified putative periodontal pathogens (Perez-Chaparro, *et al.*, 2014)

Bacterial taxa	
Anaeroglobus geminatus HOT 121	Cultivable
Archaea spp.	Cultivable
Bacteroidales [*G-2*] sp. oral taxon 274	Unnamed
Desulfobulbus sp. oral taxon 041	Phylotype
Eubacterium [*XI*] [*G-5*] *saphenum* HOT 759	Cultivable
Filifactor alocis HOT 539	Cultivable
Fretibacterium fastidiosum HOT 363	Cultivable
Fretibacterium sp. oral taxon 360	Phylotype
Fretibacterium sp. oral taxon 362	Phylotype
Mogibacterium timidum HOT 042	Cultivable
Peptostreptococcus stomatis HOT 112	Cultivable
Porphyromonas endodontalis HOT 273	Cultivable
Selenomonas sputigena HOT 151	Cultivable
TM7 [*G-5*] sp. oral taxon 356	Phylotype
Treponema lecithinolyticum HOT 653	Cultivable
Treponema medium HOT 667	Cultivable
Treponema vincentii HOT 029	Cultivable

It is well known that non-surgical periodontal treatment can decrease the amount of inflammation present in patients with chronic periodontitis. In patients with CKD, non-surgical periodontal treatment also has positive effects on periodontal as well as kidney function parameters. Most studies examining the effect of non-surgical periodontal treatment involve CKD patients with probing depths of at least 5 mm at multiple sites with oral hygiene instructions and supra and subgingival scaling and root planning (13). Non-surgical periodontal treatment has been found to be associated with a decrease in C-reactive protein and IL-6 levels in patients with periodontitis (14). Results of a recent pilot study examining the effect of non-surgical periodontal treatment on kidney function demonstrate a possibility that controlling periodontal inflammation can help to maintain kidney function, as the data showed a statistically significant increase in the median value of eGFR after treatment (15).

The impact of antimicrobial adjuncts on the results of non-surgical periodontal treatment has also been extensively studied. Locally delivered antimicrobial adjuncts to scaling and root planing include tetracycline, minocycline, metronidazole, doxycycline, and chlorhexidine. Meta-analyses of these antimicrobials in the context of periodontal treatment have generally shown a favorable result in decreasing probing depth and gains in clinical attachment level versus scaling and root planing alone (16). Another effect of the use of locally delivered antimicrobials is the reduction of periodontal pathogens within periodontal pockets. Administration of minocycline microspheres in pockets decreases the number of several bacterial species, including “red complex” bacteria, to a greater extent than scaling and root planing alone (17).

This pilot study was conducted among an underserved patient population with low access to dental care. In a study published by our collaborators, it was found that only 11% of CKD patients within the San Francisco Department of Public Health Community Health Network had

at least one outpatient dental visit in one year. Patients with CKD were also found to have a 25% lower likelihood of having a dental visit than those without CKD (18). To date, no studies have investigated the impact of intensive non-surgical treatment consisting of scaling and root planing with locally delivered minocycline on the microbiome of CKD patients. The aim of this study is to investigate the positive changes in the microbial profile in CKD patients after non-surgical treatment combined with locally delivered minocycline.

MATERIALS & METHODS

Study Population

46 patients were recruited from Zuckerberg San Francisco General Hospital (SFGH) Renal Clinic. Patients were provided a written and oral explanation of the study, which was approved by the Institutional Review Board at the University of California, San Francisco (study number: 12-09801). Patients qualified for the study based on their periodontal status and kidney function status. Criteria for patient selection were as follows: 1) age 20-75 years; 2) at least 2 estimated glomerular filtration rate (eGFR) measurements of 15-59 mL/min/1.73 m2 within the preceding 12 months and no eGFR increase by $\geq$ 50% in the preceding 6 months; 3) moderate to severe periodontal disease as classified by the Centers for Disease Control (CDC)/American Academy of Periodontology (AAP) definitions and at least 30% sites with bleeding on probing. The CDC/AAP definition for moderate periodontitis is based on the finding of at least 2 interproximal sites with clinical attachment loss (CAL) of at least 4 mm or at least 2 interproximal sites with probing depth of at least 5 mm. The definition for severe periodontitis is based on the finding of at least 2 interproximal sites with CAL of at least 6 mm and at least 1 interproximal site with probing depth of at least 5 mm. Exclusion criteria were as follows: 1) currently receiving dialysis, immunosuppressant therapy, anticoagulation therapy; 2) pregnant;

3) fewer than 6 natural teeth; 4) requiring antibiotic prophylaxis for dental procedures; 5) severe dental disease defined as having deep caries, endodontic involvement, presence of abscesses, or other dental conditions requiring immediate treatment; 6) allergies to minocycline, tetracyclines, or polyglycolate polymers (found in minocycline microspheres).

