IMPACT OF PERIODONTAL THERAPY ON METABOLIC AND INFLAMMATORY MARKERS IN PATIENTS WITH END STAGE RENAL DISEASE

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ABSTRACT

MEGGAN M.H. WEHMEYER: Impact of Periodontal Therapy on Metabolic and Inflammatory Markers in Patients with End Stage Renal Disease
(Under the direction of Steven Offenbacher)

Objective: To test whether periodontal intervention in dialysis patients with periodontal disease will result in improvement of inflammatory and metabolic markers compared to no treatment. **Methods:** Fifty-one dialysis patients with moderate to severe periodontal disease were recruited from six dialysis units. Twenty-five subjects were randomized to a periodontal treatment arm and twenty-six subjects were randomized to a control arm. Clinical periodontal parameters and serum were collected at baseline, 3 months and 6 months. Serum was analyzed for interleukin-6 and serum albumin. **Results:** Compared to untreated control, periodontal therapy was associated with a statistically significant improvement in clinical parameters of periodontal disease at 3 months, but not at 6 months and was not associated with any difference in serum interleukin-6 or serum albumin. **Conclusion:** Periodontal therapy was associated with improved clinical measures of periodontal disease, but not with improved interleukin-6 or serum albumin in this population. Diabetic status, plaque control and obesity should be tightly controlled in future studies of periodontal therapy in this population.

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BOP=bleeding on probing

BMI=body mass index

CAL=clinical attachment loss

CEJ=cementoenamel junction

CKD=chronic kidney disease

CRP=C-reactive protein

GFR=glomerular filtration rate

GI=gingival index

GM=gingival margin

Il-6=interleukin-6

hsIl-6=high sensitivity interleukin-6

PD=probing depth

PI=plaque index

SD=standard deviation

CHAPTER 1. INTRODUCTION

The burden of cardiovascular disease (CVD) is heavy among patients with chronic kidney disease (CKD). Myocardial infarction, sudden death, or stroke, remain leading causes of death and disability, especially for those patients receiving dialysis therapy [1, 2]. Traditional risk factors [3] only partially explain the burden of CVD [4]. Previous studies have explored the potential role of periodontal disease in explaining the excess burden of CVD in the CKD population. Periodontitis is an inflammatory disease caused by an infection of predominately gram negative organisms [5] and has been well-studied in other populations as a CVD risk factor [6-21]. A very high prevalence of periodontal disease in patients with various stages of CKD has been observed [22, 23]. Elevated systemic inflammatory markers is common to CVD, CKD and periodontal disease [24-32]

Treatment of periodontal patients with scaling, root planning, and flurbiprofen is associated with a trend to reduction of systemic inflammatory markers one year after therapy [28] and successful treatment of periodontitis is related to statistically significant reductions in systemic inflammatory markers CRP and IL-6 [32]. Recent data have also shown that the improved control of periodontitis achieved with local delivery of antibiotics leads to adjunctive systemic benefits [33]. The present paper presents findings from an exploratory, randomized, controlled intervention study to assess study feasibility and to determine whether the treatment of periodontal disease in maintenance dialysis patients reduces a prominent marker of systemic inflammation (serum Il-6) and improves a marker of nutrition (serum albumin)

CHAPTER 2. BACKGROUND AND SIGNIFICANCE

Periodontal Disease

Periodontal disease is a destructive inflammatory process of the supporting structures of the teeth including the gingiva, periodontal ligament and alveolar bone. Clinical measures to assess periodontal health include pocket depth (PD) and gingival recession; where PD refers to the depth of the pocket measured from the cementoenamel junction (CEJ) to the depth of the gingival sulcus, and gingival recession is the distance from the marginal gingival (GM) to the CEJ. Clinical attachment level (CAL) is calculated from the sum of PD and GM-CEJ measurements and forms the basis for the currently accepted periodontal disease classification system [34].

Chronic periodontitis is an infection of predominately gram-negative bacteria, including *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, and others (Philstrom et al 2005). Often painless and insidious in onset, it frequently is not diagnosed until it is severe and extensive. Characteristics of a healthy periodontium include pink, scalloped gingiva, well defined interdental papilla, 1-3mm probing depths, no clinical attachment loss, alveolar bone levels 1-2mm apical to the CEJ, and a lack of bleeding on probing. Characteristics of a diseased periodontium include loss of gingival stippling (indicating edema), erythema, blunting of papilla, radiographic bone height more than 3mm from the CEJ, probing depths greater than 4mm, clinical attachment loss and presence of bleeding on probing.

Periodontal disease is classified as generalized (≥30% of sites) or localized (<30% of sites) and slight (1-2mm clinical attachment loss), moderate (3-4mm clinical attachment loss) and severe (≥5mm clinical attachment loss) [34]. The prevalence of severe periodontal disease is about 5 times greater in the dialysis population (and to a lesser extent in the CKD population) than in the general population [23, 35, 36].

Chronic Kidney Disease and End Stage Renal Disease

The kidney serves several vital functions. It maintains salt/water and acid/base homeostasis and removes nitrogenous waste products. The kidney also produces hormones necessary for red blood cell synthesis and calcium absorption. Kidney disease refers to an impairment of normal kidney function. Kidney disease is often divided into two broad categories. Acute kidney injury refers to the rapid loss of renal function over days to weeks. Chronic kidney disease is a progressive loss of function occurring over several months to years.

Chronic kidney disease is categorized based on the level of impairment of the glomerular filtration rate (GFR). Stage one is early disease, while stage five represents severe disease necessitating renal replacement therapy (dialysis), and is often called end-stage renal disease (ESRD).

The burden of CVD is high in the CKD population

Cardiovascular disease continues to be a major chronic health problem in the general population. Mortality and morbidity from CVD are arguably the major chronic health problems in the CKD population.

Data from the 2006 annual report of the United States Renal Data System (USRDS) quantify the burden of CVD [1]. At the start of dialytic therapy, 31.9% of patients report congestive heart failure, 25% coronary artery disease, 8.2% acute myocardial infarction, 6.5% cardiac dysrhythmia, 9.0% cerebrovascular disease, and 13.8% peripheral vascular disease. While the USRDS relies primarily on self-reporting, recent prospective, rigorously conducted surveys seem to confirm the high prevalence of CVD. A major randomized clinical trial, the Hemodialysis (HEMO) Study revealed that 40% of prevalent hemodialysis patients have coronary artery disease, 19% have cerebrovascular disease, and 23% have peripheral vascular disease [37]. The Choices for Healthy Outcomes in Caring for End-Stage Renal Disease (CHOICE) study found that 45% of prevalent hemodialysis patient had ischemic heart disease, 18% had cerebrovascular disease, and 30% peripheral vascular disease [38].

