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Periodontal Infections, Inflammatory Markers in Chronic Kidney Disease

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Periodontal Infections, Inflammatory Markers in Chronic Kidney Disease

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DMD, University of Pennsylvania, 2006

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APPROVAL PAGE

Master of Public Health Thesis

Periodontal Infections, Inflammatory Markers in Chronic Kidney Disease

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Dedication

I dedicate this work to my wife Alma, whose love, care and support made it possible for me to overcome hardships and pursue my goals.

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I. General Introduction

A. Chronic Kidney Disease: Overview and Current Concepts

Chronic kidney disease (CKD) is a generalized term for a variety of chronic conditions that result in compromised kidney functions[1]. Under normal physiologic conditions, the kidneys serve several functions: regulation of fluid volume and the acid/base balance of plasma; excretion of nitrogenous waste; synthesis of erythropoietin, 1,25-dihydroxy-cholecalciferol, and renin; and different drug metabolism[2]. Kidney function is assessed by the glomerular filtration rate (GFR), which is estimated by the use of the following formula[3]:

$$\text{GFR (mL/min per } 1.73 \text{ m}^2) = 186 \times (S_{Cr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \\ \times (1.210 \text{ if African-American}),$$

where S_{Cr} is serum creatinine concentration in mg/dL, and age is in years.

Based on the estimated GFR, there are five stages of CKD[3] as presented in Table 1. CKD stage 5, known as End-Stage Renal Disease (ESRD), is defined by $\text{GFR} < 15 \text{ ml/min/1.73m}^2$ and characterized by bilateral, progressive, chronic deterioration of nephrons, which are the kidney functional units. The kidneys are no longer able to maintain normal homeostasis resulting in uremia caused by renal failure, retention of excretory products, and interference with endocrine and metabolic functions[4]. Stages 3 and 4 can also be characterized by uremia, but usually in lower levels than stage 5[5]. Stage 5 is caused by any condition that

could potentially destroy the nephrons, but the most common causes are diabetes mellitus, hypertension, and glomerulonephritis[6].

When the patient is diagnosed with CKD stage 5, the goals of treatment are to maintain quality of life, control disease progression and prevent further complications. At this stage, treatment focuses on dietary modification in an effort to decrease the retention of nitrogenous waste products and control fluid and electrolyte imbalances[7]. When conservative treatment fails and the number of functional nephrons is reduced to the point that the kidneys can no longer filter the blood and adequately remove nitrogen-containing compounds, either renal replacement therapy or renal transplantation is necessary[8].

Renal replacement therapy (dialysis) is a medical procedure that facilitates the removal of water and waste products such as creatinine and urea from the blood. More than 350,000 individuals receive dialysis in the United States at a cost of more than \$7 billion per year[9]. The procedure can be accomplished by either peritoneal dialysis or hemodialysis. Peritoneal dialysis involves infusion of a hypertonic solution into the peritoneal cavity 4 to 5 times per day via a permanently placed peritoneal catheter[10].

The most common type of renal replacement therapy is hemodialysis therapy (HD), with approximately 90% of dialysis patients receiving this form of treatment[9]. HD treatments are performed every two or three days, usually

three or four hours each through vascular access connected to the hemodialysis device. Vascular access is accomplished by a permanent and surgically-placed arteriovenous (AV) fistula, a graft or a venous catheter. From 1996 to 2007, AV fistula use rose from 24% to 47% among HD patients in the US[11]. Despite being a life-saving technique, HD only provides about 15% nephron function[12] and the 1-year survival rate is 78%, while the 5-year survival rate is 28%[13]. The procedure requires a substantial time commitment from the patient and potential complications include potential infection of the fistula/graft site, septicemia, infective endocarditis, cardiovascular disease, hypotension[14], hypoxemia, and arrhythmias[15]. Renal transplantation is a long-term alternative to dialysis, but in itself is also associated with a significant number of complications such as cardiovascular complications[16] and increased susceptibility to infection or malignancies[17] despite offering obvious advantages such as elimination of dialysis time commitment, fewer diet and fluid restrictions, and a better quality of life[12].