Clinical Evaluation

Patients signed an informed consent to participate in the study and allow study coordinators to access their medical charts. A comprehensive medical history was collected from each patient. Clinical measures were collected at 6 sites (mesiobuccal, midbuccal, distobuccal, distolingual, midlingual, mesiolingual) per tooth in the entire dentition. Bleeding on probing was recorded as present ("1") or absent ("0"). The distance of the gingival margin to the cementoenamel junction on each of the 6 sites were measured with a UNC-12 probe with 1 mm markings and rounded down to the nearest millimeter. Plaque was measured at the straight buccal and straight lingual surfaces and was recorded based on the plaque index of Loe and Silness (1964). Plaque index scoring was as follows: 0: no plaque in gingival area; 1: no plaque visible, but visible when a probe is scraped along gingival margin; 2: visibly covered with thin to moderately thick layer of plaque (less than 1/3 of surface); 3: heavy accumulation of soft material. Gingival index was recorded at the 6 sites of each tooth based on the visual signs of the Loe and Silness index (1964). The gingival index scoring was as follows: 0: normal gingival index; 1: mild inflammation/slight change in color/slight edema; 2: moderate inflammation/erythema, edema, loss of stippling; 3: severe inflammation/marked erythema and inflammation, tendency towards spontaneous bleeding. Measurements were recorded at baseline, 4, 8, and 12 month visits. After the baseline evaluation, those teeth deemed to be hopeless were planned to be extracted. Hopeless teeth are defined as those with two or more of the following criteria: loss of over 75%

of supporting bone, probing depths greater than 8 mm, class III furcation involvement, class III mobility, poor crown-root ratio, root proximity with interproximal bone loss and evidence of horizontal bone loss. Prognosis of these teeth were also confirmed based on a Panoramic radiograph. After baseline evaluation, patients were then randomized to either an intensive intervention cohort or a rescue treatment cohort as defined below. Since this is a pilot study to examine the effect of the intervention, patients were randomized so that twice as many patients were in the intervention group than the control group.

Intervention and Control Groups

In the intensive intervention group, patients had scaling and root planing with administration of minocycline (Arestin, OraPharma) to sites with probing depths of 5 mm or more. At months 4 and 8, debridement of the entire dentition was performed in addition to scaling and root planing and administration of Arestin at sites with persistent probing depths of 5 mm or more. 2% lidocaine with 1:100,000 epinephrine was administered as local anesthetic. Patients in the control group had hopeless teeth extracted, but no periodontal treatment was performed until 12 months after their baseline visit. Scaling and root planing would occur at months 4 and 8 only for those teeth with probing depths that have progressed 3 mm or more since screening or the prior visit. Patients in the control group who were concerned about waiting until the end of the study for treatment were given a list of clinics to receive care, but were not withdrawn from the study. At 12 months, patients in the control group received full mouth debridement with scaling and root planing and Arestin administration in sites with probing depths of 5 mm or more.

Sample Collection

Blood and urine samples collected at baseline, 4, and 12 months to assess renal biomarkers and biomarkers of systemic inflammation. Saliva, subgingival plaque, and gingival crevicular fluid

was collected from all patients at baseline and at 12 months. Whole, unstimulated saliva was collected into a 15 mL tube over 5 minutes. If the volume of saliva was less than 5 mL after 5 minutes, the subject was asked to continue to spit into the tube until 5 mL was obtained or until 10 minutes elapsed. Samples were put on ice and sent to the laboratory for processing within 2 hours. Subgingival plaque was collected at the mesial-buccal surfaces of the maxillary right first molar (tooth #3) and the mandibular left first molar (tooth #19). If the tooth was not present, the most adjacent tooth was sampled. Samples were collected by first drying the sites with a cotton roll, then placing a sterile endodontic paper point into the sulcus of the two sites for 10-15 seconds. Gingival crevicular fluid was collected from the mesial-buccal surfaces of the maxillary left first molar (tooth #14) and the mandibular right first molar (tooth #30). If the tooth was missing, the most adjacent tooth was sampled. Sites were first dried with a cotton roll, then a Periopaper ® strip was placed into the sulcus for 15 seconds, and placed into a cryovial. Both of these sets of samples were placed into one cryovial tube without transport media. Samples were immediately placed on ice, processed in the laboratory within 4 hours of collection and stored at minus 70°C until sent to the Forsyth Institute for analysis.

Bacterial Analysis

Plaque and saliva samples for all patients were sent to the Forsyth Institute for microbial sequencing analysis. An in silico hybridization process was utilized for 16s ribosomal RNA (rRNA) sequencing, better known as Human Oral Microbiome Identification using Next Generation Sequencing (HOMINGS). ProbeSeq, a basic local assignment search tool (BLAST) was used for genus- and species-level identification within the plaque and saliva samples. The process is described in Appendix I.

