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The Effectiveness of Periodontal Therapy on Renal Patients with Chronic Periodontitis

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The Effectiveness of Periodontal Therapy on Renal Patients with Chronic Periodontitis

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BACKGROUND:

Periodontal disease has been investigated as a link to conditions like Diabetes Mellitus, Cardiovascular Disease (CVD), Pre-Term Birth, as well as numerous chronic inflammatory diseases. Chronic Kidney Disease (CKD) has been linked with Diabetes Mellitus, Cardiovascular Disease and early mortality. Alone and combined, Periodontal disease and Chronic Kidney Disease affect millions of people and carry large public health implications. For this reason much research has been developed upon treatment modalities, local and systemic manifestations, prevalence, and risk factors of them both. The purpose of this study is to assess the effectiveness of non-surgical periodontal therapy in CKD with periodontitis.

Epidemiology of Periodontal Diseases:

Periodontal disease is an immune-inflammatory disease caused by the host response to a bacterial insult in the periodontium (27). It is characterized by a chronic, gram-negative infection initiated in the gingiva and leading to destruction of supporting connective tissues and alveolar bone. The host responds to the microbial/LPS challenge, with an abnormally high inflammatory response with increased levels of pro-inflammatory cytokines like IL-1, IL-6 and TNF-α. These mediators promote activation of the acute phase reactants resulting in elevated serum levels of C-reactive proteins (28).

The National Health and Nutrition Examination Survey (NHANES) (1999) survey included over 25,000 participants from 89 sites in 50 states to assess the prevalence of periodontal disease in the United States. Prevalence of Periodontal disease among dentate adults aged 20-64 years was as follows (1):

Demographic	Prevalence
Non-Hispanic White	5.82%
Non-Hispanic Black	16.81%
Mexican American	13.76%

The method used in the NHANES 1999, and 1994 survey was criticized for underestimating periodontitis prevalence by up to 50% (2) due to partial mouth screening, lack of radiographic data, the definition of disease, and the lack of distinction for previous tooth loss. A more accurate assessment is in the range of 47% (54). Eke used NHANES 2010 to assess the prevalence of mild, moderate, and severe

periodontitis. This was more accurate than the NHANES 1999 data set because full mouth probing at all six sites per tooth was employed. According to current guidelines 47% of the population (64 million people) have a form of periodontal disease. 8.7% have mild disease, 30% have moderate disease, and 8.5% have severe disease.

With regards to the prevalence of CP in a CKD population, Ioannidou and Swede (2011) published data showing an increased prevalence compared to the NHANES III data. Furthermore, they showed that there was a significant dose-response association between the prevalence of moderate CP and CKD for Non-Hispanic Blacks and Mexican Americans. In this way, Non-Hispanic Blacks and Mexican Americans with CKD are 30-60% more likely to have moderate CP than those without. The prevalence is shown below:

Demographic	**Prevalence**
Non-Hispanic White	12.9%
Non-Hispanic Black	38.9%
Mexican American	37.3%

The natural progression of periodontitis has been clearly shown to lead to edentulism (3) and compromise patient's quality of life. However, in terms of systemic consequences, periodontitis has been shown to decrease total antioxidant capacity compared to healthy controls (4). Given the established role for reactive oxygen species (ROS) in cardiovascular pathology as well as the recently established links between periodontal disease and cardiovascular disease, the reduced plasma total antioxidant capacity in periodontitis subjects warrants further investigation (4). To that end, Tamaki et al. (2010) has shown that patients with periodontitis had higher baseline oxidized LDL (a direct measure of oxidative stress) and C-reactive protein

(CRP) levels compared to healthy matched controls. Additionally, Tamaki et al. showed that nonsurgical periodontal therapy in patients with periodontitis decreased oxidized LDL and CRP serum levels to the levels of the control baseline (5).

Non-Surgical Periodontal Therapy:

There are numerous studies documenting the changes seen following non-surgical periodontal therapy (NST). The general consensus for outcomes of periodontal parameters is an increase in recession (approximately 1mm), decrease in PD (1-3mm), and a gain in CAL (1mm) (75). Additionally, there is a decrease in BoP and PS, but the ranges reported throughout the literature are less consistent. Effectively a 25% decrease can be seen with oral hygiene instruction alone, but some studies show expected outcome for Bop and PS after NST to be less than 20% (75). Shallow pockets (1-3mm) tend to lose attachment, while deeper pockets (4mm and above) tend to gain attachment (75). 4 years post treatment, Pihlstrom showed on average a 1mm attachment gain in site initially 7mm or more, and a 0.4mm attachment gain in sites that initially measured 4-6mm (83).

Dental biofilm provides an environment for bacteria to populate and thrive while being protected from the host immune response. While not in the scope of this present study, bacterial alterations have been demonstrated following NST. Among other changes, the most notable is a decrease in the red complex bacteria as demonstrated by numerous authors (75, 84).

Chronic Kidney Disease:

Under normal physiological conditions, the kidneys receive approximately 25% of the cardiac output. The kidneys serve multiple functions: control of plasma acid base balance, regulation of fluid volume, synthesis of erythropoietin, excretion of nitrogenous and water soluble waste, metabolizing varied drugs, and long-term control of blood pressure. The kidney's ability to filter the blood is measure by the glomerular filtration rate (GFR). Chronic Kidney Disease (CKD) is chiefly defined as a persistently reduced GFR (53). In 2005 Levey et al. defined CKD as a GFR <60 for 3 months or greater. The functional unit of the kidney is the nephron, and it is ultimately responsible for filtering the blood. As each unit is damaged, the kidney's ability to filter the blood diminishes until ultimately, replacement therapy (dialysis) or transplant is necessary to maintain the patient's life. Kidney function can most accurately be measured by a 24-hour urine collection (29). This method is not practical in many settings, and thus many different equations have been created in order to accurately estimate and assess kidney function.

Given the definition of CKD given by Levey et al., glomerular filtration rate (GFR) is the gold standard measure of a kidney's ability to function. One of the most universally accepted equations to estimate GFR is the Modified Diet in Renal Disease (55):

GFR = 186.3 x (serum creatinine-SCR) $^{1.154}$ X (age in years) $^{-0.203}$ X 1.212 (if patient is black) x 0.742 (if female)

There are shortcomings to this GFR estimation however and in 2009, Glassok called its accuracy into question (29). With estimated GFR (obtained by MDRD) at or greater

than 60 ml/min per 1.73m^2, the deviation compared to the measured GFR (urine collection) was greater than 30%. However, below 60 ml/min per 1.73m^2 the accuracy and precision was high independent of age, sex, race, diabetes, transplant status, and body mass index (31). Thus despite being an estimation, Glassok concluded that the MDRD equation is an appropriate and reasonably unbiased estimation of GFR. The table below presents the Chronic Kidney Disease stages based on the estimated GFR.

Stage	**Description**	**GFR (ml/min/1.73m^2)**
1	Kidney damage w/ normal or high GFR	≥90
2	Kidney damage w/mildly low GFR	60-89
3	Moderate GFR decrease	30-59
4	Severe GFR decrease	15-29
5	Kidney Failure (End Stage Renal Disease)	<15

In 2005, the National Institute of Diabetes and Digestive and Kidney Diseases estimated that by the year 2030, more than 2 million people in the United States will need dialysis or transplantation for kidney failure (stage 5). In 2005, 19 million Americans were in the "early" stages of CKD (14).

For end stage renal disease, dialysis and kidney transplantation are the only two treatment choices for kidney replacement therapy. Given the difficulties of kidney transplantation, dialysis has been important treatment choice. Dialysis was first described and used in 1854 to separate substances in aqueous solution based on different rates of diffusion through a semipermeable membrane [20]. The procedure

can be accomplished by either peritoneal dialysis (PD) or hemodialysis (HD). In peritoneal dialysis (PD), a catheter is permanently inserted into the peritoneum (lining around abdominal contents) through the abdominal wall and sterile fluid is drained in and out a few times each day [21]. Hemodialysis therapy (HD) is performed every two or three days, usually three or four hours each through a vascular access connected to the dialysis device. Vascular access is accomplished by surgically placing an arteriovenous (AV) fistula, a graft or a venous catheter. A well-functioning vascular access is essential to provide efficient dialysis therapy [22].

