Effect of non-surgical periodontal treatment on the subgingival microbiota of patients with chronic kidney disease

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Abstract: This study investigated the effect of non-surgical periodontal therapy on the composition of subgingival microbiota of patients with chronic kidney disease (CKD). Sixteen CKD pre-dialysis individuals (CKD) and 14 individuals without clinical evidence of kidney disease (C) presenting chronic periodontitis were treated by scaling and root planing. Subgingival samples were collected from each patient and analyzed for their composition by checkerboard at baseline and 3 months post-therapy. Significant differences between groups at baseline were sought by the Mann-Whitney and χ^2 tests. Changes over time were examined by the Wilcoxon test. At baseline, the CKD group had significantly lower counts of *E. faecalis* compared to the C group ($p < 0.05$). After treatment, the levels of a greater number of species were reduced in the C group. Higher levels of *A. israelii, C. rectus, F. periodonticum, P. micra, P. nigrescens, T. forsythia, N. mucosa*, and *S. anginosus* ($p < 0.05$) were found in the CKD group compared to the C group. Also, non-responsive sites in CKD individuals harbored significantly higher levels of pathogenic species (*T. forsythia, P. gingivalis, T. denticola, Fusobacterium* spp., *D. pneumosintes*, *E. faecalis* and *S. aureus*; $p < 0.05$) than sites that responded to therapy, as well as non-responsive sites in the C group. The periodontitis-associated subgingival microbiota of CKD and systemically healthy individuals was similar in composition. However, high levels of pathogenic species persisted in the subgingival microbiota of patients with CKD after treatment.

Descriptors: Periodontitis; Dental Scaling; Root Planing; Microbiology; Kidney Failure, Chronic.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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Introduction

Chronic kidney disease (CKD) is a public health problem worldwide,[1] and its prevalence has been increasing.[2] CKD is defined as glomerular filtration rate (GFR) reduction or kidney damage, reflected as abnormal urine sediment or abnormalities in the renal anatomy.[1] Periodontitis has emerged as a non-traditional risk factor and a prediction model for CKD.[3] The link between periodontal disease and CKD may be due to concomitant infection and inflammation.[4] The periodontal inflammatory state may add to the chronic inflammation present in CKD,[5] decreasing renal function.[6] Periodontal therapy reduces inflammation and improves endothelial function,[7] leading to more effective kidney microcirculation

and filtration. CKD patients with periodontitis can also be effectively treated by periodontal mechanical therapy.[8,9] In a previous study, we demonstrated that non-surgical mechanical treatment led to periodontal clinical improvement, as well as an improvement in GFR levels.[8] However, limited data are available regarding the subgingival microbial profile of pre-dialysis CKD patients and the effect of therapy on the oral microbiota. Therefore, the aim of this study was to investigate the effect of mechanical non-surgical periodontal therapy on the subgingival microbiota of CKD patients with chronic periodontitis.

Methodology

The present study was a two-arm monocentric clinical trial with a 3-month follow-up period. The study was conducted according to the principles outlined in the Declaration of Helsinki on experimentation involving human individuals, and was approved by the Human Research Committee/Institute for Community Health Studies at the Federal University of Rio de Janeiro, protocol 113/2006. To be enrolled, patients were informed about the nature of the proposed treatment, its risks and benefits, and signed informed consent forms. The sample population has been described in a previous paper.[8] From January to November 2006, 16 pre-dialysis (CKD) patients were recruited from the Nephrology Division of Clementino Fraga Filho University Hospital, and 14 systemically healthy individuals (C) were recruited from the General Medicine department of the same hospital. This study used the GFR to define the groups, according to the renal function stages proposed by the National Kidney Foundation.[1] The CKD group consisted of patients with clinical diagnosis of renal failure, having GFR between 89 and 15 mL/min, and receiving conservative treatment (pre-dialysis). The C group consisted of patients seeking general medical care without signs and symptoms of renal disease and having GFR > 90 mL/min. All study participants were between 35 and 76 years of age, presented at least 15 teeth, and were diagnosed as having chronic periodontitis, i.e., the presence of ≥ 4 sites in 3 different teeth with clinical attachment level (CAL) ≥ 4 mm and bleeding on probing (BOP). A detailed medical history was obtained from all participants at baseline, as well as information about age, gender, ethnicity, and smoking habit. Exclusion criteria included HIV infection, pregnancy, lupus erythematosus, rheumatoid arthritis, need for antibiotic prophylaxis for periodontal procedures, periodontal treatment, and/or use of antibiotics in the preceding 6 months. A full-mouth periodontal clinical examination was performed at 6 sites per tooth (excluding third molars) by one calibrated examiner (C.O.S.) at both visits. Periodontal assessment included probing depth (PD) and CAL, measured to the nearest millimeter with a periodontal probe (UNC-15, Hu-Friedy, Chicago, USA), the presence or absence of BOP, supragingival biofilm (VP), gingival marginal bleeding (GB), and suppuration (SUP). Intra-class correlation coefficients > 0.90 were obtained for PD and CAL. The periodontal treatment was performed by a single experienced periodontist (H.P.C.A.). Both groups received non-surgical periodontal therapy consisting of oral hygiene instructions, and supra- and subgingival scaling and root planing with hand instruments (Gracey curettes; Hu-Friedy®) under local anaesthesia. One sextant was instrumented at each dental visit (1- to 2-hour session), and the therapy was completed within 6-8 weeks.