Historically, CVD mortality is approximately 10-20 times higher among ESRD patients compared to the general population [27]. Cardiovascular mortality accounts for nearly 50% of overall mortality in the dialysis population [1]. The overall death rate was 229.4 per 1000 patient years; CVD accounted for 107.6 events (CVD deaths defined as AMI, atherosclerotic heart disease, cardiomyopathy, cardiac arrhythmia, cardiac arrest, pulmonary edema, congestive heart failure, and cerebrovascular disease). The next largest cause of death was septicemia (25.0 events per 1000 patient years).

An editorial by the editor of *Arteriosclerosis, Thrombosis and Vascular Biology*, noted that "The conventional risk factors for atherosclerosis are well understood, but they can account for only about 50% to 70% of atherosclerotic events in the general population" [39]. Risk factors for CVD observed in the general population do not necessarily extrapolate to the

CDK populations, especially those with ESRD. In fact, conventional risk factors may account for even lower percentage of atherosclerotic events. Again, both the HEMO and CHOICE studies revealed that traditional risk factors were not associated with CVD [37, 38, 40]. Interestingly, the CHOICE study made a case for inflammation confounding the association of a traditional risk factor, elevated serum cholesterol, with CVD [40]. Periodontal disease is a source of inflammation and an avenue for bacteremia. A report by Ishani, Collins et. al reported that septicemia appears to be an important and potentially preventable cardiovascular risk factor in dialysis patients [41]. In addition, vascular calcification as a consequence of altered phosphate and calcium balance are proving to be a novel risk factor for CVD in this population.

Periodontal disease is an independent risk factor for CVD

Periodontal disease contributes to the development of the systemic inflammatory response and accelerates atherosclerosis. Inflammation and infection are putative risk factors for atherosclerosis. Periodontal disease is a candidate risk factor that results in a vigorous localized inflammatory, as well as systemic inflammatory response.

Biofilms in the oral cavity on soft and hard tissues contain greater than 10^{10} organisms [5]. Many of these organisms are pathogenic and, upon evasion of local host defenses, elicit local inflammation as well as gain access to the circulation they also induce systemic inflammation. Within the local periodontal tissues, monocytes, dendritic cells and other immune cells recognize lipopolysaccharides in the bacterial cell wall and other toll-like receptor (TLR)-agonists and secrete various inflammatory mediators, including prostaglandin

E_2 (PGE_2), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNFα) [29, 42].

Evidence from human studies demonstrate that periodontal disease can induce a systemic inflammatory response and is associated with cardiovascular risk factors including acute phase proteins, CRP, and plasma fibrinogen [14, 43, 44]. Using data from the NHANES III and the ARIC studies, individuals with periodontitis were found to have increased systemic levels of CRP and fibrinogen. Levin et al (2004) note that the single most used marker for inflammation is C-reactive protein (CRP). It has been linked epidemiologically to nutritional state and its markers [24, 45, 46], cardiovascular risk factors [22, 47], cardiovascular disease [30, 40, 48-51], and morbidity and mortality [25, 31, 40, 48, 52-55] in patients with chronic kidney disease (CKD), most notably in CKD stage five or end-stage renal disease (ESRD).

These associations remained statistically significant after adjustment for multiple periodontal and CVD risk factors. Case control studies have shown that CRP levels were elevated in patients with periodontal disease as compared to periodontally healthy individuals [28, 56, 57]. In addition, pilot results indicated that periodontal treatment may reduce CRP levels [58]. Treatment of periodontal patients with scaling, root planning, and flurbiprofen is associated with a trend to reduction of CRP levels one year after therapy [28]. Successful treatment of periodontitis is related to statistically significant reductions in CRP and IL-6 [32].

The effects of systemic dissemination of bacteria from periodontitis results in hepatic activation of the acute phase response with increases in CRP and IL-6 [59-61]. Moreover, these bacteria are also capable of invading the endothelial lining of the major elastic arteries [59-61]. Both PCR nucleotide signals and viable bacteria are detectable within atheromatous

plaques [62, 63]. Consequently, these bacteria in combination with inflammatory cytokines and acute phase reactants are believed to contribute to an acceleration of systemic atherosclerosis [64-66] and the development of clinical CVD. In experimental models, periodontal pathogens have been shown to promote platelet aggregation, foam-cell formation, and the development of atheromas [67-69]. The mechanism by which this may occur remains elusive but may be a consequence of the production of antibodies to periodontal bacteria, including antibodies to bacterial heat shock proteins (HSPs) that cross-react with HSPs of the heart and may contribute to atheroma formation [70], a decrease in anti-atherogenic potency of HDL cholesterol by periodontitis, or enhanced macrophage activation by mean of increased serum lipopolysaccharide production [18, 71, 72].

Chronic kidney disease strengthens the association of periodontal disease and preclinical atherosclerosis

Data from the Dental ARIC study demonstrates how underlying kidney disease strengthens the relationship of periodontal disease and preclinical atherosclerosis [23]. A cross-sectional study of 5702 middle-aged black and white men and women was conducted. Periodontitis was determined by a clinically-derived definition [73], and categorized as healthy/gingivitis, initial, and severe. Renal insufficiency was defined as glomerular filtration rate (GFR) < 60 ml/min/1.73 m^2. Compared to mild/none periodontal disease, severe periodontal disease was associated with intima media thickness (IMT) in a group of participants with GFR < 60 ml/min. The odds ratio, with 95% C.I. was 3.2 (1.5-6.9, p=0.004). In individuals with GFR > 60 ml/min, there was no association observed between periodontal disease and carotid IMT.

Periodontal disease is associated with CVD morbidity and mortality During the last 15 years a substantial number of population-based, clinical, laboratory, and animal studies have been published that reported findings of a possible periodontal disease and cardiovascular disease relationship. The bulk of evidence suggests an association between periodontal disease and heart disease [6-21]. Yet because these studies were performed primarily in the general population, we feel that the strength of associations would be at a level considered moderate. In the CKD population, especially in the ESRD population, the burden of CVD is high enough to expect a stronger association. Whether treatment of periodontal disease reduces risk of cardiovascular events still remains unclear [74-76].

Moderate to severe periodontal disease predicts cardiovascular mortality among ESRD patients

A recent study investigated mortality (CVD and all-cause) over 18 months by periodontal disease status [77]. One-hundred patients had mild or no periodontitis; 68 had severe-to-moderate disease. During the follow-up period there were a total of 22 deaths, 14 of which were from cardiac causes. Adjustment for age, center, dialysis vintage, smoking, diabetes mellitus, and hypertension did not diminish the strength of association between periodontal disease and mortality, hazards ratio of 4.8 (95% C.I. 1.3-17.7).