Oxidative Stress and CKD:

Oxidative stress takes place when the production of oxidants exceeds local antioxidant capacity leading to tissue injury (12). This imbalance of reactive oxygen species (ROS) leads to damage of cell lipids, protein, and DNA, inhibiting normal function (14). Oxidative stress has been linked to systemic inflammation, onset of periodontal destruction, CKD, and CVD (12-14).

Free radicals are produced normally during daily metabolic processes. The electron transport chain involved in respiration within the inner membrane of the mitochondria is perhaps the most ubiquitous (44). In a single day, a single cell can generate between 1 to 3 billion ROS, most of which are cleared by antioxidants. Furthermore, free radicals are generated by neutrophils and macrophages during their respiratory bursts in an effort to kill bacteria (14). Additionally, there are extrinsic sources of radical formation: heat, trauma, ultrasound, ultraviolet light, ozone, smoking, exhaust fumes, radiation, infection, excessive exercise, and even some drugs (43).

Directly, ROS have the ability to damage DNA, lipid membranes, promote apoptosis, and deplete antioxidant stores (10,12,32, 35-40). The unpaired electrons are highly oxidizing and can attack carbon bonds, changing the tertiary structure of proteins. These changes can disrupt function, lead to protein accumulation, and ultimately "age" the cell more rapidly (10). Indirectly, ROS damage cells by exerting effects on the DNA and creating alterations in cellular activity through genetic disregulation (49).

Chronic kidney disease is characterized by a state of chronic inflammation and oxidative stress that has been associated with high mortality rates (50). The progressive loss of kidney function results in the accumulation of uremic toxins like cysteine, and homocystiene. Secondary to this, uremia (as well as hemodialysis) causes an increase in inflammatory cytokines and oxidants (16). Cytokine induced inflammation is implicated in the malnutrition-inflammation-atherosclerosis (MIA) syndrome that is also associated with frequent hospitalization and high cardiovascular mortality rates in CKD patients (50). The MIA syndrome is defined by the interaction between increased pro-inflammatory cytokine levels, malnutrition and atherosclerosis in CKD patients (50). Elevated levels of inflammatory markers such as CRP and IL-6 are known predictors of cardiovascular outcomes in the general population as well as in the CKD population (Rao et al. 2004), where they are linked to hypoalbuminemia, malnutrition, erythropoietin resistance and increased mortality (51, 52). There are many sequelae associated with CVD, but it has been shown that the accumulation of reactive oxygen species leads to damage of the endothelium. This endothelial damage regardless of genesis, is ultimately the pathogenesis of CVD.

Conditions, which promote or increase chronic inflammation and oxidative stress exacerbate the risk for CVD in CKD patients. Libetta et al. showed that greater than 50% of CKD patients die from CVD, namely by CVA. In an effort to assess the CVD risk, Cheung et al. showed that a 45 year old CKD patient is 100fold more likely to die from a CVA compared to an age, sex, lipid profile matched non-CKD control. CKD patients

have lower antioxidant capacity and their condition further increases the ROS burden. In 2002, Himmelfarb reported high correlation between CVD mortality and increased levels of oxidative stress (16).

Uremic patients are characterized by an accumulation of waste products normally filtered out by the kidneys. Uremic oxidative stress is characterized biologically by an increase in lipid peroxidation products and reactive aldehyde groups as well as by increased retention of oxidized thiols (49). Thus, before dialysis is instituted, uremic patients are already experiencing high oxidative stress levels.

A substantial body of evidence has accumulated to suggest that MPO is involved in inflammation and oxidative stress in patients with kidney disease (49). Catalytically active MPO can be released during the hemodialysis procedure, and 3-chlorotyrosine, an oxidative stress biomarker highly specific for MPO- catalyzed oxidation through hypochlorous acid, has been demonstrated in the plasma proteins of dialysis patients but not in that of healthy subjects. Furthermore, important antioxidants such as Thiols, and Glutathione are both depleted in absolute concentration and hyporeactive due to oxidation by way of the cystiene waste present in dialysis patients (49).

Studies demonstrate that there are significantly elevated serum concentrations of C-reactive protein and F2-isoprostane levels in hemodialysis patients compared with patients with normal kidney function (62). Handelman showed in a study with 25 ESRD on HD and 23 healthy controls that the average total esterified F2-isoprostanes

in the ESRD patients was 1.62 vs. 0.27 ng/mL in controls (P =0.001), with no overlap between patients and controls. Interestingly, plasma F2-isoprostanes in diabetic ESRD patients were similar to F2-isoprostanes in patients with other causes for renal failure. In the 13 patients in whom Diabetes was responsible for ESRD, the F2-isoprostanes measured 1.53ng/mL. In the 12 other patients, ESRD was caused by hypertension and other causes; in these patients, F2-isoprostanes were 1.71ng/mL. This was not statistically significant between groups (63).

Handelman also showed that in a subset of 10 ESRD patients who were re-evaluated eight months after the initial measurement, plasma-esterified F2-isoprostanes were not altered by an individual dialysis session (63). This is an important documentation because it clarifies the ability for one to follow F2-isoprostane trends over time as long as there are no changes to dialysis treatment. If the F2-isoprostanes were not as consistent their usage as a biomarker would be more limited.

Himmelfarb argues that the reduced bioavailability of NO and abundant formation of ROS within the vascular wall detected in uremic patients seem to be the key determinants of endothelial dysfunction (16). Thus, oxidative stress plays a key role in pathogenesis of atherosclerosis and CVD. This is particularly pertinent for CKD patients because CVD is the leading cause of death as they experience a significantly increased rate of atherosclerotic complications and prevalence of cardiovascular disease compared to normal controls (15).

F2 Isoprostanes are a family of Arachodonic Acid metabolites that resemble prostaglandins and are regarded as a sign of oxidative stress secondary to systemic inflammation (13). We have chosen, F2 Isoprostanes because they are considered as one of the most reliable and consistent in vivo markers of oxidative stress (9).

They have been shown to be elevated compared to controls in patients with periodontal disease (7). F2 Isoprostanes are formed in vivo and are present in detectable amounts in all normal tissues and biological fluids and do not exhibit normal diurnal variation. Lastly, they are known to substantially increase in animal models of oxidant injury. Considering these elements, F2 Isoprostanes seem to be one of the best available markers of in vivo oxidative stress (8, 10).

Oxidative Stress and Chronic Periodontitis:

It is widely understood that ROS are produced in the periodontal lesion (32-34). ROS can promote bone loss, directly through cellular injury and indirectly through gene transcription alterations (34-42). ROS production is an essential protective mechanism against diseases (Tomofuji 2009), and periodontitis is no different.

Neutrophils and macrophages present in the periodontal lesion produce ROS via the oxidative burst, which is released into the extracellular environment. Unfortunately, ROS are not target-specific, and subsequent damage to host tissue occurs, confirming the critical role the host plays in the pathogenesis of periodontitis.

As mentioned before, oxidative stress is the consequence of imbalance between oxidants (ROS, Free Radicals, etc.) and antioxidants (Glutathione, Ascorbic Acid, etc.). In 2001 Ebersole showed that in deep periodontal lesions Treponema *denticola* (*T. denticola),* Aggregatibacter *actinomycetemcomitans (A.a)* metabolize glutathione, a primary antioxidant to produce hydrogen sulfide (Ebersole 2001) resulting to decrease levels of local antioxidants. Consequently, in periodontal lesion the mechanisms promoting oxidative stress include increasing oxidant production and decreasing antioxidants (40).

This double-pronged attack has been shown to affect more than just the periodontal lesion itself. Multiple authors have, clearly shown increases in systemic oxidative stress in periodontitis patients when compared to periodontally healthy controls.