Microbiological assessment

Microbial analyses were performed at baseline and 3 months after therapy. The presence and levels of 49 bacterial species were determined in the subgingival biofilm samples by genomic DNA probes and the checkerboard DNA-DNA hybridization method.[10] The species *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) serotypes a, b, and c, and *Propionibacterium acnes* 1 and 2 were pooled into two DNA probes for the two species, respectively. Eight enteric species (*Enterobacter aerogenes*, *Enterobacter cloacae, Enterobacter gergoviae, Enterobacter sakazakii, Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca,* and *Pantoea agglomerans,* formerly *Enterobacter agglomerans*) were pooled into one enteric probe. After removal of supragingival biofilm, subgingival biofilm samples were taken from 6 sites with the deepest PD per individual by means of in-

dividual sterile Gracey curettes (Hu-Friedy®), and were placed in individual tubes. Bacterial cells underwent lysis, and denatured DNA was fixed on a nylon membrane (GE Healthcare Life Science, São Paulo, Brazil) using the checkerboard slot blot device (Minislot 30, Immunetics, Cambridge, USA). Digoxigenin-labeled (Roche Applied Science, São Paulo, Brazil) whole-genomic DNA probes were hybridized at 90° to the lanes of the plaque samples in the slot blot device (Miniblotter 45, Immunetics). After hybridization, the membranes were washed at high stringency, bound probes were detected using phosphatase-conjugated antibody to digoxigenin (Roche Applied Science), and fluorescence was captured by an imaging system (Storm™ 860, GE Healthcare Life Science). Signals were evaluated visually by comparison with the standards at 10^5 and 10^6 bacterial cells for the test species on the same membrane. They were recorded as:

- 0, not detected;
- 1, < 10^5 cells;
- 2, approximately 10^5 cells;
- 3, 10^5 to 10^6 cells;
- 4, approximately 10^6 cells; and
- 5, > 10^6 cells.

The sensitivity of the assay was adjusted to permit the detection of 10^4 cells of a given species by adjustment of the concentration of each DNA probe. The microbiological test was read by a single calibrated examiner (C.M.S.B.).

Statistical analysis

Statistical analyses were performed with SPSS software (SPSS, release 17.0, Chicago, USA). Microbial data were presented as mean levels (× 10^5 bacterial cells). The levels (scores 0 to 5) of each species in a sample were converted to absolute numbers, and the mean counts were computed for each patient and averaged within the groups. Moreover, the mean counts of bacteria in sites that did not improve or presented disease progression, i.e., PD increase and/or attachment loss at 3 months post-therapy, were compared to sites that improved (PD reduction and CAL gain) after treatment. Significant differences in demographic, clinical, and microbiological parameters between groups were determined by the Mann-Whitney and χ^2 tests. Differences in clinical and microbiological changes between groups over time were evaluated by the Wilcoxon Signed-Rank test. The level of significance for all analysis was 5%.

Results

The majority of individuals in both groups were females (9 in the CKD and 10 in the C group), white, and non-smokers; however, no significant differences for these parameters were observed between groups. In contrast, those in the CKD group were significantly older (58.8 ± 10.8 years) than those in the C group (52.0 ± 3.3 years; p = 0.014, Mann-Whitney test). Regarding the clinical features of the sampled sites (Table 1), there were no significant differences between groups for all periodontal

Table 1 - Periodontal clinical parameters (mean ± SEM) of the sites sampled for microbiological analysis in the two clinical groups, at baseline and 3 months after therapy.