The burden of periodontal disease is high in the CKD population

Data suggest that the burden of periodontal disease is high in the CKD population, especially among those with ESRD. Studies suggest that the prevalence of periodontal

disease and severity of kidney disease are positively related. One such study was performed in the Third National Health and Examination Survey to examine the association of periodontal disease and kidney disease [35]. Because of the nature of the periodontal exam (half-mouth exam in NHANES III versus full mouth in D-ARIC), a slightly different definition of periodontal disease was used. The definition, periodontal pocketing depth (PD) status, and was divided into three categories: *none* (0 sites with pocket depth, PD, 4+ millimeters), *moderate* (1 to 8 sites with PD 4+ mm), or *extensive* (>8 sites with PD 4+ mm). Renal insufficiency was defined as high serum creatinine $\geq$ 1.3 mg/dl. Extensive pocketing was associated with increased odds of high serum creatinine (1.36, 95% C.I. 1.07-1.60) compared to no pocketing, after adjusting for demographic and medical covariates.

An additional study performed using D-ARIC data [78] examined whether serum antibodies to oral periodontal pathogens were associated with renal insufficiency. Serum antibodies represent better evidence of invasive periodontal disease compared to the standard measures of attachment loss and/or pocket depths. Oral biofilm microbial composition was performed by DNA checkerboard and serum antibody IgG titers to specific bacteria by immunocheckerboard. Levels of serum IgG to specific oral bacteria were categorized by quartiles as high titer (comparing upper versus lower three) and eGFR<60 as the dependent variable in logistic regression models. High levels of serum IgG to selected periodontal pathogens including *Porphyromonas gingivalis, Treponema denticola* and *Aggregobacter actinomycetemcomitans* were associated with an increased risk for eGFR<60mL/min/1.73 m^2 when adjusted for race, center, age, gender, education, diabetes, hypertension, LDL cholesterol, HDL cholesterol, triglycerides, BMI (adj OR 1.6-1.7, P<0.05).

Inflammation/malnutrition in the CKD population

Inflammation and malnutrition are common in the ESRD population. The prevalence of elevated inflammatory markers is high, between 30% and 60% of North American and European dialysis patients [51, 54, 55, 79]. Protein energy malnutrition affects from 23% to 76% of hemodialysis patients, and 18% to 50% of peritoneal dialysis patients [40, 79]. Importantly, inflammation may be highly episodic. For example, both acute phase proteins CRP and albumin vary over time [45], albumin considerably less so than CRP.

The potential causes of the high inflammatory burden and malnutrition are not known but likely to be multifactorial: possibly related to dialysis technique (i.e. exposure to dialyzer membrane), or to the accumulation of pro-inflammatory compounds in renal failure (AGE's, beta2 microglobulin) or to the loss of defense mechanism to oxidative stress.

Clinically apparent and occult infection are common in the dialysis population, yet are not often considered as potential sources of inflammation. Periodontal disease, one of the major types of infection-driven inflammation, has not hereunto been extensively studied in hemodialysis/ESRD patients. Preliminary evidence suggests further investigation because of the abundance of this disease in the dialysis population, and the fact that it is associated with an important marker of inflammation, nutrition, and mortality.

Despite a poor understanding of the potential mechanisms/causes of inflammation, these markers are strongly predictive of mortality and morbidity in the dialysis population. Hypoalbuminemia and CRP have been demonstrated to be strong predictors of death [46, 80-82]. Severe periodontal disease is strongly associated with low serum albumin among ESKD patients. In a study [83] of 154 patients undergoing maintenance hemodialysis, thirty-five

subjects had severe periodontitis. Severe periodontitis was associated with low serum albumin, odds ratio = 8.20 (p=0.01; 95% Confidence Interval: 1.61-41.82) compared to individuals without severe periodontitis disease after adjustment for age, gender, race, diabetes mellitus, hypertension, body mass index, smoking, study site, total cholesterol, serum calcium, serum phosphorus, and normalized protein catabolic rate. Serum albumin is one of the key indicators of dialysis quality [84]. Hypoalbuminemia has been demonstrated to be a strong predictor of death [85].

Summary

The model of periodontitis, a plaque-induced inflammatory disease, contributing to systemic inflammation and accelerated atherosclerosis and clinical CVD is well described. There is also ample evidence that inflammation and CVD are common in the CKD population. Complications from CVD continue to be the leading cause of death in the ESRD population. The underlying causes of CVD in this population are still not fully understood. The primary objective of this study was to conduct a randomized interventional small exploratory study to test the hypothesis that periodontal intervention in dialysis patients who have both periodontal disease and a historically high burden of cardiovascular disease will result in a reduction of inflammatory markers and an improvement in metabolic markers. These markers are known to predict mortality [46, 80-82].

CHAPTER 3. MATERIALS AND METHODS

Subjects

Hemodialysis and peritoneal dialysis patients were recruited from the University of North Carolina Hospital Affiliated Dialysis Units at Carrboro, Pittsboro, Siler City and Fresenius Medical Care Units in Carrboro, Pittsboro, Siler City and Durham, North Carolina from July 2008 through December 2009. All subjects met the following inclusion criteria: presence of moderate to severe periodontal disease (2 or more teeth with at least 6 mm CAL and at least 1 site with PD > 5 mm), receiving dialysis for at least three months, presence of end stage renal disease (GFR $\leq$15ml/min/1.73m^2), English speaking, ability and willingness to give written informed consent for participation in the study, age 18 to 80 years, and presence of twelve or more teeth.

Subjects were excluded if they had the following: any severe co-morbid conditions likely to affect life expectancy within 1 year (for example, metastatic cancer), any condition that would, in the judgment of the clinician or patient's physician, be a contraindication to dental treatment, dementia, pregnancy or lactation, inability to take oral medications, allergy or intolerance to minocycline, tetracyclines or polygycolate polymers, allergy to both penicillin and clindamycin, severe dental caries, pulpal or mucosal disease that would interfere with periodontal therapy, inability or unwillingness to follow the study protocol. Written informed study consent and HIPAA consent were obtained from all subjects prior to study inclusion. The study was approved by the Biomedical Institutional Review Board of the University of North Carolina.

Subjects were blindly randomized (1:1) to either the treatment or control group using randomization codes generated by the study statistician. The treatment group received periodontal therapy following baseline, while the control group received periodontal therapy at the conclusion of the study (6 months). All subjects were appointed for a baseline visit at the General and Oral (GO) Health Clinic, a clinical research facility at the dental school in Chapel Hill, NC. All study appointments occurred on the subject's inter-dialysis day with the exception of peritoneal subjects.