D'Aiuto (2011) showed that patients with severe periodontitis had higher oxidative stress, and lower total anti-oxidant capacity when compared to periodontally healthy controls. In the same study, the patients were corrected for all confounders such as age, gender, ethnicity, and lipid profiles, allowing direct comparison with regards to oxidative stress. Ultimately, patients with severe periodontitis had an odds ratio of 3.6 for having increased oxidative stress compared to periodontally healthy controls.

In 2009, Tamaki evaluated the systemic oxidative stress in a prospective interventional trial treating mild to moderate chronic periodontitis with non-surgical therapy. 19 patients with PPD of 4mm or greater on 4 teeth were given scaling and root planing as non-surgical therapy with oral hygiene instructions, while 19 controls were given oral hygiene instruction only. At baseline, the two groups showed statistical difference in regards to BoP, PPD, CAL, and ROM levels. In patients with periodontitis, the plasma ROM level was positively correlated with PD, CAL, and the percentage of sites with BOP but not with plaque level. One month after therapy ROM levels were close to the periodontally healthy, age matched controls being reduced by approximately 30%. Ultimately, the results showed that there was a dose dependant response with the decrease of Reactive Oxygen Metabolites (ROM) in regards to the decrease in BoP.

Again, in 2010 Tamaki evaluated systemic oxidative stress in patients with chronic periodontitis. Blood samples were taken from all 44 patients and were evaluated for total antioxidant capacity (Ox-INDEX), ROM, and oxidized LDL (oxLDL). At baseline

the periodontally involved patients were statistically different in regards to BoP, PPD, OxLDL, and Ox-INDEX. 1 and 2 months after therapy clinical parameters showed a positive response, BoP decreased from 30% to 5%, percentage of sites with 4-6mm PPD decreased from 20% to 11%. Ox-INDEX was positively correlated with decrease in BoP as well as a decrease in oxLDL. The levels after therapy did not reach the levels of the healthy controls but it did decrease by 37%, more than previously reported by Montebugnoli in 2005 (47).

BOP is a clinical parameter used to evaluate the level of inflammation present within the periodontal lesion, and as such is a surrogate marker of effectiveness of therapy (48). These studies by Tamaki have shown, in a prospective fashion, that treating periodontitis can positively affect systemic oxidative stress levels in otherwise healthy patients. Both studies showed sufficient changes in clinical parameters indicating that non-surgical therapy was performed well. It is particularly interesting that Tamaki found, in both instances, that BoP was correlated with an improvement in oxidative stress. However, the way that the increased circulating ROS from the periodontal lesion affect general health in patients with periodontitis is still unclear. Beyond directly causing tissue damage oxidative stress can cause changes in gene expression that indirectly leads to tissue damage. Ekuni (2009) used a rat model to experimentally prove that oxidative stress caused by a periodontal lesion could have affects in the vasculature both directly, through tissue damage and indirectly through gene regulation. 8 animals had ligature induced for periodontitis for 4 weeks, while 8 were left untreated. Molars and aorta were resected from both experimental and control

groups to be evaluated for hexanoyl-lysine, ROS and lipid deposits. Descending aorta samples in the experimental group showed lipid accumulation, with increased expression of ROS and oxidative stress-related genes compared to normal controls. There was increased expression of hexanoyl-lysine, ROS and oxidative stress-related genes (including nitric oxide synthases 2 and 3), whereas the superoxide dismutase 1 gene level was down regulated. These findings were unique to the experimental periodontal and aortic tissues. This model showed prospectively the induction of periodontitis leads to increased lipid peroxidation, a measure of oxidative stress, both locally and systemically emphasizing the common biologic link between periodontitis and atherosclerosis.

Diabetes As A Confounding Systemic Disease:

Diabetes mellitus (DM) encompasses a heterogeneous group of disorders with the common characteristic of altered glucose tolerance or impaired lipid and carbohydrate metabolism. DM develops from either a deficiency in insulin production (IDDM/Type 1) or an impaired utilization of insulin (NIDDM/Type 2).

Numerous studies have corroborated the fact that DM II and CP have a bidirectional relationship (66). In 1993 a study by Ternoven, showed that well-controlled diabetic subjects had 2.5% of sites with probing depths ≥ 4 mm compared to 11.2% of sites in poorly controlled diabetic subjects (69). This indicated that poor diabetic control lead to a worsening periodontal condition in regards to clinical parameters. There are a number of biologic complications as to why this may be. Impairment of neutrophil function, either in chemotaxis, adherence, and phagocytosis have all been shown to be present in diabetic patients when compared to healthy controls (70). Neutrophils are the primary response cell in the acute periodontal lesion and their impairment has the obvious consequence of an impaired host response and thus more severe destruction.

Besides the host defense, tissue healing is impaired as well in diabetics. The hyperglycemic state induces glycosylation of proteins, which can impair their function (71). The glycosylation of the basement membrane proteins has garnered much attention as it is the focus for the underlying susceptibility to CVD seen in diabetic patients (72). In regards to periodontal wound healing, the changes in vascular permeability have been hypothesized to impede oxygen diffusion, metabolic waste elimination, PMN migration, and diffusion of serum factors including antibodies (66,

71, 72). Thus the changes in vascular anatomy due to glycosylation decrease immune function and impede healing. With all of this considered it is clear that periodontal status of a diabetic patient is different from a non-diabetic.

With regards to response to therapy, Tervonen et al showed a similar response between diabetic and non-diabetic controls to non-surgical therapy (73). This study had over 75 patients and the reevaluation was completed between 3-4 months after therapy. He showed approximately a 50% reduction of bleeding on probing, and a 33% to 50% reduction in the number of pockets with probing depths of 4 to 5 mm. Ultimately, there was no statistically significant difference in responses between the controlled diabetic and non-diabetic individuals in the study.

When looking at long-term response to therapy, again there has been evidence showing that diabetic patients are able to respond like non-diabetic patients. Lindhe et al. evaluated 20 diabetic and 20 non-diabetic age, gender-matched patients in regards to their response to non-surgical therapy and then again after surgical therapy up to 5 years later (74). Re-examinations regarding plaque, gingivitis, probing depth and probing attachment level were performed 12, 24, and 60 months after the baseline examination. Surgery was performed at sites with remaining 5mm or greater probing depths with bleeding on probing after non-surgical therapy. Interestingly, the frequency of sites that exhibited signs of recurrent disease was similar in the two study groups.

The findings from the examinations disclosed that diabetics and non-diabetics, treated for moderately to advanced forms of periodontitis, during a subsequent 5-year period, were able to maintain healthy periodontal conditions.

Although many studies evaluated the effect of SRP in subjects with DM II, only two studies were found which examined the outcomes of SRP in CKD patients (67, 68). In 2010 Artese et al. (67), evaluated the response of 21 pre-dialysis subjects with CP to SRP and compared that to 19 systemically healthy patients with CP. These groups were matched for gender, BMI, ethnicity, and smoking status. The CKD patients responded similarly to CP (only) patients. Specifically, a significant decrease in gingival bleeding, visual plaque, suppuration, BoP, PD, and AL with no difference between the groups was seen at 3 months post-SRP. In addition, statistically increased GFR was found for both groups. A key distinction in this study is that the CKD subjects were in the pre-dialysis stage. It is reasonable to theorize that these subjects were yet to develop the severe uremic state seen in dialysis patients, and thus were not fully compromised in regards to immune responses and wound healing. Unfortunately, this study did not include F2-isoprostane measurements as an outcome. Thus while showing that we could expect clinical parameters to respond in regards to periodontal parameters and GFR, we don't know have any further knowledge of the oxidative status. Theoretically this could be shown with oxidative status as well.

The other study to investigate the response to NST for CKD was by Graziani et al. (2010). Like Artese, they focused on the renal parameter of GFR and they showed a

significant increase following NST. In regards to the periodontal parameters they showed a mean CAL gain of approximately 1mm with a mean PD reduction of just under 2mm. BoP lowered about 50% and PS lowered, but still remained above 50%. Furthermore, they report these results are consistent 180 days post therapy. As explained previously, these numbers are very consistent with what can be seen in a systemically healthy population. It should be noted that in this study there were no systemically healthy or intervention controls (81).