Statistical Analysis

Several comparisons were made in this study. Within both the control and intervention groups and sample sources, comparisons were made. For example, in the control group, the microbial composition was analyzed in the plaque samples at baseline and 12 months, in the saliva samples at baseline and 12 months. The same comparisons and analyses were performed for the intensive treatment group. The samples were analyzed for individual bacterial species as well as grouped into the red, orange, and newly identified species as outlined by Perez-Chaparro. Comparisons were also made between the control and intensive treatment groups at baseline, at 12 months, and between baseline and 12 months. For comparisons within the two treatment groups, statistical analysis was performed using the Wilcoxon signed-rank test. For comparisons between the control and intensive treatment groups, statistical analysis was performed using the Mann-Whitney test. Statistical significance was obtained when $p < 0.05$.

RESULTS

Of the targeted 51 CKD patients, 46 patients completed the study, 32 patients in the intervention group and 14 patients in the control group. In both groups, patients were an average of 59 years old. Both groups were predominately male. The average eGFR of patients in the intensive treatment group was 42 and in the control group, it was 45. These values signify that the patients studied had stage 3 chronic kidney disease, which is a mild to moderate loss of kidney function. Smokers comprised about 23% of the control group and 28% of the intervention group. (Table 2).

Table 2. Patient demographics in the intervention and control groups.

	Intervention (n = 29)	Control (n = 13)
Age	59 ± 7.87	59 ± 9.36
Gender	Male: 19 Female: 13	Male: 11 Female: 3
eGFR	42.13 ± 11.90	45.21 ± 8.28
Current smokers	8	3

Overall, the patients in this study had low access to dental care. 78% of the patients did not have a dentist. With regards to their last dental visits, 52% of the patients had not seen a dentist in more than 2 years. The main reason that patients could not get dental care when they needed it was because they could not afford it.

When the bacterial species were grouped into their complexes (red, orange, and other periodontal pathogens), there was no statistically significant differences found between the control and intensive treatment groups at baseline or at 12 months. When the levels of the bacterial complexes were compared between baseline and 12 months, there was statistically significant differences found for only the intensive treatment group for all the complexes. A trend for decreasing subgingival microbial load in the intensive treatment group is evident, as shown in figure 1. On the other hand, in the control group, there was only a change in the levels of orange complex species, but not in the levels of red complex or other periodontal pathogens group (figure 2). There was no statistically significant change between baseline and 12 months for the bacterial complexes of the control group. No trend was noted in the saliva samples.

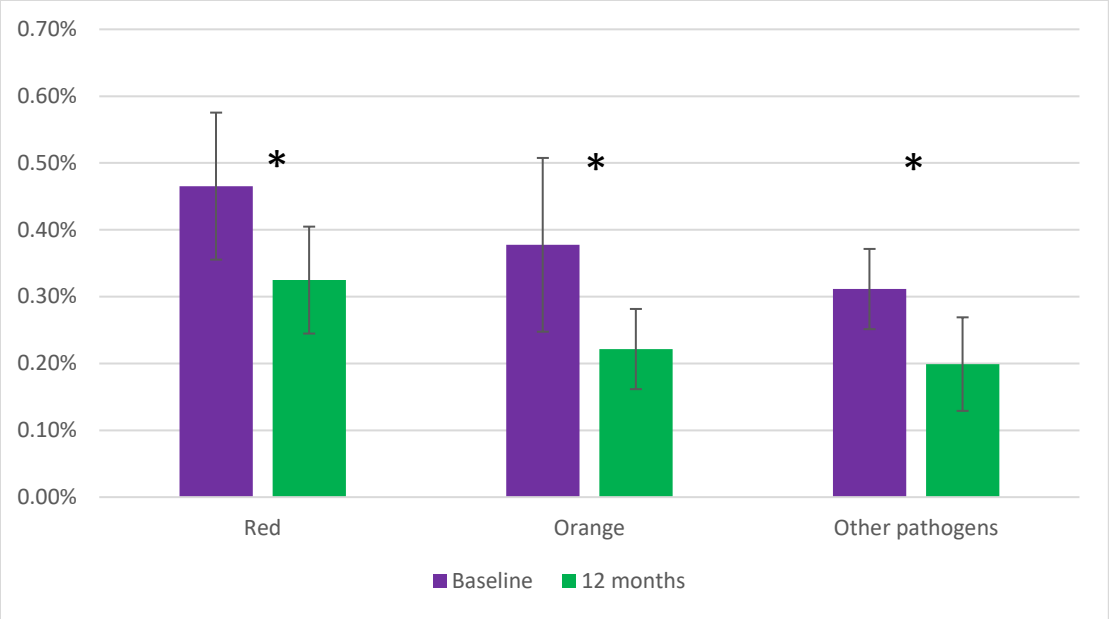

Figure 1. Means of microbial complexes from baseline to 12 months in the intensive treatment group (in % frequency ± SEM). * Refers to significant difference; $p < 0.05$; Wilcoxon signed-rank test.