Study Groups

Those randomized to the periodontal treatment group received periodontal therapy following baseline. Those randomized to the control group received delayed periodontal therapy at the conclusion of the 6 month follow up appointment. Systemic oral antibiotics (2mg amoxicillin or 600mg Clindamycin if patient had an allergy to amoxicillin) were administered by the examiner prior to the periodontal therapy appointment only in order to minimize the effect of any transient bacteremia on the patient's dialysis catheter. Antibiotics were not administered at any other appointment. Periodontal treatment consisted of the removal of periodontal organisms and other dental deposits by intensive supra- and subgingival scaling and root planning under local anesthesia by quadrant using hand and ultrasonic instruments as needed. Adjunctive subgingival antimicrobial therapy (Arestin®, FDA approved and marketed by J&J) that provides controlled release of microsphere-encapsulated, biodegradable minocycline was administered to all sites with >5 mm probing depths. Any teeth deemed hopeless (gross decay, severe bone loss, abscess, etc.) were extracted at the time of scaling and root planing in the quadrant. Treatment was done in one

or two appointments by one of three providers (two hygienists and one periodontology resident).

Control group subjects received follow up examinations at 3 and 6 months after baseline; treatment group subjects received follow up examinations at 3 and 6 months after completion of therapy. Those in the treatment group received administration of Arestin® to any site with residual PD >5mm at 3 and 6 month follow up appointments.

Participant Training

All subjects received verbal and written oral hygiene instruction (downloaded from ADA website at http://www.ada.org/public/topics/cleaning_faq.asp on February 4, 2008) during each visit. Each subject was instructed to use a soft bristled toothbrush two times per day using the modified Bass sulcular brushing technique and to floss once per day using the c-wrap technique. A demonstration was given using a large scale dental model and toothbrush and floss as well as in the patient's mouth using a hand mirror. Each subject was asked to demonstrate their understanding by replicating the modified Bass brushing technique and the C-wrap flossing technique in their own mouth.

Clinical Parameters

Examiners were calibrated for accuracy and repeatability against a gold standard. An initial calibration with all examiners was performed followed by yearly re-calibrations. Percent agreement with the Gold Standard was above 90% for pocket depth and attachment loss measurements and Kappa scores were above 0.90. Medical histories were reviewed and

updated at every appointment. The following parameters were assessed on all subjects at baseline, 3 months and 6 months.

Plaque Index (PI): The plaque and stain index was scored for three facial surfaces (distofacial, facial, mesiofacial) and the direct lingual surfaces of each tooth using the Silness and Löe index. The tooth surface to be scored was air dried and not disclosed.

Gingival Index (GI): The gingival index was scored on three facial surfaces (distofacial, facial, mesiofacial) and the direct lingual surfaces using the Löe and Silness Index following a 1mm subgingival sweep.

Probing Depth (PD): A manual periodontal probe (UNC-15) was used to measure probing depth (PD) at 6 sites per tooth for all teeth present in the mouth. PD was measured from the free gingival margin to the base of the pocket, and was recorded in whole millimeters (rounded down to the nearest millimeter.)

Clinical Attachment Level (CAL): Clinical attachment level was calculated for 6 sites per tooth on all teeth present in the mouth using the formula probing depth measurement minus gingival margin to CEJ measurement (where a gingival margin coronal to the CEJ is recorded as a positive number and a gingival margin apical to the CEJ is recorded as a negative number, rounded down to the nearest millimeter.)

Bleeding on Probing (BOP): BOP was assessed and recorded after probing measurements and CAL are taken for each quadrant. BOP was recorded for six sites per tooth for all teeth present in the mouth.

A 14-radiograph periapical series was obtained to assess additional pathology and referrals for treatment were made as needed.

Serum Albumin and IL-6 measurements

Venous blood (2 vials of 5-7 ml each) was collected under sterile technique. Blood was processed into serum within 2 hours after collection: the whole blood was kept at room temperature for 30-45 minutes to allow a clot to form, and then centrifuged for 12 minutes to separate the serum from the clot. Serum was then aliquoted into barcode labeled microfuge tubes and quickly frozen at -80°C and stored until analysis. Serum was blindly analyzed for hsIl-6 by the UNC GCRC BAC lab, and serum albumin was blindly analyzed by the UNC Hospital McLendon Lab. Interleukin 6 was assayed by high-sensitivity enzyme-linked immunofluorescent assay (R&D Systems, Minneapolis, MN). The ELISA results (hsIL-6) were obtained with a SpectraMax M2 from Molecular Devices (Sunnyvale, CA). Serum Albumin was analyzed via colorimetric assay on a dry slide (Ortho Johnson and Johnson, Rochester, NY).

Statistical Considerations

A sample size of 25 per treatment group at six months follow-up was calculated to provide 80% power to detect statistically significant differences in the mean levels of the primary outcomes between the two treatments that are as small as seventy-nine percent of the applicable standard deviation, using two-sided 0.05 significance tests. The estimated standard deviation of serum albumin is 0.53, and the minimum detectable difference is 0.42. These power calculations were based upon a simple two group comparison of a single outcome variable at the six month follow-up visit with the independent t-test.

The main purpose of this study was to compare serum albumin and Il-6 for treatment vs. no treatment in patients with periodontal disease and receiving renal dialysis for at least 3

months. Additional variables considered included age (years), gender (male or female), BMI (kg/m^2), diabetic status (diabetic or not), dialysis type (hemodialysis or peritoneal dialysis), smoking status (current smoker or non-smoker), and a personal medical history of heart disease (known history or no known history).

Statistical analysis was performed using the SAS program (SAS 9.1.3, SAS Institute Inc., Cary, NC, US). Exploratory univariate data analyses were conducted to examine the distributions of periodontal disease parameters, co-morbid variables (diabetes, smoking, BMI, age, race, dialysis vintage and gender), and the outcome variables and to inspect for outliers and missing data. This was done by means of plots (horizontal bar charts, box plots, and normal probability plots) and test statistics. Outliers were detected and checked for accuracy. Analysis with outliers (more than two standard deviations from the mean) excluded did not significantly alter conclusions for any outcome parameter. Analysis included means and standard deviation for continuous variables and percents for categorical variables. Baseline comparisons between treatment groups were done using chi-squared tests and t-tests, as appropriate.

A general linear model (GLM) was used to compare variables for the treatment group to the control group at 3 months and at 6 months, adjusting for baseline. P-values <0.05 were considered statistically significant

.