RATIONALE:

Given the medically compromised status of CKD patients, the goal of this study is to evaluate the effectiveness of initial periodontal treatment in CKD patients with periodontitis. Moreover, we will assess the predictors of periodontal outcomes in this medically compromised population in a multivariate regression model.

Further, we aim to evaluate the effect non-surgical therapy on systemic oxidative stress. Ultimately this will help to elucidate the mechanisms by which CKD and CP may affect each other.

SPECIFIC AIMS/HYPOTHESIS:

Hypothesis:

In this study, we hypothesize that CKD patients with CP will show a positive response to non-surgical periodontal therapy as compared to a control group of CKD with CP that received only supragingival prophylaxis.

Specific Aim:

1) to compare the change of periodontal parameters in a within and between group analysis.

2) to assess the predictors of periodontal outcomes in the test and control groups

STUDY ELEMENTS:

Design:

Single blind randomized controlled trial to evaluate the effect of periodontal therapy on serum inflammatory and oxidative stress markers in CKD subjects.

Population:

The study was approved by the Institutional Review Board (IRB) (#10-092-02). Patients were recruited from the University of Connecticut Dialysis Center (UCDC), the Newington Dialysis Center in Newington, Connecticut, Fresenius Dialysis Center in Forestville, Connecticut, the Springfield Dialysis center in Springfield, Massachusetts, Heritage Dialysis Center in Chicopee, Massachusetts

The nephrologist in each dialysis unit presented the study to the potential participants. IRB approved prescreening forms were filled for each patient. Following this, the study coordinator had a meeting with each potential participant to explain and discuss the IRB-approved consent form. In the session, the patient had the opportunity to ask questions to the study coordinator related to the study. If the patient was willing to participate in the study, informed consent for participation was obtained. The patients were explained that they had to participate in the study for approximately two months, which included the follow-up time period. The study involved a total of five visits, which included consent forms, blood draws, treatment and follow up visits. Only for the dialysis patients in Massachusetts, visits 2 and 3 were combined into one visit. Additionally, the Massachusetts patients were recruited, treated, and re-evaluated all at the dialysis unit in order to minimize attrition rates.

At enrollment, patients were then scheduled for a comprehensive periodontal exam; subsequently they were randomly assigned to the test (scaling and root planing) and control (oral prophylaxis) groups. Consequently patients were then scheduled for a 2 month follow up visit where the comprehensive periodontal examination was repeated and blood samples were taken to evaluate any change as a result of intervention. At the therapy and re-evaluation visits patients were financially compensated for their time.

Inclusion criteria:
1) A minimum of 15 teeth
2) No history of use of antibiotic within the last month
3) No history of periodontal treatment within the last year, and
4) No history of vascular access infection or clotted access within the last month (Hemodialysis patients)

Exclusion criteria:
1) Severe co-morbid conditions likely to affect life expectancy within 1 year (for example, metastatic cancer)
2) Dementia, Pregnancy or lactation
3) Inability or unwillingness to follow the study protocol
4) Smoking
5) Inability to meet any of the inclusion criteria

F2 Isoprostane Quantification:

Plasma F2-isoprostanes were measured by gas chromatography/negative-ion chemical ionization mass spectrometry as described by Morrow et al.(54) The assay is commercially available and it's precision is +/-6%, with an accuracy of 96%. Data are expressed in nanograms per milliliter (ng/mL).(56)

Periodontal Data Collection:

The patients received a full mouth periodontal examination, which includes clinical attachment level (CAL), pocket depth (PD), bleeding on probing (BOP), and plaque

score (PS) at six sites on all teeth (mesio-buccal, buccal, disto-buccal, disto-lingual, lingual and mesio-lingual). Probing depth (PD) was measured from gingival margin to the base of pocket at 6 sites per tooth on all teeth. Recession was measured with respect of the gingival margin position in relation to the cementoenamel junction at six sites per tooth. The averaged whole-mouth number of periodontal lesions, the score for full- mouth gingival bleeding on probing (the number of sites with gingival bleeding on probing divided by the total number of sites per mouth, multiplied by 100), and the score for full-mouth plaque (the number of sites with detectable supragingival dental plaque divided by the total number of sites per mouth, multiplied by 100) were calculated for each patient.

The criteria for periodontitis were:

1. Minimum of 1 site with 5mm or greater PD and
2. Minimum of 2 sites with 6mm or greater CAL.
    - OR
        i. at least 2 interproximal sites with clinical attachment loss (CAL) ≥ 4 mm not on the same tooth
        ii. at least 2 sites with probing depth (PD) ≥ 5 mm not on the same tooth

<u>Systemic markers:</u>

Baseline medical and demographic data will be obtained at the start of intervention. This data will include diabetic status, albumin level, dialysis vintage, age, gender, and ethnicity.

In addition, blood samples were drawn from the arterial end of the vascular access immediately prior to HD initiation for quantification of serum F2 Isoprostane. The samples were spun immediately for 10 minutes in a centrifuge and kept on ice for transportation.

Blood samples were drawn from both treatment groups in the same fashion repeated in 2 months after baseline quantifying the above markers.

Blood samples were drawn at baseline for the periodontally healthy patients prior to HD initiation and any oral manipulation.

CKD patients enrolled with periodontitis were randomly assigned in two groups. Randomization was performed with the use of a computer-generated software in random permuted blocks of randomly distributed varying sizes. The blocks were stratified in the test and control group. The study coordinator was responsible for enrolling and then randomly allocating the patients to test or control groups. All participants and study personnel, including the dentist rendering the treatment, were unaware of the study assignments until the day of the procedure. The provider assessing the final outcomes will be blinded to the treatment group in order to minimize bias. However, for the Massachusetts patients, the provider was not blinded at the time of re-evaluation.

Intervention - Test/Control:

The test group received oral hygiene instructions as well as full mouth scaling and root planning with local anesthesia in two appointments.

The control group received oral hygiene instructions and full mouth supragingival debridement (7). This type of therapy was shown to have no impact on serum markers (7). The control group received full mouth periodontal treatment at the end of 2-month follow-up period.

The subjects will be recalled at 2 months time point, as described before (5, 6). At this point, the medical history will be updated. Additionally, subjects received full mouth periodontal examination by a blinded examiner. Also, blood samples were collected for the quantification of serum F2 Isoprostane levels, prior to oral manipulations.

Primary outcomes:

The change of the clinical periodontal parameters from baseline to 2 months (PD, BOP, PS and CAL) will be measured to evaluate the effectiveness of periodontal treatment in this population.

Secondary outcomes:

Predictors of response to treatment as derived from a multivariate regression model.

Data Analysis:

All data sets were tested for normality using the Shapiro Wilk test. When data was parametric, we used the unpaired student t-test. When the data was non-parametric

the Mann Whitney test was used. A Pearson's correlation will be run with the clinical parameters and baseline medical and demographic data.

In the future an unpaired student's t test will be run, using serum F2 Isoprostanes as the dependent variable and the treatment (test or control) as an independent variable to evaluate the effect of the treatment outcome. Primary response variables were pocket depth, clinical attachment levels, bleeding on probing and plaque index.

For clinical measurements, a patient level response variable was calculated for each parameter by computing the mean scores per patient at baseline and after therapy. The change Δ for every clinical parameter was calculated as the difference between the pre- and the post-treatment value. A p-value of ≤ 0.05 was considered statistically significant.