CKD is a major global health issue. It is estimated that up to 19 million Americans have CKD and 350,000 are currently on dialysis[18]. Each year, approximately 79,000 new cases of stage 5 CKD are diagnosed—a rate of 1.3 in 10,000 persons. Approximately 60,000 Americans die annually as a result of stage 5 with cardiovascular disease being the main cause of mortality in this population[19].

B. Inflammation: Role in Chronic Kidney Disease and Outcomes

Inflammation is the biological response of tissue to harmful stimuli such as pathogens or irritants[20]. Research has shown inflammation to be a common feature in patients suffering from CKD. 30-50% of dialysis patients showed evidence of an elevated inflammatory response as indicated by high levels of serum C-reactive protein (CRP) [21]. Other studies have found similar results in regards to CRP levels in ESRD patients[22-24].

Identifying the sources of inflammation in CKD patients is still under investigation. Evidence supports the idea that deterioration of renal function is associated with increased serum cytokine levels[25], while other studies showed that increased levels of advanced glycation end products due to decreased renal clearance are contributory factors to vascular inflammation [26]. Persistent infections such as *Chlamydia pneumoniae*[27, 28], clotted access grafts[29], and dental infections[30, 31] have also been suggested as possible sources of inflammatory responses in CKD patients.

Over the past decade, inflammation has been found to play a strong role in the pathogenesis of atherosclerosis in patients with ESRD[32] and associations between inflammation and endothelial dysfunction have been documented[21]. Inflammatory markers such as high sensitivity C-reactive protein (hsCRP) and

the pro-inflammatory cytokine interleukin-6 (IL-6) are elevated in ESRD patients with cardiovascular disease (CVD)[33]. CRP is a powerful risk predictor for the development of CVD[34-36], and this inflammatory burden has been suggested to be a significant contributor to the high CVD-induced mortality rate in dialysis patients[37].

In addition, inflammatory markers including serum CRP and IL-6 have been documented as strong predictors of HD poor outcome. Elevated CRP levels have been associated with increased mortality in both hemodialysis[35, 38, 39] and peritoneal dialysis patients[40], as well as otherwise healthy adults[41]. Elevated serum IL-6 levels have also been shown to predict myocardial infarction in non-renal patients, as well as mortality in the elderly[42, 43].

c. Periodontitis: Association with Systemic Inflammation

Periodontal diseases are chronic, predominantly Gram-negative infections of the oral cavity that are initiated in the gingiva and, if untreated, lead to alveolar bone destruction and eventual tooth loss[44, 45]. Collectively, periodontal diseases affect over 70 percent of the adult population[46].

During the past two decades, a field of periodontal research known as “periodontal medicine” has emerged[47], investigating the link between periodontal disease and other systemic diseases. Recent evidence suggests

that there may be an association between periodontal infections and several systemic conditions including diabetes[48, 49], pneumonia[50], cardiovascular disease[51-54], and adverse pregnancy outcomes[55, 56], although other investigators have found conflicting results[57, 58].

For more than a century, clinical researchers have been investigating the biologic mechanism that could explain the link between periodontal infections and systemic inflammatory diseases. In 1900, Hunter proposed the “Focal Infection Theory”[59], which supports the role of bacterial by-products from a chronic, localized infection that could be disseminated throughout the body and cause disease in other organs[60]. This idea led to preventative full-mouth extractions in patients assessed susceptible to any diseases. This practice was based on anecdotal evidence. Full mouth tooth extraction often failed to prevent systemic diseases[61] confirming that the hypothesis was not valid. As a result, the Focal Infection Theory collapsed by the 1950s[62]. Recently, periodontal research has focused on systemic conditions such as diabetes mellitus, cardiovascular disease, and CKD that have been shown to have an inflammation-linked pathogenesis [33, 63, 64]. The underlying hypothesis is that periodontitis may contribute to overall systemic inflammation and affect the existing systemic disease status. Evidence has shown elevated serum levels of CRP as well as inflammatory cytokines such as IL-6 in patients with periodontal disease [65-67]. The idea that periodontal infections can contribute to systemic inflammation is further validated by evidence indicating that treatment of periodontal disease can

reduce systemic levels of CRP, IL-6[68-70], and improve endothelial function[63, 70-72].