CHAPTER 4. RESULTS

A total of 342 dialysis subjects were screened for study eligibility; of these, sixty-eight subjects (20%) met all study criteria. Reasons for non-participation, including exclusion criteria are given in **Figures 1 and 2.** Twenty-seven percent (80 patients) of those approached for study screening were not interested in participation and were not screened for eligibility. Interested subjects were verbally screened and consent for an intra-oral screening was obtained for those who met verbal criteria. Of those screened verbally and (if eligible), by intra-oral examination, 26% met all inclusion criteria and agreed to participate in the study. Thirty-seven percent had less than 12 teeth and 10% did not meet age criteria (18-80 years). Seventeen subjects failed to present for the baseline appointment (25% of those who initially agreed to participation). Fifty-one subjects were randomized (15% of approached patients). Twenty-five subjects were randomized to the periodontal treatment group and twenty-six subjects were randomized to the control (delayed treatment) group. Full clinical parameters and serum samples were obtained for fifty-one subjects at baseline, for forty-six subjects at three months and for forty-one subjects at six months (**Figure 1**). Three subjects withdrew because they received kidney transplants, one subject became medically unstable (uncontrolled hypertension), five subjects failed multiple appointments and were dismissed and one subject died due to complications from general anesthesia during cardiac surgery. Twenty-eight total adverse events were reported among twenty-three subjects over the course of the study. Thirteen events were among control subjects and fifteen events were among treatment group subjects. Twenty-four events were unrelated (for example: subject diagnosed

with hypothyroidism). Two mild events were probably related (dentinal sensitivity). Two events were possibly related (increase in probing depth, catheter infection). Ten unrelated events were classified as severe.

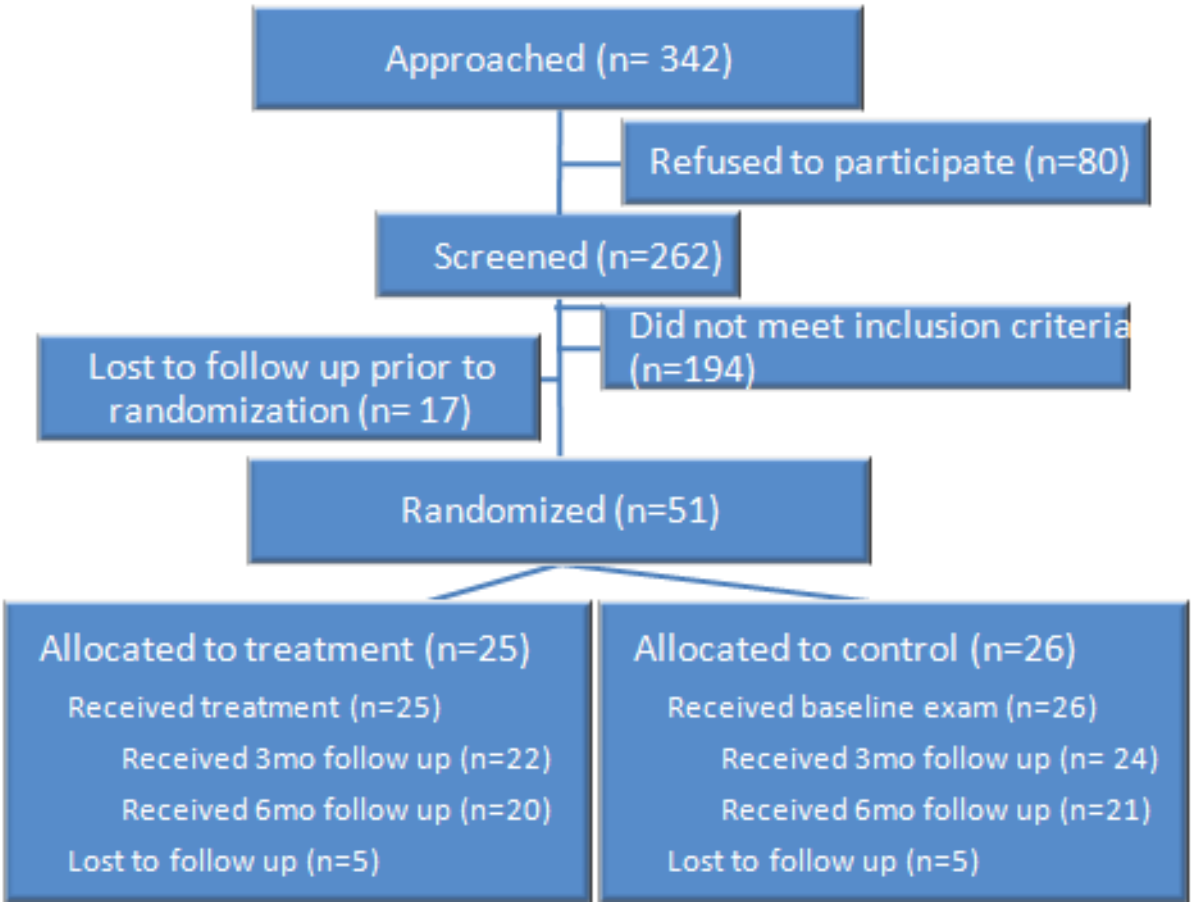

Figure 1. Study summary. All data collected was used in analysis.