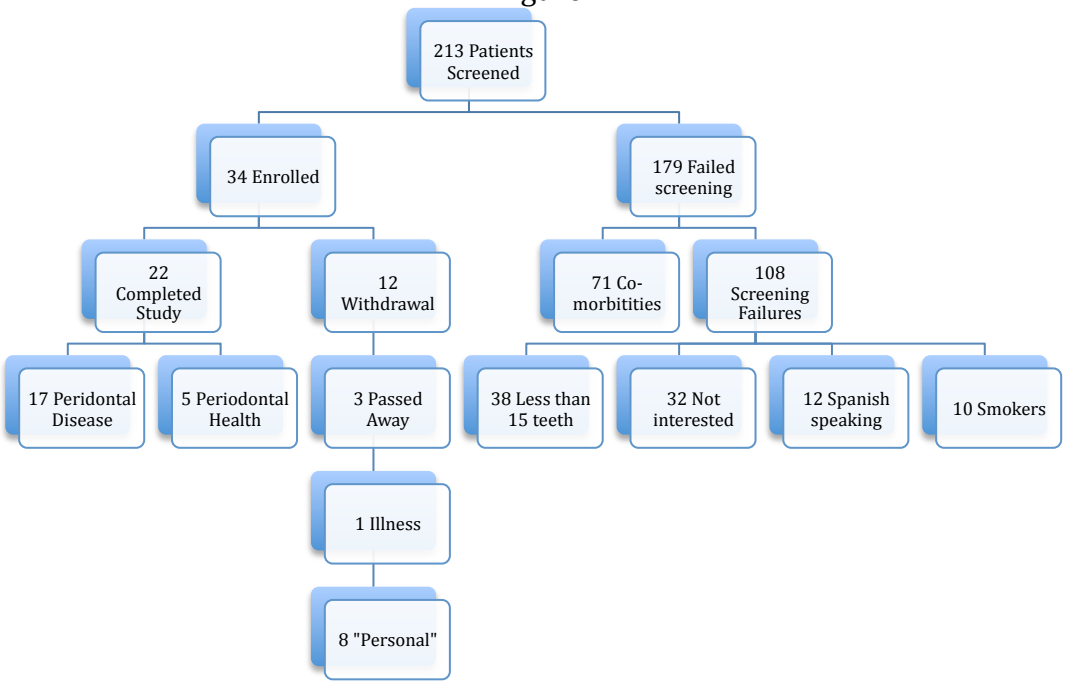
For all analyses the statistical software package (SPSS 18.0, SPSS Inc.) was used.

RESULTS:

Baseline Demographic Data:

The charts of 213 end-stage renal disease patients (ESRD) were screened and 34 were enrolled in the study. 22 HD patients ultimately completed the study. Of these patients, 17 had periodontal disease and 5 were periodontally healthy. The main reason for prescreening failure was co-morbidities and/or failing health, 71 patients. Of the remaining 108 patients who failed screening the reasons were: having less than 15 teeth, 38 (35%), not being interested after learning about the study, 32 (32%), not being proficient in English, 12 (10%), smoking, 10 (5%). The patient recruitment flow chart is shown in Figure 1.

Figure 1



Subject mean age was 59 years (range 44-80) with a median dialysis vintage of 36 months (range 5-84 months). 70% of the subjects were male, 65% of subjects self reported their race as white, 18% as blacks and 17% subjects as Hispanics. 59% subjects had diabetes.

More specifically, the mean age in the test and control groups was 64±14 and 59±17 y/o, respectively. 56% of the test group was comprised of male patients, while 87% were in the control group. Diabetes was 67% of the test and 50% of the control group. Mean tooth number was 24 ±4.5 for both test and control groups. Thus, at baseline when comparing the demographic characteristics between the two groups, there were no statistically significant differences observed.

Baseline Periodontal Data:

We compared the periodontal parameters at baseline by using the Mann Whitney test and Independent t-Test depending on the normality of the distribution as evaluated by the Shapiro-Wilk test of normality. There was no statistically significant difference between the groups at baseline regarding PD, CAL, BoP, PS, and number of teeth (Table 1). The distribution of gender, diabetes, ethnicity, albumin, and dialysis vintage was not significantly different between groups (Table 2). The results and p-values are presented in Tables 1 and 2 below:

Table 1: Baseline Periodontal Data

Baseline Data	Test	Control	p-value
Mean BoP (%)	40	28	0.26
Mean PS (%)	72	75	0.39
Mean CAL (mm)	3.8	3.4	0.63
Mean PD (mm)	3.1	2.8	0.18
% of sites PD > 5mm	11	8	0.92
Mean Teeth Number	24	24	0.88

Table 2: Baseline Demographic Data

Baseline Data	Test	Control	p-value
Age	64	54	0.21
%Male	55	87	0.15
% Diabetes Mellitus	67	50	0.49
% White	78	50	0.13
% Hispanic	0	37	0.13
% Black	22	13	0.12
Albumin (g/dL)	4.0	4.1	0.18
Dialysis Vintage	2.7	3.4	0.44

Within Group Analyses

Test group:

When comparing the periodontal data of the two time points, we found a statistically significant improvement for all parameters. Table 3 below shows the levels of significance reached. With this representation, it becomes clear that non-surgical therapy was effective in improving the clinical parameters. BoP, PS, CAL, PD, and percentage of sites with PD > 5mm were statistically lowered after intervention. No teeth were lost during this treatment phase. This is an expected outcome for scaling and root planing and corroborates with numerous authors (5, 66, 67, 73, 74). It is important to note that while PS did significantly decrease, the absolute post-treatment score, 45% is not a satisfactory outcome of non-surgical periodontal treatment and certainly shows limited patient compliance (80).

Table 3: Within Group Analysis; Test Group

Re-evaluation	Baseline	Post-tx	p-value
Mean BoP (%)	41	23	**0.02**
Mean PS (%)	72	45	**0.02**
Mean CAL (mm)	3.8	3.2	**0.01**
Mean PD (mm)	3.1	2.6	**0.01**
% of sites PD > 5mm (%)	11	3.4	**0.01**
Mean Teeth	24	24	0.38

Control group:

When comparing the periodontal data of the two time points, the only parameter that showed a statistically significant difference was BoP. PS did show a positive trend, however, it did not ultimately reach significance. The trend and change in these two parameters is expected due to all supragingival plaque deposits removal during prophylaxis. Regardless of reaching significance, a PS of 50% is generally not considered acceptable for maintaining periodontal health. The data for pre- and post-therapy along with its significance is shown below in Table 4.

Table 4: Within Group Analysis; Control Group

Re-evaluation	Baseline	Post-tx	p-value
Mean BoP (%)	28	15	**0.02**
Mean PS (%)	75	50	0.09
Mean CAL (mm)	3.4	3.52	0.26
Mean PD (mm)	2.8	2.81	0.84
% of sites PD > 5mm (%)	8	6	0.59
Mean Teeth	24	24	0.35

Between Group Analysis:

After intervention both test and control parameters were tested for normality prior to running statistics. Paired t-test was run for parametric distribution while Wilcoxon Ranks Test was run for non-parametric distribution. Two parameters reached significance after intervention between the test and control groups, Mean CAL (p=0.01) and Mean PD (p=0.04). The test group gained significantly more CAL and significantly decreased PD compared to control. Mean percentage of sites with PD>5mm did show a positive trend, but did not reach significance (p=0.16). Mean BoP, PS, and Teeth Number all did not reach significance, nor showed any trend to favor test or control. Evaluation of the clinical parameters showed an effective non-surgical therapeutic intervention. Table 5 below shows the values of the test and control parameters after intervention.

Table 5: Reevaluation Data

Reevaluation Data	Test	Control	p-value
Mean BoP (%)	23	15	0.85
Mean PS (%)	45	51	0.94
Mean CAL (mm)	3.2	3.52	**0.01**
Mean PD (mm)	2.6	2.82	**0.04**
% of sites PD > 5mm	3	6	0.16
Mean Teeth Number	24	24	1.0

Subsequently analysis was carried out comparing the values of changes within the groups. After testing for normality, the unpaired student t-Test and Mann Whitney tests were used to compare the values of change between the Test and Control group. ΔPD and ΔCAL showed a significant difference between the test and control groups. This confirms the data shown in Table 5, where ΔMean CAL and PD reached significance between the groups (p=0.01, p=0.04, respectively). All other changes in clinical parameters did not statistically change between the groups. Non-surgical therapy therefore showed a significant decrease in PD and gain in CAL as compared to prophylaxis. The values and their levels of significance are shown in Table 6.