Clinical parameters	CKD (n = 16)		C (n = 14)	
	Baseline	3 months	Baseline	3 months
Pocket depth (mm)	4.5 ± 0.2	1.8 ± 0.2*	4.5 ± 0.4	1.7 ± 0.2*
Clinical attachment level (mm)	5.3 ± 0.4	4.3 ± 0.4*	5.3 ± 0.4	4.1 ± 0.3*
% of sites with				
Supragingival biofilm	78.7 ± 4.5	55.2 ± 7.7*	69.2 ± 7.8	46.4 ± 7.4*
Gingival bleeding	33.6 ± 7.2	25.0 ± 6.4	26.9 ± 6.0	20.2 ± 4.3
Bleeding on probing	77.8 ± 5.2	58.3 ± 6.6*	67.4 ± 6.3	53.5 ± 4.9
Suppuration	12.7 ± 3.6	8.3 ± 4.3	9.3 ± 4.4	2.4 ± 1.6

CKD: chronic kidney disease pre-dialysis individuals; C: systemically healthy individuals; *Refers to significant differences between baseline and 3 months post-therapy within the groups (p < 0.05, Wilcoxon test).

parameters at baseline and at 3 months post-therapy ($p > 0.05$, Mann-Whitney test). Both groups showed significant clinical improvement in those sites for PD, CAL, and VP after treatment ($p < 0.05$, Wilcoxon test). The CKD group also showed significant improvement in BOP. The subgingival microbial profiles of both groups at baseline and 3 months after therapy are depicted in Figure 1. In general, the C group showed absolute higher levels of many tested species, especially members of the green and orange complexes. However, only the species *Enterococcus faecalis* was detected in significantly higher mean counts in C compared to CKD individuals at baseline ($p = 0.025$; Mann-Whitney test). Levels of most species decreased significantly after treatment in the C group, whereas, in the CKD group, significant reductions were observed only for the species *Actinomyces gerencseriae, Actinomyces oris, A. actinomycetemcomitans, Fusobacterium nucleatum polymorphum, Streptococcus constellatus, Leptotrichia buccalis, Dialister pneumosintes*, *Enterics*, and *Staphylococcus aureus*. Moreover, a significant increase in mean counts was observed for *Prevotella nigrescens* in the CKD group ($p < 0.05$, Wilcoxon test). At 3 months post-therapy, significantly higher levels of *Actinomyces israelii, Campylobacter rectus, Fusobacterium periodonticum, Parvimonas micra, Prevotella nigrescens, Tannerella forsythia, Neisseria mucosa,* and *Streptococcus anginosus* were found in the CKD group compared with the C group ($p < 0.05$, Mann-Whitney test). The subgingival microbiota of sites that did or did not show clinical improvement after treatment were also analyzed. A total of 42 sites (35.6%) from 13 members of the CKD group and 31 sites (28.2%) from 12 members of the C group presented increases in PD