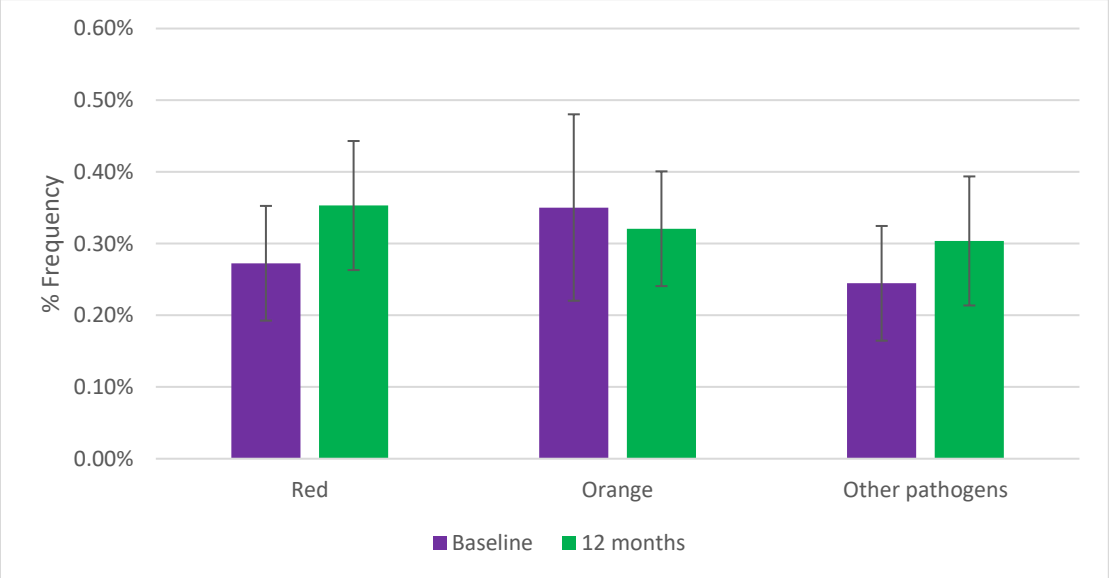

Figure 2. Means of microbial complexes from baseline to 12 months in the control group (in % frequency ± SEM).

A comparison at the complex levels between control and intervention is shown in figure 3. Overall, the intervention group showed decreases in percent frequency of the red complex, orange complex, and newly identified pathogens group. However, only the orange complex and newly identified pathogens group showed statistically significant reductions.

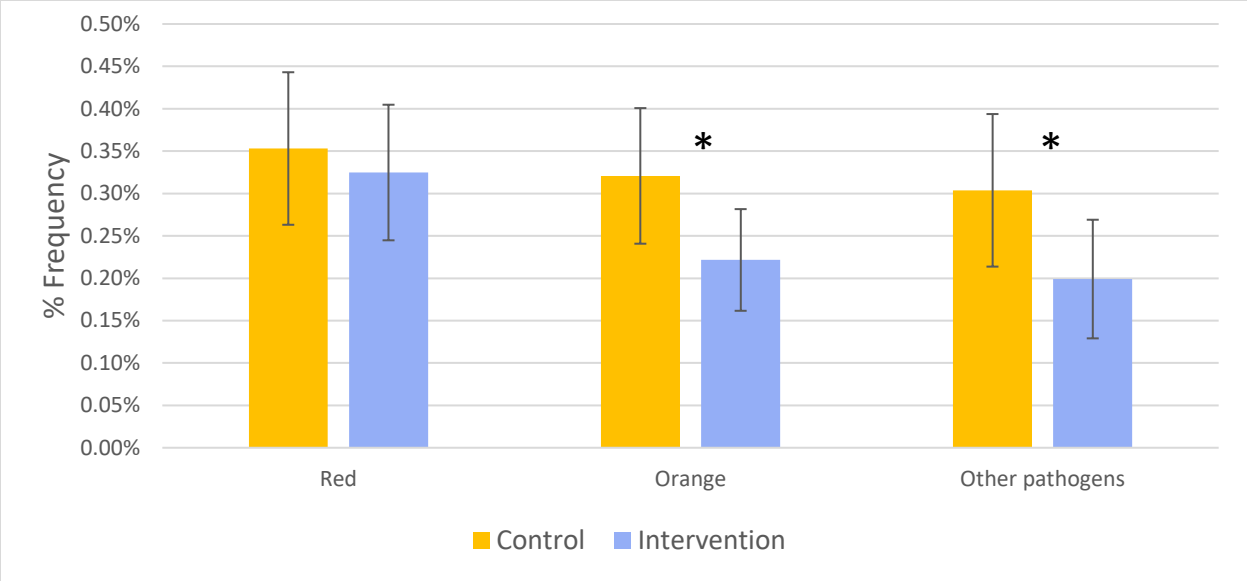

Figure 3. Comparison between control and intervention groups at 12 months in % frequency ± SEM. * Refers to significant difference; $p < 0.05$; Mann-Whitney test.

The subgingival microbial profiles in plaque samples from both groups at baseline and 12 months are shown in Figure 4 and 5. Although not shown in the figures, the species found with highest frequency in both control and intensive treatment groups at both timepoints was *Streptococcus* species and *Rothia mucilaginosa*. The reduction in levels of *R. mucilaginosa* was statistically significant in both the control and the intensive treatment groups.