Table 6: Reevaluation Data: Test v. Control

Re-evaluation	Test	Control	p-value
ΔMean BoP (%)	-17%	-14%	0.85
ΔMean PS (%)	-26%	-25%	0.94
ΔMean CAL (mm)	-0.6mm	0.1mm	**0.01**
ΔMean PD (mm)	-0.5mm	0.03mm	**0.04**
ΔMean PD > 5mm (%)	-8%	-1%	0.16
ΔMean Teeth	0	0	1.0

The mean PD for the test group decreased (Δ=-0.5mm) to a mean of 2.6mm, but increased for the control group (Δ=0.05mm). This difference between groups was also statistically significant (p=0.04), see Fig. 2. The test group showed an average ΔCAL gain of 0.6mm, to a mean of 3.2mm and this was statistically significant as compared to

both pre-therapy and the control group (p=0.011, p=.01 respectively), see Fig 3. Additionally we ran a Pearson Correlation to substantiate these descriptive statistics. Mean ΔCAL was highly significant (p=0.01), and Mean ΔPD trended (p=0.06). ΔBoP reached significant differences for both test and control (p=.02, p=.015, respectively) post intervention. However, the difference between the two groups did not meet statistical significance (p=0.85). The significant decrease in the pocket depth and increase in clinical attachment levels present in the test group alone confirms the efficacy of the non-surgical therapy in this medically compromised population.

Figure 2.

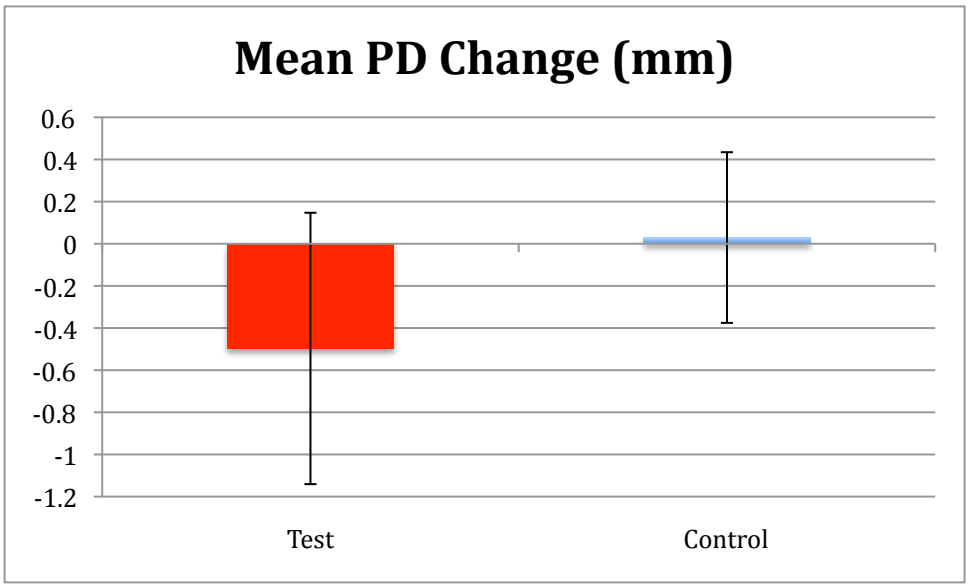
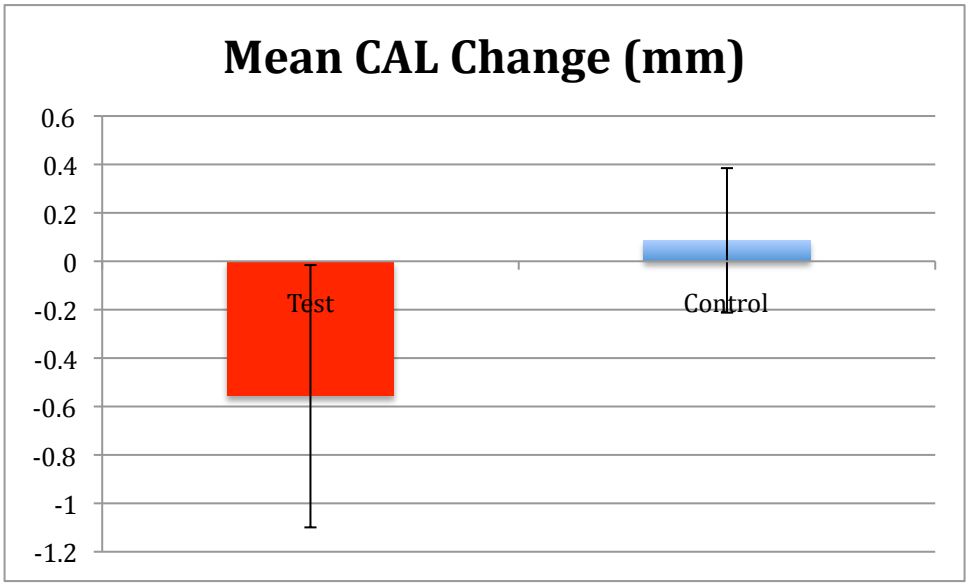


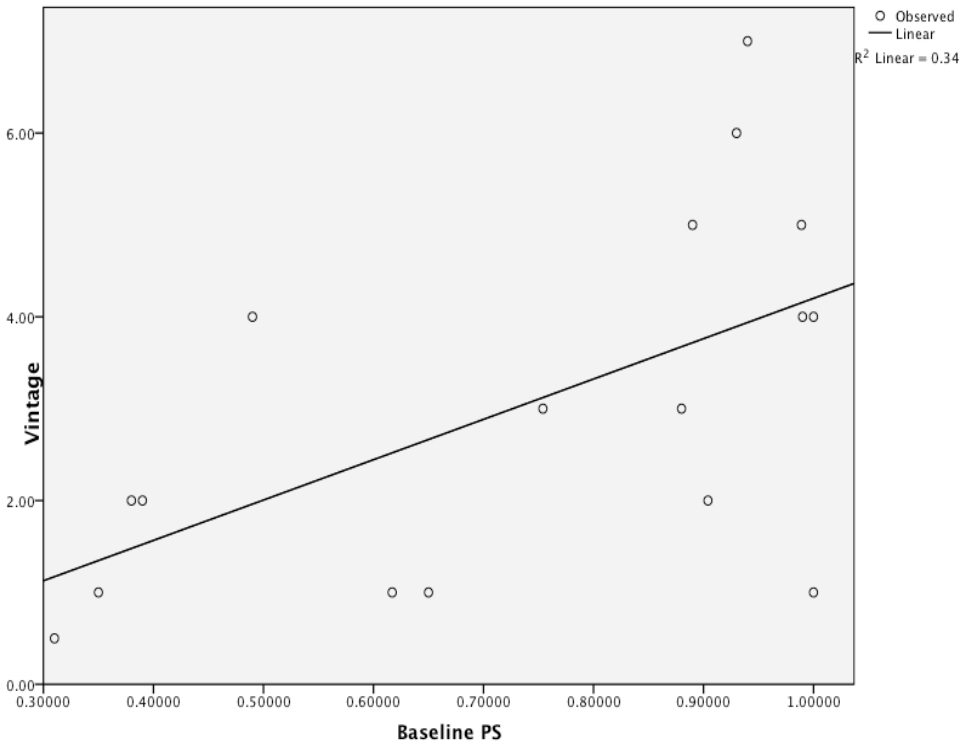
Figure 3.