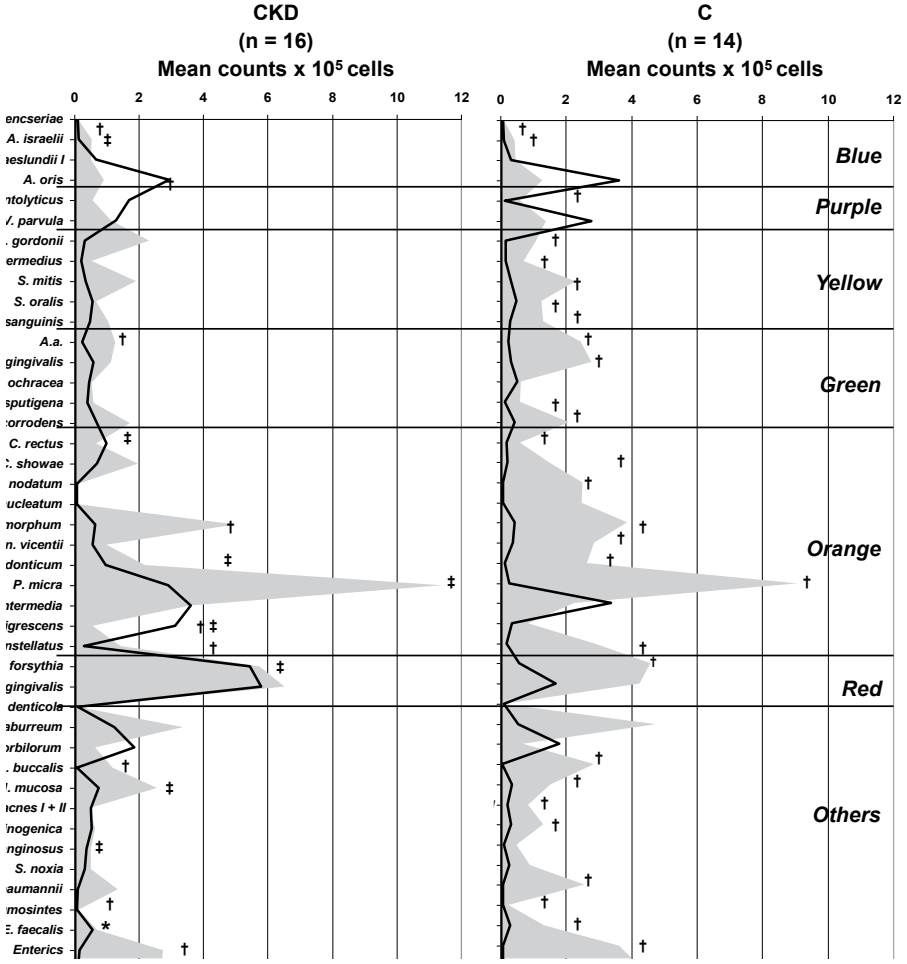

Figure 1 - Mean levels (x 10^5 cells) of bacterial species in the two clinical groups (CKD: chronic kidney disease pre-dialysis individuals; C: systemically healthy controls) at baseline and 3 months post-therapy.

A.a.: *Aggregatibacter actinomycetemcomitans*;
*refers to significant difference between groups at baseline ($p = 0.025$, Mann-Whitney test);
†refers to significant differences within groups over time ($p < 0.05$, Wilcoxon test);
‡refers to significant differences between groups at 3 months post-therapy ($p < 0.05$, Mann-Whitney test).

and/or loss of clinical attachment in spite of treatment. The number of non-respondent sites did not differ between groups ($p > 0.05$, χ^2 test; data not shown). Figure 2 shows the microbial composition of successfully treated and non-responsive sites in both groups at 3 months post-therapy. Those in the CKD group had significantly higher levels of several species in sites that did not respond to therapy compared with sites that did, particularly the pathogenic species *T. forsythia, Porphyromonas gingivalis, Treponema denticola, Fusobacterium* spp., and non-oral species including *D. pneumosintes*, *E. faecalis*, and *S. aureus* ($p < 0.05$, Wilcoxon test). Conversely, no significant differences regarding the levels of all tested species between sites that did or did not respond to therapy were observed in the C group (Figure 2). Comparisons of non-responsive sites between groups demonstrated significantly lower levels of *Veillonella parvula, Streptococcus intermedius, Capnocytophaga sputigena, Eikenella corrodens, Campylobacter showae, F. periodonticum, P. micra, T. forsythia, Eubacterium saburreum, L. buccalis, Prevotella melaninogenica,* and *Selenomonas noxia* in individuals in the C group than in those in the CKD group ($p < 0.05$, Mann-Whitney test).

Discussion

Limited data are available regarding the composition of the periodontal microbiota of individuals with CKD, as well as the impact of mechanical periodontal therapy on their microbiota. In this investigation, we showed that the periodontal microbiota of persons with chronic periodontitis and CKD and that of systemically healthy individuals was similar in composition, except that *E. faecalis* was found in higher counts in the C group. Although not considered a periodontal pathogen, *E. faecalis* produces various virulence factors that may be related to periodontal inflammation, tissue destruction, and neutrophil impairment.[11] Moreover, this species is a biofilm-forming bacterium, capable of adhering and