At baseline, the only species that showed a statistically significant difference between the control group and the intensive treatment group were *Treponema denticola* and *Treponema vincentii* ($p < 0.05$).

In the control group from baseline to 12 months, there was a decrease in levels of *Fusobacterium nucleatum nucleatum, Fusobacterium nucleatum polymorphum, Fusobacterium nucleatum vincentii, Prevotella nigrescens, Streptococcis constellatus, Fretibacterium fastidiosum, Mogibacterium timidum, Selemonas sputigena,* and *Treponema lecithinolyticum*. These reductions were statistically significant for *Fusobacterium nucleatum nucleatum, Fusobacterium nucleatum polymorphum, Fusobacterium nucleatum vincentii, Prevotella*

nigrescens, and *Selemonas sputigena* ($p < 0.05$). There was a statistically significant increase in *Fusobacterium periodonticum* and *Peptostreptococcus stomatis* ($p < 0.05$). (Figure 4)

In the intensive treatment group, from baseline to 12 months, there was a decrease in levels of *Porphyromonas gingivalis, Tannerella forsythia, Fusobacterium nucleatum nucleatum, Fusobacterium nucleatum vincentii, Streptococcus constellatus, Anaeroglobus geminatus, Eubacterium saphenum, Fretibacterium fastidiosum, Mogibacterium timidum, Peptostreptococcus stomatis, Porphyromonas endodontalis, Selenomonas sputigena, Treponema lecithinolyticum,* and *Rothia mucilaginosa*. These reductions were statistically significant for *Fusobacterium nucleatum nucleatum, Fusobacterium nucleatum vincentii, Eubacterium saphenum, Fretibacterium fastidiosum, Mogibacterium timidum, Porphyromonas endodontalis, Selenomonas sputigena,* and *Treponema lecithinolyticum* ($p < 0.05$). There was also a statistically significant increase in *Treponema denticola* and *Fusobacterium periodonticum* ($p < 0.05$). (Figure 5)

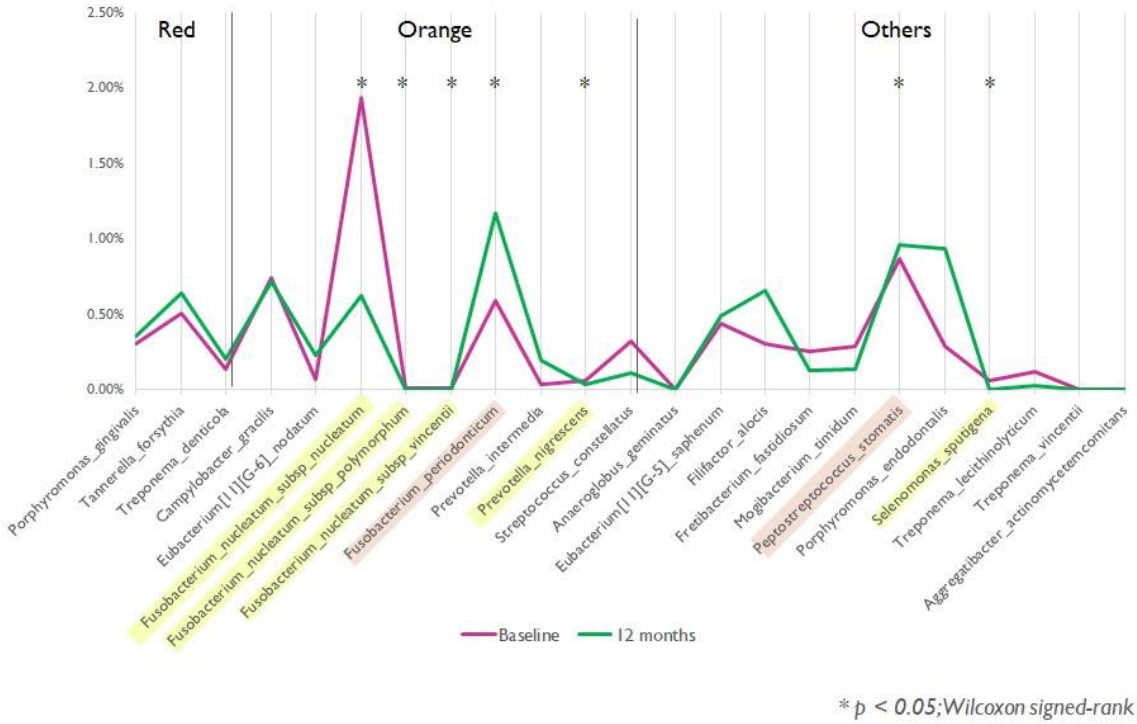

Figure 4. Change in microbial profile between baseline and 12 months in the control group (in % frequency). Yellow highlights are species that showed a significant reduction; red highlights are species that showed a significant increase in levels.