Bivariate Correlation

Additionally, a Pearson's Correlation model was run to assess the relationship between clinical periodontal parameter and renal parameters. Interestingly, it was found that dialysis vintage was significantly correlated with PS at baseline ($r=0.58$, $p=0.01$). This corroborates with the concept that patients with CKD (and other chronic illnesses) have lower overall compliance. Effectively, the longer a patient has been on HD, the worse their plaque accumulation was. This is shown graphically in the Figure 4 below:

Figure. 4



Albumin, a surrogate marker of HD control, was positively correlated with Mean PD (r=0.5, p=0.04) and Mean CAL change (r= 0.53, p=.0.03). This parallel's the concept seen with DM whereas better control usually leads to more beneficial outcomes to therapy compared to poorly controlled subjects. No subjects were below 3.7g/dL in our population, thus they would generally be considered adequately controlled. Our results show that better HD control, as demonstrated by higher albumin, leads to statistical change in Mean PD (Figure 5) and CAL change (Figure 6).

Figure 5.

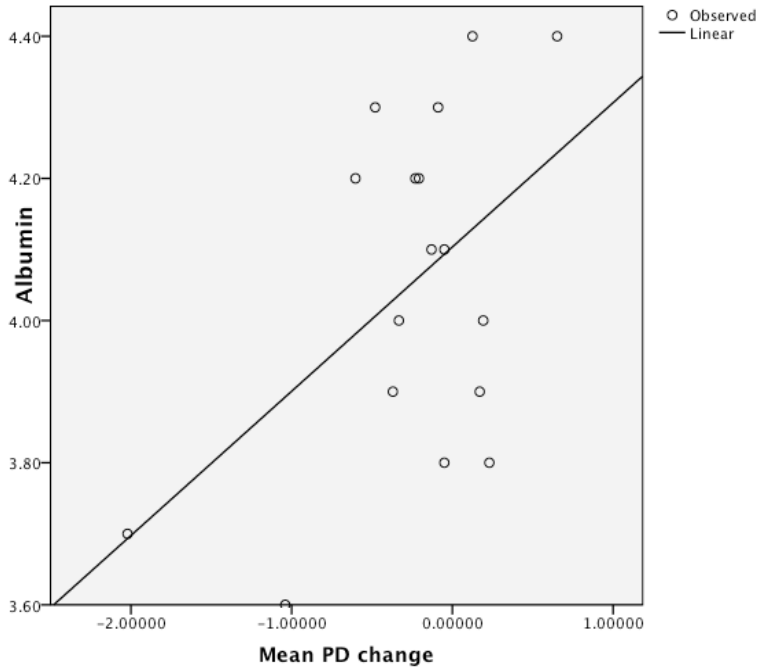
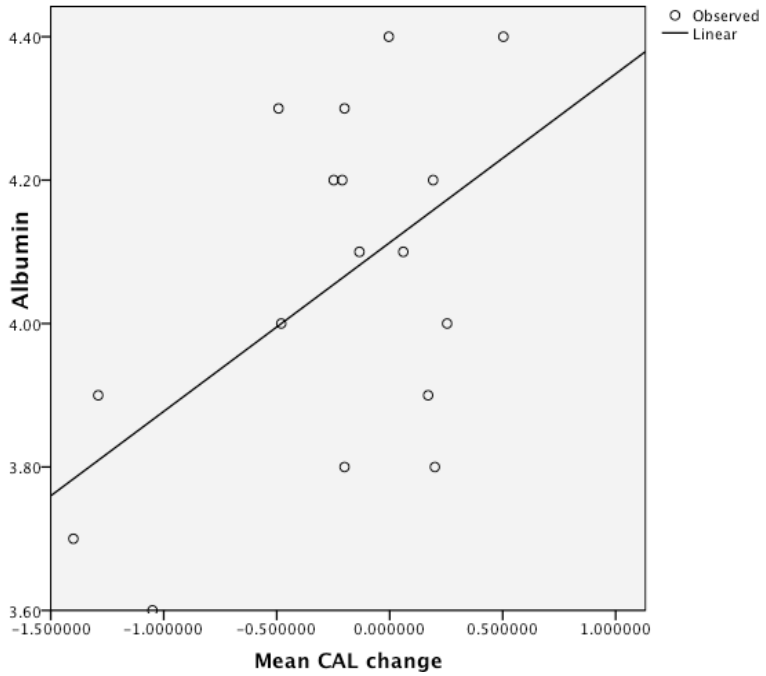


Figure 6.



The change in percentage of sites with PD>5mm had a statistical correlation with many variables. This was fitting as PD>5 is an important parameter. One can assume that with no sites at 5mm or more PD, the patient is a controlled subject with no active disease. Change in percentage of sites with PD>5mm was negatively correlated with Mean PD (r=-0.8, p =0.0001), Mean BoP (r=-0.51, p=0.036), and Mean PD>5mm (r=-0.93, p=0.0001) at baseline. Change in percentage of sites with PD>5mm was positively correlated with the change of Mean PD (r=0.87, p=0.0001), Mean CAL (r=0.75, p=0.001), Mean BoP (r=0.5, p=0.037) at re-evaluation.

DISCUSSION:

The goal of non-surgical periodontal therapy is the removal of etiologic factors in order to achieve reduction of pocket depth and gain of attachment. When this goal is achieved, therapy is considered to be effective. PD is considered as an indicator of existing periodontitis, whereas attachment loss represents cumulative past and present disease activity (12). Thus regaining attachment and decreasing pocket depth are main therapeutic goals when treating periodontal disease. Supragingival debridement or prophylaxis has been shown to not improve clinical attachment levels as compared to scaling and root planing (57). However, previous investigators have found that supragingival debridement does help to address marginal tissue health with decrease in BoP and PS (75).

This trial has shown that non-surgical periodontal therapy can be a safe and effective method in decreasing pocket depth and increasing clinical attachment for CKD patients. Interestingly, there were no adverse events during any treatments and all patients tolerated treatment and post-operative healing without complication, although CKD and DM patients are at higher risk for infections (70, 72, 73). However, this has yet to be understood and fundamentally articulated in literature. It is known that non-surgical therapy and supragingival prophylaxis both induce a transient bacteremia (76). Non-surgical therapy induces bacteremia approximately 40% of the time while prophylaxis (which includes flossing) induces it 30% of the time (76). The investigation used 30 patients with CP and no systemic disease. In our study over 50% of the CKD patients had DM, confirming immunosuppression. Despite this, none of the

population reported acute onset of illness of complications of any kind after either therapy. Furthermore, none of the patients were given antibiotic prophylaxis to guard against such a bacteremia and still there were no reported or recorded adverse events.

Scaling and root planing has been shown to reduce PD, gain CAL, and remove subgingival deposits (57-62). Decrease in PD has been in the range of 1-3mm and a gain of CAL of approximately 1mm (57-62). Generally, deeper initial PD showed greater reduction in PD and gain in CAL compared to more shallow pockets. Prophylaxis, by comparison has been repeatedly shown to have a nominal affect of PD, CAL, and gingival indices (57, 58). Generally, as a result of non-surgical periodontal therapy, we can expect at most a 0.5mm decrease in PD, no gain in CAL, and a 25% decrease in bleeding on probing (57-59).

After non-surgical periodontal therapy we gained 0.6mm of CAL and decreased PD 0.5mm. This has not reached the expectations of previous authors. However, given the complex medical profile of CKD patients, we did not anticipate to reach or to achieve the end points that have been observed in systemically healthy subjects. It is important to note that these numbers are average values. So while 0.5mm may not be clinically significant, we can see from the decrease in PD more than 5mm that the therapy is still effective. It has been shown that deeper pockets tend to respond more favorably to non-surgical therapy (75). In this way, the CKD population, despite their systemic complications, responded parallel to the healthy population. This response has also been shown with DM patients (74).

What is implied with this information is that these patients still require further treatment as there are still pocket depths remaining at 5mm. To that end, Lindhe has shown that well controlled diabetics are capable of maintaining stable clinical attachment level after surgical periodontal therapy. However, with such a high remaining PS, further intervention is not warranted (80).