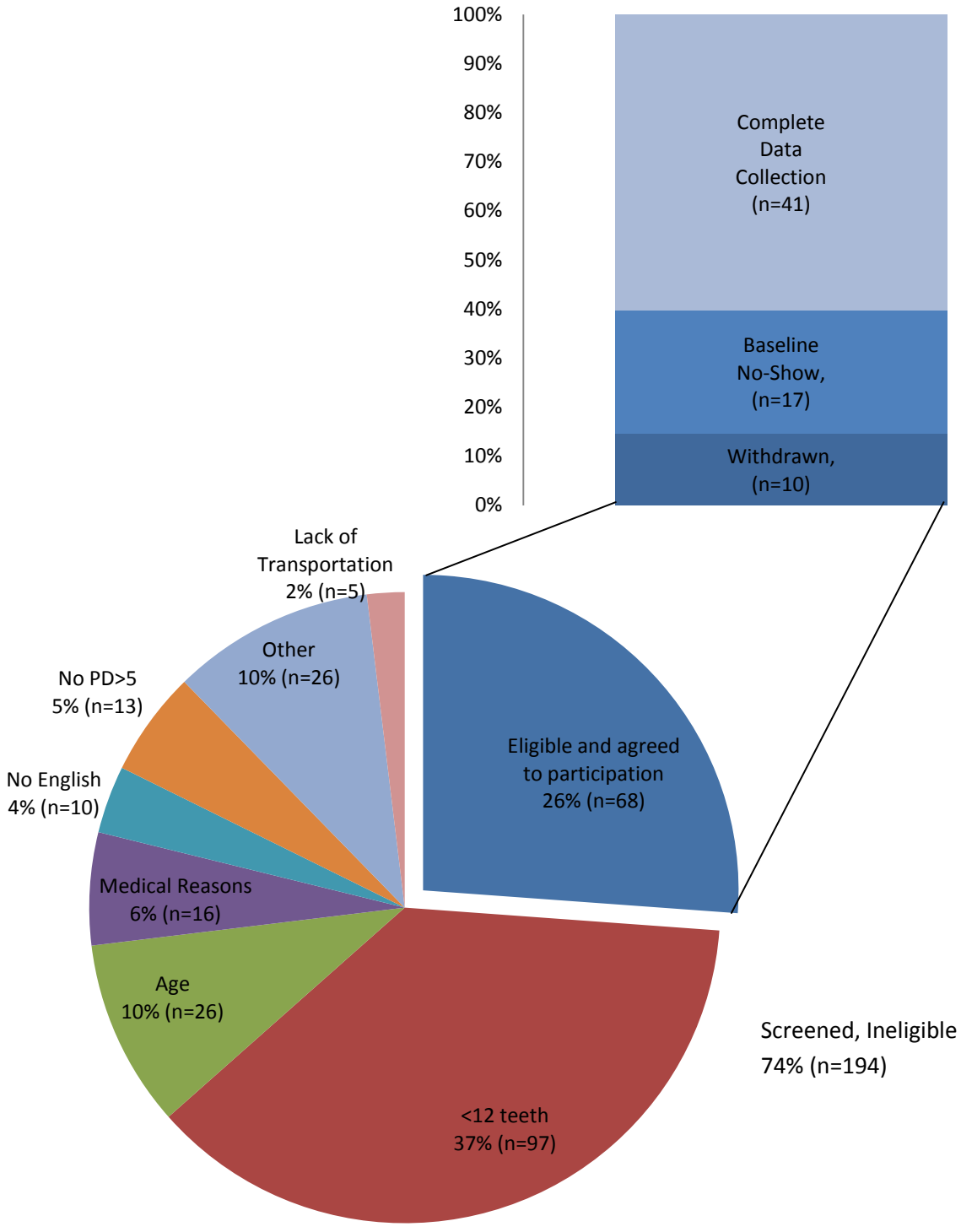

Figure 2. Status of screened subjects

The mean age for participants was 54.1 (±9.7) years and ranged from 30-75 years old. Treatment and control groups did not differ significantly at baseline by any variable measured (**Table 1**). Thirty-eight percent overall were female. Eleven percent of subjects were normal weight by body mass index (BMI), 47% were overweight and 42% were obese (WHO 2004). About one-third of subjects overall reported a history of heart disease. Mean time on dialysis was 42.5 months (about 3.5 years), with a range of 3 months to 176 months. Only 11% were current cigarette smokers. Eighty-five percent were on hemodialysis (versus peritoneal dialysis). The most common cause of kidney failure was hypertension, followed by diabetes. About half were diabetic overall, and it appears the diabetics were unevenly distributed between the control and treatment groups, with the treatment group containing 64% of the diabetics and the treatment group containing only 36%. In other words, 73% of the treatment group were diabetic, but only 39% of the control group were diabetic (p=0.06). This uneven distribution approaches statistical significance and may have played a major role in the results. There were no significant differences between treatment and control group for any other characteristic at baseline.

The overall mean number of teeth per subject was 23 (s.d. 5), mean extent bleeding on probing was 44 % (s.d. 23) and mean extent of plaque score >1 was 90% (s.d. 18). On average, extent clinical attachment loss ≥3 was 41% (s.d. 26). Seventy five percent had 4 or more sites with ≥5mm probing depth. Mean baseline serum albumin was 4.14 (s.d. 0.12) for control group and 4.37 (s.d. 0.11) for the treatment group. Mean hsIL-6 was 6.35 (s.d. 1.54) for the control group and 10.2 (s.d. 3.19) for the treatment group. There were no significant differences between clinical periodontal parameters or serum markers at baseline, however

there were three serum albumin outliers. Excluding the outliers did not change the conclusions.

Table 1. Baseline characteristics of control and treatment group subjects*

Characteristic	Overall Mean (SD) or % (n=51)	Control Group Mean (SD) or % (n=26)	Treatment Group Mean (SD) or % (n=25)	p-values*
Age (years)	54.1 (9.7)	53.9 (10.5)	54.3 (9.15)	0.90
% Female	38	35	42	0.63
% African American	87	87	88	0.48
BMI	31.9 (7.6)	32.3 (6.7)	31.5 (8.42)	0.73
% Diabetic	54	39	73	0.06
% Hemodialysis	85	87	83	0.12
% Current Smoker	11	9	13	0.86
% Past Heart Disease	30	30	29	0.92
Dialysis Vintage (months)	42.5 (43.8)	45.7 (50.7)	38.8 (36.8)	0.59

*Significance tests for comparisons between treatment and control group based on 2-sample t-test for continuous patient characteristics and Pearson's chi-square test for categorical patient characteristics. None were statistically significant at $p<0.05$

Clinical Parameters for treatment vs. control at 3 and 6 months

Table 2 summarizes the clinical periodontal parameters for control versus treatment group at baseline, 3 months and 6 months. GLM models were used at each time point to adjust for baseline values. At 3 months, there was a statistically significant improvement for the treatment group compared to the control group, adjusted for baseline, for the majority of the periodontal parameters: extent of probing depths ≥4mm ($p=0.003$), extent gingival index ≥1 ($p=0.003$), extent plaque index ≥1 ($p=0.006$), mean attachment loss ($p=0.01$) and extent attachment loss ≥3mm ($p=0.01$). By 6 months, however, the difference between groups was no longer present for most variables. Only extent attachment loss ≥3mm was maintained at 6

months for the treatment vs. control group. Extent bleeding on probing and extent plaque score ≥1 were not statistically significantly different between the two groups at any time point. Adjusting for BMI, diabetic status, plaque index, disease level and response to therapy did not significantly alter results.

Table 2: Mean (StdErr) clinical measures for each time point by treatment group

Characteristic	Group	Baseline* (n=51)	3 Months** (n=46)	6 Months** (n=41)	p-value B/L-3m	p-value B/L-6m
Mean PD	Control	2.96 (0.20)	2.85 (0.09)	2.71 (0.09)		
	Treatment	2.84 (0.14)	2.43 (0.10)	2.50 (0.09)	**0.003**	0.10
Extent PD ≥4mm	Control	25.1 (4.72)	21.8 (2.18)	18.5 (2.10)		
	Treatment	23.2 (3.94)	12.1 (2.24)	12.8 (2.05)	**0.003**	0.06
Extent BOP	Control	40.4 (5.66)	38.6 (4.46)	35.7 (4.48)		
	Treatment	46.9 (4.22)	27.9 (4.57)	27.7 (4.37)	0.10	0.21
Extent GI ≥1	Control	95.2 (2.27)	98.3 (2.72)	93.2 (3.29)		
	Treatment	96.6 (1.74)	86.9 (2.78)	89.0 (3.21)	**0.006**	0.36
Extent PI ≥1	Control	87.1 (4.56)	89.5 (3.56)	86.5 (3.65)		
	Treatment	92.3 (3.20)	84.3 (3.64)	86.4 (3.56)	0.32	0.98
Mean CAL	Control	2.72 (0.26)	2.77 (0.13)	2.67 (0.15)		
	Treatment	2.52 (0.20)	2.29 (0.13)	2.35 (0.14)	**0.01**	0.13
Extent CAL ≥3mm	Control	41.9 (5.53)	45.5 (3.61)	44.5 (3.80)		
	Treatment	40.4 (5.40)	31.5 (3.70)	33.9 (3.71)	**0.01**	**0.05**

*No significant differences were found between groups at baseline
**GLM Models adjusting for baseline values.