Based on the above, the underlying hypothesis of this research project was developed as shown below: CKD subjects compared to healthy controls have higher prevalence of periodontal infections that alter systemic inflammatory status leading to poor renal outcome.

II. Periodontitis in CKD Patients

A. Objectives

1. **Hypothesis:**

CKD subjects have higher prevalence of severe periodontitis than healthy controls.

2. **Specific Objectives:**

Using a population of stable CKD patients and healthy controls, we aimed to:

- a) Assess the prevalence of severe periodontitis in HD and pre-dialysis patients and compare to healthy controls.
- b) Assess the relationship between periodontal status and renal function.

B. Introduction:

Evidence on the prevalence of periodontitis in the CKD population is conflicting. In a cross-sectional study of 45 dialysis patients, Naugle and co-workers[73] found that 100% of subjects displayed some form of periodontal disease, although the majority of patients (64%) had mild periodontitis or gingivitis. Conversely, Marakoglu et al[74] found no difference in clinical parameters of periodontal disease between 36 chronic renal failure patients on hemodialysis and 36 age and gender matched systemically healthy controls. The authors concluded that chronic renal failure did not seem to be a risk factor for more severe periodontal destruction. More recent studies have reported more severe periodontal destruction in renal failure patients compared to the general population[75]. In addition, CKD subjects are characterized by some well-established risk factors of periodontitis such as poor oral hygiene[76] and diabetes[77]. Because of the conflicting available evidence, it was the goal of this study to further explore the prevalence of periodontitis and its role on systemic inflammation in CKD patients.

C. Materials & Methods:

1. Subject recruitment

Based on studies by Borawski et al.[75] and Chen et al.[76], our power analysis estimated that 27 HD, 27 pre-dialysis CKD and 58 systemically healthy control patients would need to be recruited in order to identify a difference in periodontitis prevalence between HD, CKD and systemically healthy. However, since this was a one-year pilot feasibility study, we limited our sample size.

Twenty-one CKD patients, including twelve hemodialysis (HD) and nine stage 3-4 (pre-dialysis) CKD patients were recruited from the University of Connecticut Health Center (UCHC) Dialysis Unit and Nephrology Clinic after consultation with their nephrologist. In addition, thirteen non-CKD patients were recruited from UCHC personnel and served as healthy controls. Smokers were excluded from the study. The inclusion criteria were as follows: 1) a minimum of 15 teeth 2) no use of antibiotics within the past month 3) no periodontal treatment within the past year 4) absence of systemic infection 5) for the HD patients, no history of vascular access infection within the past month. The study was approved by the UCHC Institutional Review Board (IRB). All patients signed a consent form to enter the study.

2. Data Collection

Medical Data

Medical information of the CKD patients was extracted from hospital records using a standardized extraction form (see Appendix) that included age, gender,

ethnicity, weight, height, diabetes status, history of cardiovascular disease and hypertension, primary diagnosis, medication history including use of anti-hyperlipidemic or non-steroidal anti-inflammatory agents. Additionally, the most recent biochemical data were extracted from the charts including serum albumin, blood urea nitrogen (BUN), creatinine, ferritin level, total iron binding capacity (TIBC), total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and triglycerides. For HD patients, dialysis vintage was recorded. A detailed medical history questionnaire (see Appendix) was used in the control group.