A positive correlation was found with albumin value and the mean PD Change and mean CAL change. It is generally accepted that albumin levels are to be kept above 3.7g/dL in CKD patients on HD (77). Within reason, higher albumin levels are an indicator of better control in HD therapy, whereas hypoalbuminemia is correlated with higher morbidity and mortality (56, 78). Change in PD and CAL are two important clinical parameters to see effectiveness of therapy. The results infer that better HD control correlates with greater change in PD and CAL, meaning better response to therapy.

The change in percentage of sites with PD>5mm had a statistical correlation with many variables. PD>5 is an important parameter, because one can assume that with no sites at 5mm or more PD, the patient is a controlled subject with no active disease. Most periodontal therapy is aimed at not just decreasing pocket depth, but also decreasing the pockets to less than five millimeters (27, 74, 75). A five millimeter pocket is the cut off point by which therapy is either considered complete, or if more intervention is required (74). The Change in PD>5mm was negatively correlated with Mean PD, BoP

and percentage of sites with PD>5mm at baseline. The negative correlation with Mean PD corroborates with classic literature showing that shallow pockets do not decrease in depth as much as deep pockets (78). The negative correlation with BoP hasn't been described previously, but a plausible biologic explanation is that a subject with a baseline higher mean BoP may be more susceptible to the plaque burden. At first the negative correlation with mean PD>5mm at baseline was a quandary. However, when looking at how the data was collected an explanation came. All PD above 5mm are grouped together. It is generally understood that an 8mm pocket will not respond to therapy by decreasing to 4mm or less. Thus when understanding that a subject with many PD>5mm may in turn have numerous 7-9mm pockets, and these pockets cannot expect to be reduced to a 4mm pocket. It is reasonable then to understand that this is the reason for the negative correlation. This is particularly poignant in this population because we have shown that the gain in CAL and reduction in PD following NST is of a lesser magnitude compared to the historical data.

Mean PD, CAL, and BoP change all correlated positively with the change of PD>5mm. This evidence is consistent with previous reports. Deeper pockets have greater reduction in PD and greater gain in CAL (74, 78). Decrease in BoP is an expected outcome of non-surgical therapy (75). In our study this provides further evidence that the benefits of non-surgical periodontal therapy seen in a CKD population will parallel what is to be expected in a healthy population.

Prophylaxis corroborated with what has been reported. There was approximately 25% decrease in bleeding on probing. There was a small increase in PD, and loss in CAL, although both were not clinically or statistically significant. This contrast from the non-surgical therapy is well documented and thus was expected (75).

Another interesting finding was the statistically significant BoP reduction in both groups, did not significantly correlate with the changes in PS for the control group ($p=0.09$). As both groups received the same, standardized oral hygiene instructions, and had the same baseline plaque score and teeth numbers, we naturally presumed that they would equally improve. PS did trend positively, though, and ultimately it did decrease the same magnitude in both groups. Although not statistically significant, the gender difference between groups could explain these differences. It has been contested in the literature that men tend to have less compliance with oral hygiene (58). Perhaps in this population, the males did practice less oral hygiene, and this explains the discrepancy between the two groups.

Bleeding on probing decreased after treatment for both test and control; however the difference was not significant between the two groups. Plaque score and bleeding on probing typically parallel each other and are surrogate markers for patient compliance. Prophylaxis eliminates the supragingival plaque leading to a healthier marginal gingiva, however this is also accomplished during scaling and root planing. While plaque score did not reach significance, it did trend positively and decreased (in absolute value) similarly to the test group.

Normally, PS is used as a surrogate indicator for patient compliance. While it was not recorded, the general accumulation was severe and it became clear that home care was not given tremendous priority. Oral hygiene instruction was given to each patient and the importance of home care was stressed. However, lack of compliance characterizes individuals with chronic conditions such as CKD, DM etc (79). Hence, lack of compliance in this population was not surprising. Furthermore a positive correlation was found between dialysis vintage and PS. The longer a CKD patient was on HD the higher the PS would be. This further corroborates what has been found in literature that subject with chronic illnesses tend to become less compliant over time (75, 79).

Generally, BoP follows PS because bleeding is a consequence of the bacterial insult. BOP is an indicator of gingival inflammation and active disease (13). Despite a positive trend, without the mean PS dropping significantly we can hypothesize that the bio-burden remained constant and the inflammatory insult did not change. PS for the control group did trend positively, but this result was not expected. One can theorize that BoP did significantly decreased after both test and control intervention, because perhaps both interventions were adequate in maintaining marginal tissue health (59). During the duration of this study, there was a very low enrollment rate (approximately 15%). Approximately 30% of the qualified population was uninterested in the study generally citing difficulty of transport, general health complications, and lack of time as the main reasons for not participating. This has been documented previously in literature where Sergeyeva et al. identified that physical limitations and fatigue

prevent this population from independent transportation, and these are major factors for low research study participation in this population (79). Despite our small available population, our 15% enrollment rate is not much different from the 12% enrollment rate seen by Sergeyeva, who screened 6,276 HD patients.

The results show that amongst patients willing to enroll, the most common reason for disqualification was the lack of an adequate number of teeth, approximately 35%. While there was no data kept on total tooth count per patient who failed pre-screening, it was our observation that many patients were completely edentulous. Tooth loss can be from many reasons, however, the ultimate outcome of advanced periodontal disease is tooth loss. In a population with such heavy co-morbidities and extensive systemic disease, one could reasonably theorize that lack of care, both personal and professional, coupled with a predisposition to periodontal disease could result in extensive tooth loss.

Furthermore, there was a relatively large attrition and withdrawal rate, as 35% of the patients withdrew (12 of the 34). Once enrolled, declining health and mortality were the most common reasons for attrition, 33% (3 patients passed away and 1 patient became too sick to travel). Factors such as socio-economic status, transportation, and dialysis scheduling decrease a patient's ability to participate in the RCT. When performing RCTs in medically compromised populations, one should be prepared to face issues of subject recruitment and retention affecting the study timeline and sample size achievement.

Limitations:

One limitation of our study is the bias, which was introduced by the fact that for patients Massachusetts, the therapy was given in the dialysis unit, not in a dental operatory as done of the individuals recruited in Connecticut. Non-surgical therapy is a tedious and time-consuming labor (57). Specific operational factors could have interfered with the final treatment outcome. First and foremost, the absence of dental chair with a dental light, absence of dental assistant make it significantly more difficult to perform non-surgical therapy. Furthermore, coordinating with the nurses and technicians during the HD session interrupts workflow. Lastly, patient factors such as patient anxiety and discomfort during their dialysis treatment due to fluctuations of electrolytes, glucose, and iron (79) could have interfered with rendering treatment. Cumulatively, all of these factors could have potentially interfered with treatment outcome.

Another limitation is our sample size. With a population of 17 we cannot assume normal distribution for our data points. While tests for normality were employed prior to choosing a parametric or non-parametric test, it is generally accepted that larger sample sizes give more reproducible results and thus more powerful statistics. Given the difficulties already mentioned regarding recruitment, the only way to address this is to extend the enrollment period and obtain enough patients to have a larger sample size.

Future Directions:

Upon obtaining the oxidative stress biomarker, F2-isoprostane, analysis can be done to quantify the affect non-surgical periodontal therapy has on systemic oxidative stress. F2-isoprostane is an excellent biomarker to detect oxidative stress for a HD population because it has been shown to be consistent, not only through the day, but more importantly between HD sessions (from above). Using this information along with the five periodontally healthy CKD subjects we will be able to get a better sense of the background oxidative stress that CP contributes to these patients.

CONCLUSIONS:

1. Non-surgical therapy is a safe and effective treatment in the CKD population.
2. Non-surgical therapy results in significant decrease in PD, BoP, and PS as well as an increase in CAL however, not in the magnitude of a systemically healthy population.
3. Change in PD>5mm correlates negatively with mean PD, BoP, and PD>5mm at baseline.
4. Dialysis vintage correlates positively with baseline PS.
5. Change in PD>5mm correlates positively with the change in mean PD, CAL, and BoP.
6. Albumin values correlate positively with the change in mean PD and CAL implying that better HD control is correlated with better periodontal outcomes.

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