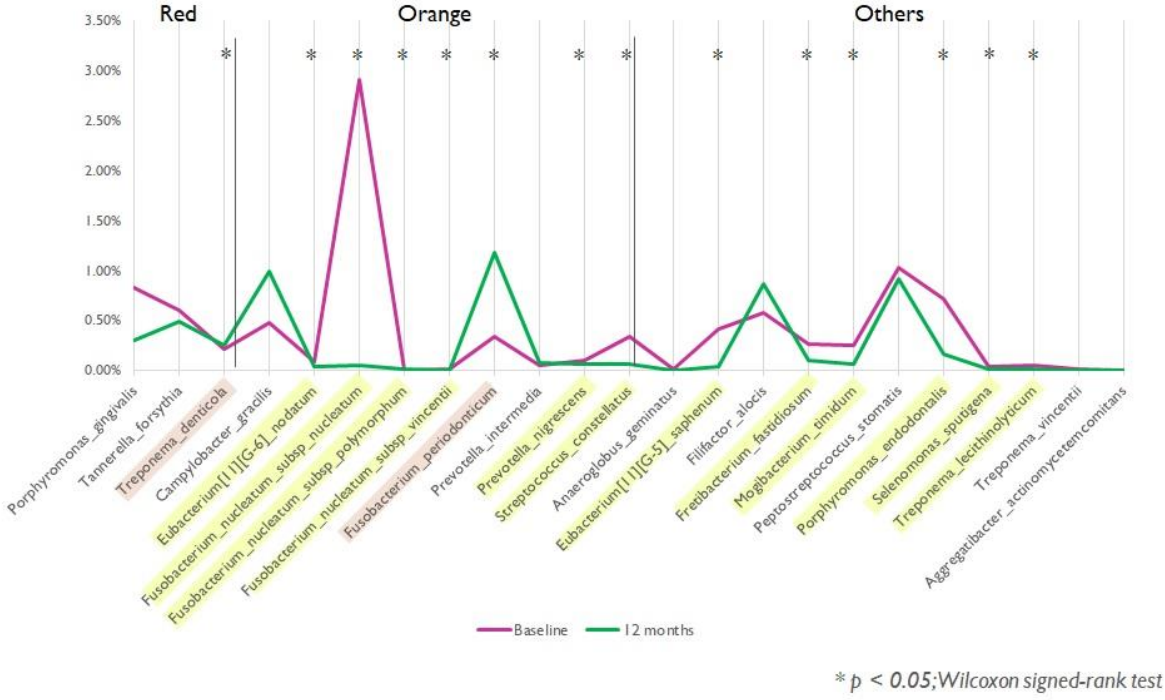

Figure 5. Changes in microbial profile between baseline and 12 months in the intensive treatment group (in % frequency). Yellow highlights are species that showed a significant reduction; red highlights are species that showed a significant increase in levels.

A comparison of the bacterial levels between control and intensive treatment group at 12 months reveal several significant differences. The intensive treatment group had significantly lower levels of *Tannerlla forsythia, Prevotella intermedia, Streptococcus constellatus, Fretibacterium fastidiosum,* and *Peptostreptococcus stomatis* ($p < 0.05$) than the control group. On the other hand, the control group had a statistically significant lower level of *Filifactor alocis* than the intensive treatment group ($p < 0.05$). (Figure 6)