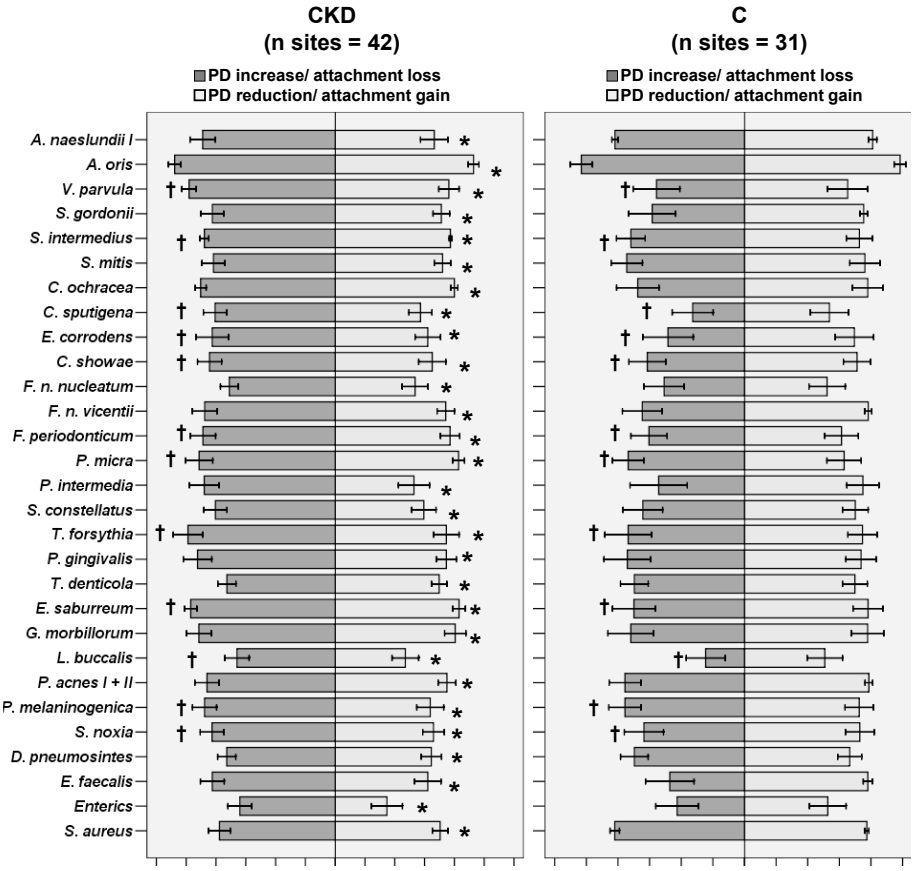

Figure 2 - Bar chart of bacterial species that differed significantly in mean levels (in log 10 ± SEM) at 3 months post-therapy between sites that responded (pocket depth reduction/attachment gain) or not (pocket depth increase/attachment loss) to periodontal therapy in the two clinical groups (CKD: chronic kidney disease pre-dialysis individuals; C: systemically healthy controls).

*Refers to significant differences between sites that responded or not to therapy within the CKD group ($p < 0.05$, Wilcoxon test); †refers to significant differences between groups regarding sites that did not respond to therapy ($p < 0.05$, Mann-Whitney test).

invading soft tissues, which enables this organism to co-aggregate with many oral species.[12,13] *E. faecalis* may also enhance pathogenicity in mixed infections with anaerobic bacteria.[14] Previous studies by our group have shown an association between *E. faecalis* and chronic periodontitis.[15,16] However, it is difficult to explain why this species was detected in higher levels in the periodontitis biofilm of systemically healthy individuals compared with those with CKD. Due to the immunosuppression usually present in CKD patients,[17] as well as the common association of *E. faecalis* and kidney infections, one would expect to find this species in higher levels in the CKD group. Changes in the bacterial levels from baseline to 3 months after therapy showed that a larger number of species diminished significantly in the C compared with the CKD group. In addition, one species of the orange complex, *P. nigrescens*, showed a significant increase in the CKD group after treatment. However, few species differed between groups at 3 months, including species of the red and orange complexes, which were found in higher levels in the CKD group. Further analyses comparing responsive and non-responsive sites showed that individuals with CKD presented higher levels of many species, such as *T. forsythia, P. gingivalis, T. denticola, Fusobacterium* sp., *D. pneumosintes*, *E. faecalis,* and *S. aureus,* in sites with PD increase and attachment loss after therapy, whereas no differences between those sites were observed for those in the C group. Moreover, non-responsive sites in the CKD group presented higher counts of *V. parvula, S. intermedius, C. sputigena, E. corrodens, C. showae, F. periodonticum, P. micra, T. forsythia, E. saburreum, L. buccalis, P. melaninogenica,* and *S. noxia* than non-responsive sites in the C group. Species of the red complex and *D. pneumosintes* have been recently associated with treatment failure or periodontal attachment loss, as well as non-responsive sites in generalized aggressive periodontitis.[18,19] The persistence of high levels of many pathogenic species in CKD patients compared with systemically healthy individuals after treatment could be related to the immunocompromised state associated with uremia in CKD patients.[20] Conceivably, the uremia can cause an indirect effect on the microbiota by modifying the host inflammatory or immune response, and by changing the host-parasite balance, favoring a rapid re-colonization by pathogenic species after mechanical therapy. One should consider, however, that this was a short-term post-therapy study consisting of a small sample population. Further longitudinal investigations are needed to evaluate how the persistence of high levels of periodontal pathogens will affect the efficacy of mechanical periodontal treatment of individuals with both CKD and chronic periodontitis.

Conclusions

The microbial composition of the periodontitis-associated subgingival biofilm of individuals with CKD was very similar to that of systemically healthy individuals. Nevertheless, fewer bacterial species were affected by mechanical periodontal therapy in the CKD than in the C group. In addition, pathogenic species persisted in high levels in non-responsive sites of CKD individuals compared with C patients.

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