Serum Albumin for treatment vs. control at 3 and 6 months

Table 3 summarizes the serum parameters for control versus treatment group at baseline, 3 months and 6 months. GLM models were used at each time point to adjust for baseline values. There was no difference between the groups for serum albumin or serum Il-6 at any time point, when adjusted for baseline values. Adjusting for BMI, diabetic status,

disease level and response to therapy did not significantly alter results. No subjects improved from a serum albumin of less than 4 to greater than 4 during the course of the study.

Table 3. Mean (StdErr) Serum Markers for Each Time Point by Treatment Group

	Group	Baseline* (n=51)	3 Months** (n=46)	6 Months** (n=41)	p-value B/L-3M	p-value B/L-6m
Serum Albumin (g/dl)	Control	4.16 (0.11)	4.11 (0.07)	4.03 (0.07)		
	Treatment	4.39 (0.10)	4.10 (0.07)	4.01 (0.07)	0.98	0.83
Serum Il-6 (pg/ml)	Control	6.28 (1.36)	7.38 (1.27)	7.39 (1.14)		
	Treatment	10.2 (3.19)	7.25 (1.27)	7.17 (1.14)	0.94	0.89

*No significant differences were found between groups at baseline
**GLM Models adjusting for baseline values.

Since diabetic status was not evenly distributed between the treatment and control group (p=0.06), further analysis was performed to compare treatment vs. control in diabetic subjects vs. non-diabetic subjects (**Tables 4 and 5**). Baseline serum albumin was significantly higher in the treatment vs. control group among the non-diabetics (p=0.001). No other baseline serum markers were significantly different. For both diabetics and non-diabetics, in the treatment and control groups, there was a non-significant decrease in serum albumin. For serum hsIl-6, while there were no statistically significant differences when separated by diabetic status, the pattern of response is notably different. Specifically, for diabetics, hsIl-6 increased from 3-6 months among the treatment group, but decreased among the controls. Conversely, for the non-diabetics, hsIl-6 decreased in the treatment group and increased in the control group.

Table 4. Mean (StdErr) Serum Markers for Each Time Point by Treatment Group (Non-Diabetics)

	Treatment Group	Baseline	3-Months**	6-Months**	p-value B/L-3M	p-value B/L-6m
Serum Albumin (g/dl)	Control (n=14)	4.16 (0.12)	4.29 (0.09)	4.29 (0.10)		
	Treatment (n=8)	4.90 (0.14)&	4.17 (0.14)	3.95 (0.15)	0.54	0.11
Serum IL6 (pg/ml)	Control (n=14)	7.12 (2.45)	6.54 (1.76)	8.13 (1.58)		
	Treatment (n=8)	8.44 (4.67)	9.23 (2.33)	6.00 (2.33)	0.37	0.46

&p=0.001
**GLM Models adjusting for baseline values.

Table 5. Mean (StdErr) Serum Markers for Each Time Point by Treatment Group (Diabetics)

	Treatment Group	Baseline*	3-Months**	6-Months**	p-value B/L-3M	p-value B/L-6m
Serum Albumin (g/dl)	Control (n=9)	4.11 (0.24)	3.95 (0.13)	3.88 (0.11)		
	Treatment (n=16)	4.10 (0.09)	3.99 (0.10)	3.91 (0.09)	0.82	0.87
Serum IL6 (pg/ml)	Control (n=9)	5.15 (1.10)	8.27 (1.46)	6.16 (1.63)		
	Treatment (n=16)	11.1 (4.27)	6.44 (1.13)	7.70 (1.21)	0.34	0.46

*No significant differences were found between groups at baseline
**GLM Models adjusting for baseline values.

CHAPTER 5. DISCUSSION

In this study, a round of intensive periodontal therapy (quadrant scaling and root planing with local delivery antibiotics) was not associated with an improvement in Il-6 or serum albumin levels at 3 or 6 months, despite improvements in periodontal therapy at 3 months. Most benefits of therapy were lost by 6 months. Only extent attachment loss ≥3mm was maintained at 6 months for the treatment vs. control group. Control subjects also exhibited some improvement in clinical parameters, likely attributable to the Hawthorne effect.

Initial power calculations were based on 25 subjects per group, however, by 6 months, only 20 subjects remained in the treatment group and 21 remained in the control group. *Post hoc* analysis of our data shows that in our sample, the power of detecting a 0.15 observed difference is 0.14. Thus, this study did not have adequate power to detect differences between the groups in serum albumin; the results of this study serve as preliminary and feasibility data and provide insight into future study design.

Patient characteristics may further explain the lack of treatment effect in our study. The ESRD patient population overall suffers from very high levels of multiple chronic diseases. Despite high levels of obesity in this sample, the assembled group appears to be relatively healthy. The average baseline serum albumin for both groups is greater than 4 mg/dl, which is commonly regarded as the goal serum albumin value. Further improvements in albumin levels beyond this goal would likely be clinically irrelevant and difficult to demonstrate. Previous studies have shown an inverse relationship of periodontal disease

severity and serum albumin [83], however, very few subjects in this sample presented with low (<4mg/dl) serum albumin. The high albumin levels may also be a marker for confounders that were not investigated in this study (for example, socioeconomic variables), and may also contribute to the relative healthiness of the population. All participants had the ability to attend multiple dental appointments, which is not necessarily the case for all dialysis patients.

Furthermore, cytokine levels have been demonstrated to be influenced by both age and ethnicity. While still controversial, several studies have concluded that there is an increase in Il-6 with increasing age [86-88] as well as higher levels of Il-6 in African Americans as compared to whites [88]. Our sample had an average age of 54.1 years (s.d. 9.7) and was 87% African American and potentially carry a higher inflammatory burden.

While improvements in periodontal parameters were statistically significant at 3 months, resolution of disease to levels generally considered to be consistent with maintenance of health was limited. Subjects had generally poor home care as indicated by high levels of plaque and BOP at baseline and 3 and 6 months. Improvements in periodontal health gained from treatment were detected at 3 months, but were lost by 6 months as the biofilm re-established. This is likely related to a lack of periodontal maintenance and may account in part for lack of any detectable systemic response in this population, although stratifying by treatment response did not alter results. Positive treatment effects may have been seen at a shorter time interval when plaque and BOP levels were more likely to have been reduced. For future studies, a strict maintenance recall and shorter time interval for assessment may increase the likelihood of detecting treatment differences, which would underscore the critical role of professional biofilm disturbance. Furthermore, subjects were

instructed in the Bass Sulcular brushing technique, which is a relatively complicated technique to master. Use of a simpler brushing technique or use of an electric toothbrush may be a more realistic approach to home care in this population.