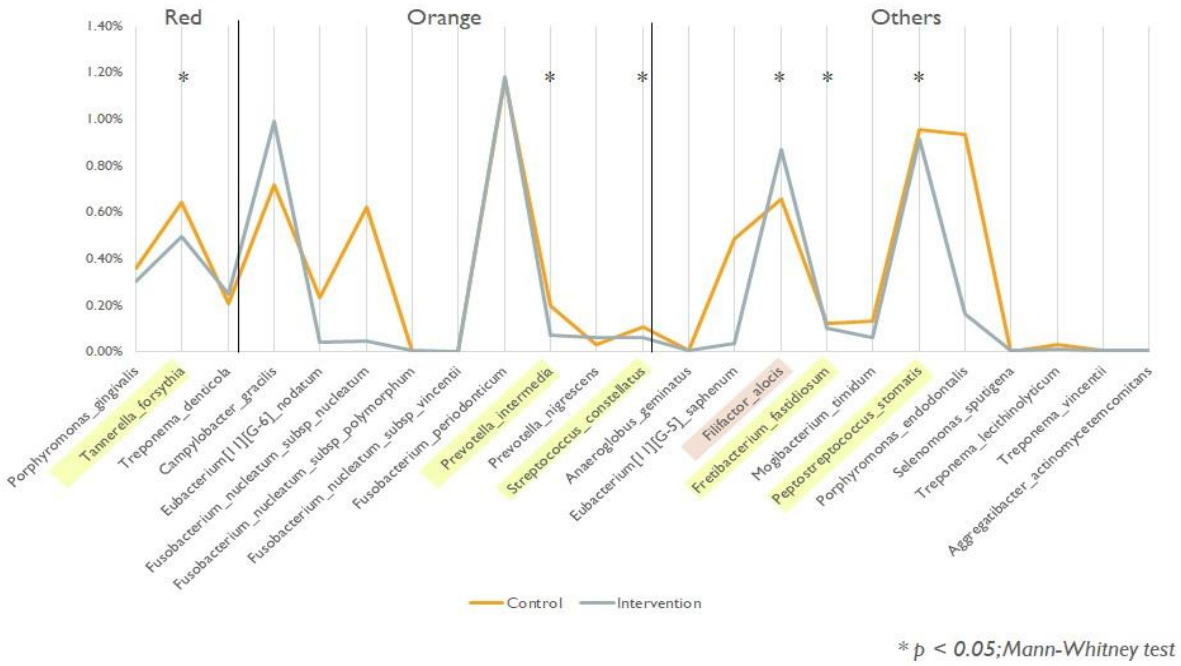

Figure 6. Comparison of levels of individual bacterial species at 12 months in the control and intensive treatment groups (in % frequency). Yellow highlights are species that showed a significant reduction; red highlights are species that showed a significant increase in levels.

DISCUSSION

There is limited data in the literature regarding the subgingival microbial profile of CKD patients and the impact of periodontal therapy on their oral microbiome. There are no studies that also include use of a locally delivered antibiotic in the non-surgical periodontal therapy of this group of patients. In this study, it was found that in the subgingival plaque samples, there is a

statistically significant trend for decreasing levels of red and orange complex species as a group, as well as in the group of newly identified periodontal pathogens. On a species level, more species in the intensive treatment group showed a statistically significant decrease in levels from baseline to 12 months.

In subjects with periodontitis, Socransky and Haffajee found a significantly higher proportion of members of the orange and red complexes in subgingival biofilms (10). In chronic kidney patients with periodontitis, analysis of subgingival plaque has shown that the microbial profile of these patients do not differ significantly from non-CKD patients. One study identified *T. denticola, T. forsythia, and P. micros* as frequently detected pathogens (19). Another study found that chronic periodontitis patients with CKD had a higher frequency of red-complex pathogens than those without CKD (20). In this study, the microbiome in the CKD patients were not compared with those without CKD. Interestingly, it was found that *Rothia muciloginosa* as well as several of the orange complex species were found with the highest frequency both at baseline and at 12 months. Recent studies have found that *R. mucilaginosa* is a Gram-positive bacteria that is the most predominant oral Rothia species in the oral cavity (21). This species is part of the normal flora in the oral cavity and pharynx. Infections of the face and oral cavity by *R. mucilaginosa* is relatively rare and usually are opportunistic infections (22). To date, no studies have investigated the role of this species in periodontal disease.

Few studies have explored the effect of non-surgical periodontal therapy on the subgingival microbiota in CKD patients. A Brazilian study found that in comparison to CKD patients, systemically healthy patients showed a reduction in the levels of a greater number of species after non-surgical periodontal therapy. CKD patients showed higher levels of *A. israelii*, *C. rectus*, *F. periodonticum*, *P. micra*, *P. nigrescens*, *T. forsythia*, *N. mucosa*, and *S. anginosus*.

Patients with CKD also had significantly higher levels of several species that included the "red complex" species in sites that did not respond (pocket depth increase or attachment loss) to non-surgical periodontal therapy. In general, the subgingival microbiota is very similar between CKD patients and systemically healthy patients. However, it was found that there are higher levels of pathogenic species that remain after periodontal therapy in CKD patients (23). In our study, CKD patients that were treated with Arestin during multiple scaling and root planing visits showed significantly decreased levels of more species than the control group, specifically of the species in the orange complex and of the newly identified periodontal pathogens group. Patients in our control group did not demonstrate a decrease in red complex species. The intervention group showed a decrease in two of the red complex species, *P. gingivalis* and *T. forsythia*, while there was a slight, but statistically significant increase in the levels of *T. denticola.*

Knowledge of microbial parameters assists in determining appropriate periodontal treatment for patients. Failure of traditional non-surgical therapy to treat periodontal disease may lead to trying other strategies such as local or systemic antibiotic therapy.

Within both the control and intervention groups, smokers made up 23% and 28% of the control and intervention groups, respectively. Multiple studies in the literature have documented the relationship between smoking and increasing severity of periodontal disease. However, the effect of smoking on the oral microbiota is less clear. Studies have failed to find significant differences between smokers and non-smokers in the prevalence of the groups of putative periodontal pathogens examined (24, 25). Renvert studied the effect of non-surgical periodontal therapy on clinical and microbiological parameters and found that the change in microbial profile was more related to the change in clinical parameters rather than to smoking status (26). On the other hand, other studies have found that smokers had significantly elevated levels of several

periodontal pathogens (27, 28). When scaling and root planing was combined with administration of minocycline microspheres, it was found that the combination treatment reduced red and orange complex bacteria numbers and proportions to a greater extent than SRP alone (29). This study on CKD patients did not conduct an analysis on the difference in microbial composition between smokers and non-smokers, so a comparison to these studies cannot be made.

Limitations to this current study include population size, number of sites sampled, and distribution of patients. After taking into account patients who dropped out of the study, the population size of this study was fairly small, total of 46 patients. The original goal of 51 patients was a random population size chosen because to our knowledge, there is no existing data of the anticipated effect size of periodontal treatment to inform sample size calculations. However, a larger cohort size may have provided more microbial data to analyze and find more statistically significant changes in the microbial profiles of these patients. Additionally, patients were assigned in a 2:1 ratio, with more patients intentionally assigned to the intensive treatment group. This provided more microbial sample data for the intensive treatment group than for the control group because the goal of the original study is to primarily examine the effect of periodontal treatment on renal biomarkers. Only two sites per patient were sampled in this study, possibly leading to the low percentage of bacterial species found in this cohort. Increasing the number of sampled sites could have increased the bacterial count found in the analysis and possibly have led to more statistically significant changes.

Future directions for oral microbiome studies regarding this cohort involve making changes and additions to the study design. One such addition to the study design could be to perform the same intervention and rescue treatment to a systemically healthy population and

compare the changes in microbiome between the CKD and the healthy groups. Along the same lines, in this cohort of CKD patients, microbial profiles of smokers and diabetics could also be compared with those without these conditions. It would also be interesting to correlate the bacterial species found with pocket depths to determine if there is a difference between the microbiome found in deeper pockets and that found in shallower pockets, then compare it to a systemically healthy cohort with periodontitis.

Overall, this study found that intensive non-surgical periodontal treatment consisting of scaling and root planing and Arestin administration every 4 months in a dentally underserved CKD population had a positive effect in changing the oral microbiome of these patients. The study population consisted of patients who have low health literacy and limited access to dental care. Despite these factors, the oral microbiome of these patients showed a trend of decreasing levels of species of the red complex, orange complex, and newly identified periodontal pathogens in the intensive treatment group.

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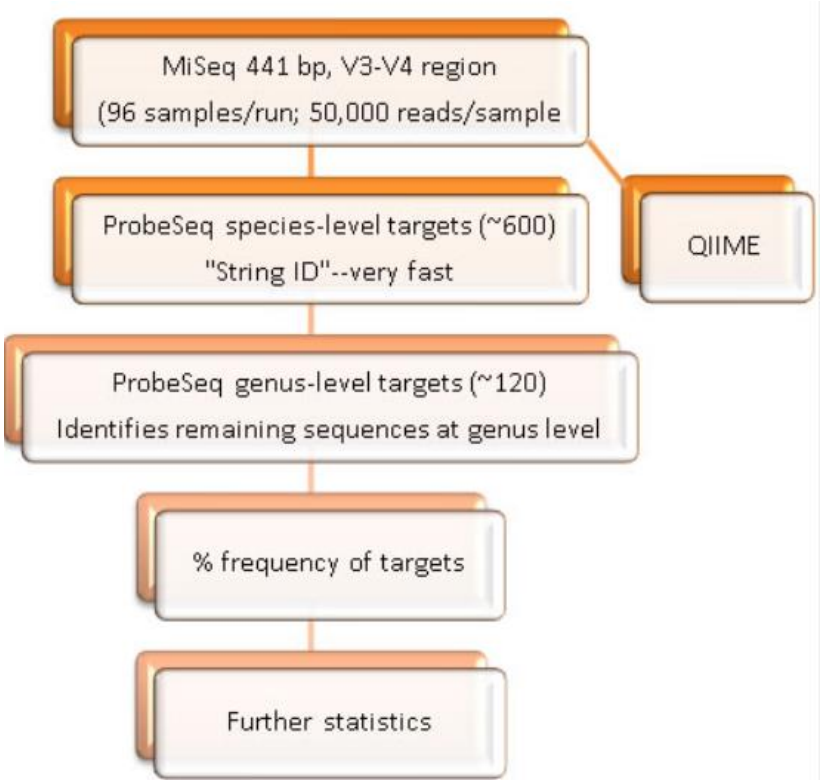
Appendix I: Schematic representation of microbial sample analysis



Schematic representation of protocol for analysis (homings.forsyth.org)

An average of >50,000 sequences are obtained in a MiSeq run of about 90 samples. Bad reads are removed from the analysis. Species-specific, 16S rRNA-based oligonucleotide probes are used in a Basic Local Alignment Search Tool (BLAST) to compare nucleotide or protein sequences to sequence databases. In this case, the program ProbeSeq was used to determine the frequency of oral bacterial targets. 598 oligonucleotide probes of 17-40 bases target individual oral bacterial species. An additional panel of 94 genus-specific probes is used.

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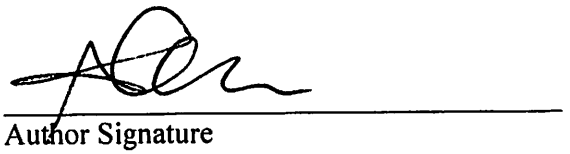

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