A recent study examined the impact of periodontal therapy on estimated glomerular filtration rate in healthy subjects with generalized severe periodontitis [89]. In this study, subjects with initially high plaque (65%) and bleeding scores (53.5%) were able to maintain a low (9%) plaque score and low bleeding score (7% and 5%, respectively) following scaling and root planing at 90 and 180 days with supragingival debridement at 1, 7, 30, 90 and 180 days. In addition, subjects were given pre-therapy OHI and not provided with scaling and root planing until their plaque scores were below 20%. No subjects had renal dysfunction and there was a significant improvement in cystatin C- an alternative estimate of GFR [90] after 30 days. This study suggests periodontal therapy may have the capacity to impact kidney function. However, the change in cystatin c is small and other estimates of kidney function such as creatinine did not change. Furthermore, it suggests that good plaque control and strict maintenance in addition to scaling and root planing may be necessary to elicit changes. Interestingly, this group, similar to the dialysis population, did not demonstrate long-term improvement in systemic markers of inflammation (CRP) following therapy. In contrast, D'Aiuto (2005) did detect changes in systemic inflammatory markers (Il-6) in healthy subjects with generalized severe periodontitis using a similar treatment regimen.

The Periodontitis and Vascular Events (PAVE) study [76] used a similar treatment regimen and found that any periodontal therapy (including community provided therapy) compared to no treatment showed a significant reduction in the percentage of people with elevated CRP at 6 months. However, when the results were adjusted for obesity, the

association was no longer significant. Change in CRP among obese individuals was not observed. Evidence suggests that obesity itself is a chronic inflammatory state, indicated by increased expression, production, and release of inflammation-related adipokines, including tumor necrosis factor-α, Il-6, plasminogen activating factor-1, haptoglobin, and leptin in obese individuals [91-94]. Adipose tissue is one of the main sources of inflammatory mediators in the body [95] and several studies have shown elevated Il-6 levels in obese patients [92]. BMI may also be associated with albumin levels [96-98] and so may also mediate changes in serum albumin. In dialysis patients, BMI is used as a marker of energy nutritional status (energy stores) and is a complementary indicator of nutritional status to serum albumin. It has been independently associated with survival in dialysis patients. The presence of obesity may negate any positive impact of periodontal therapy [76]. Only 11% of subjects in our study were normal weight, with 47% overweight and 42% obese. Eleven percent of subjects had a BMI between 35-39.99 (WHO Class II obesity) and 11% had a BMI ≥40 (WHO Class III obesity). Interestingly, studies show that both male and female dialysis patients in all age groups actually have a lower BMI as compared to a gender- and age-matched general population [99]. Given the abundance and severity of overweight and obese subjects in our study population, it is possible that this interfered with any reduction in Il-6.

Diabetic status is an additional factor which may have played a role in the results. Diabetes is often referred to as one of the most important systemic disease risk factors for periodontitis. Diabetes and periodontal disease have a complex interrelationship, with each impacting the other [100]. An American Academy of Periodontology Commissioned Review [101] notes that "Over 200 articles have been published in the English literature over the past

50 years examining the relationship between these two chronic diseases." Diabetes induces changes in immune cell function and stimulates up-regulation of pro-inflammatory cytokines. This predisposes to chronic inflammation, progressive tissue breakdown, and diminished tissue repair capacity [102]. Reduced wound healing ability has a profound impact on periodontal disease expression as well as on response to periodontal therapy. The level of control of diabetes may also play a role in response to periodontal therapy. Diabetics were not assessed for level of diabetic control for this study. Diabetic subjects were unevenly distributed between the treatment and control groups, with more diabetics in the treatment group than in the control group. Given their diminished wound healing ability, diabetics tend to have a poorer response to periodontal therapy and may have dampened any treatment effects. When stratified by diabetic status, some interesting trends emerge. These trends are not statistically significant, but do show differences that potentially explain why the overall study did not show differences. Baseline serum albumin levels were significantly different between the non-diabetic treatment and control groups and the trends in serum marker levels differ when subjects are stratified by diabetic status. The uneven distribution of diabetic subjects results in more stability for the diabetic treatment group, and the non-diabetic control group. Overall, serum albumin shows a non-significant decrease in all groups, with a slightly larger decrease in the non-diabetic treatment group. Kshirsagar et. al found an association between severe periodontitis and low serum albumin among patients on maintenance hemodialysis therapy, eight individual subjects had low ($\leq$3.5g/dl) serum albumin at least one time point during the study (17%). Four subjects in this study had low serum albumin (<3.5g/dl) at baseline, five subjects had low serum albumin at 3 months, and seven had low serum albumin at 6 months. Three subjects had low serum albumin at every

time point, two subjects had low serum albumin at two time points and three subjects had low serum albumin at only one time point. To our knowledge, no other studies have investigated changes in serum albumin levels following periodontal therapy.

Interestingly, Il-6 also has a non-significant decrease overall. In apparently healthy patients, average Il-6 level was 1.77pg/ml with a range of 0.45-9.96pg/ml . Our sample had an average Il-6 of 6.28pg/ml (s.d. 1.36) in the control group and 10.2pg/ml in the treatment group, which is higher than the average, potentially reflecting an increased systemic inflammatory burden among these subjects. When the treatment and control groups are stratified by diabetic status, there are some conflicting results. Among the diabetics, Il-6 initially fell in the treatment group by 3 months, but then rebounded by 6 months; while among the non-diabetics, Il-6 initially increased in the treatment group by 3 months, and then decreased by 6 months. Other studies show a transient elevation of Il-6 and inflammatory markers following periodontal therapy [103] up to 120 minutes, but longer term studies have conflicting findings on the impact of periodontal therapy on Il-6 and other inflammatory markers in healthy and medically compromised populations [32, 76, 104-107].

Periodontal disease is only one potential source of inflammation in this population with multiple co-morbidities. While periodontal disease is potentially a modifiable contributor to the systemic inflammatory burden, it is likely to require a strict plaque control maintenance protocol and potentially more invasive therapy to improve periodontal status to a level consistent with periodontal health. Furthermore, the influence of other chronic inflammation-related disease may overwhelm the impact of periodontal disease; thus control of systemic factors including obesity and diabetes is critical in assessing the impact of

periodontal therapy on systemic markers of inflammation and metabolism in the dialysis population.

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