Parameters of Periodontal Status

All patients received a full-mouth periodontal examination, which included clinical attachment loss (CAL), probing depth (PD), bleeding on probing (BOP), and plaque score (PS)[78] at six sites on all teeth. Based in part on work from Armitage[79] and Offenbacher[80], severe chronic periodontitis was defined as a minimum of 2 sites with pocket probing depth (PPD) ≥ 5 mm, associated with ≥ 5 mm CAL with $\geq 30\%$ of sites exhibiting BOP. A subject was considered periodontally healthy if all of the following criteria were met: 1) full-mouth BOP $< 30\%$ of sites, 2) fewer than 2 sites with PPD > 4 mm and 3) fewer than 2 teeth per quadrant with evidence of CAL ≥ 5 mm.

3. Statistical analysis

All continuous variables were tested for normality. When a continuous variable was not normally distributed it was treated with logarithmic transformation to achieve normality. Data were analyzed using Student's t test, ANOVA, and post-hoc Bonferroni testing. A p value of ≤ 0.05 was considered statistically significant. A computer software program¹ was used for the statistical analyses.

D. Results:

1. Population characteristics

Population demographics of the test and control groups are presented in Table 2. The control group had more female and younger participants compared to the test group, although these differences did not reach statistical significance. The test group contained significantly more diabetics than the control group (35.0 percent versus 7.6 percent, $p=0.0001$).

2. General periodontal findings

Periodontal findings between CKD and control subjects are listed in Table 3 and Figures 1-3. Severe periodontitis was found in 47.4 percent of CKD subjects, compared to 15.4 percent of controls ($p=0.02$). Control subjects had significantly

¹ SPSS 16, SPSS Inc.

fewer missing teeth than CKD subjects (mean 4.62 ± 0.56 versus 8.09 ± 3.23 , $p=0.01$). Compared to healthy controls, CKD subjects had significantly higher mean PD (2.66 ± 0.09 mm versus 2.4 ± 0.10 mm, $p=0.03$) and CAL (3.21 ± 0.18 mm versus 2.47 ± 0.10 mm, $p=0.004$) (Figure 2). As shown in Figure 3, CKD subjects had significantly more sites with $CAL \geq 5$ mm (mean 16.80 ± 4.22 percent versus 2.55 ± 1.5 percent, $p=0.001$). There were no significant differences in mean PS and BOP between CKD and control groups, although both parameters tended to be higher in the CKD group.

3. Periodontal findings and dialysis status

The periodontal data of the study population stratified into three groups based on CKD stage (CKD 3-4/Pre-dialysis, CKD 5/HD, Healthy) are shown in Table 4 and Figures 4-6. Although severe periodontitis was more prevalent in the hemodialysis and pre-dialysis groups compared to controls, the difference did not reach statistical significance. There were significant differences in PD ($p=0.049$), CAL ($p=0.002$), $CAL \geq 5$ mm ($p=0.016$), and missing teeth ($p=0.030$) among the groups. Pre-dialysis patients had significantly more missing teeth than healthy controls. HD subjects had significantly higher mean PD (2.80 ± 0.11 mm versus 2.4 ± 0.10 mm, $p<0.05$), CAL (3.32 ± 0.19 mm versus 2.47 ± 0.10 mm, $p<0.05$), and $CAL \geq 5$ mm (22.01 ± 6.49 percent versus 2.55 ± 1.5 percent, $p<0.05$) compared to control subjects. Pre-dialysis subjects also had higher PD and CAL compared to controls, but this difference only reached significance for CAL (3.06 ± 0.244 mm

versus 2.47 ± 0.10 mm, $p < 0.05$). There was no statistical difference in mean BOP or PS among the three groups ($p > 0.05$).

E. Discussion

In our study, severe periodontitis was prevalent in 47.4 percent of CKD patients, compared to 15.4 percent of controls. This difference was statistically significant. Additionally, when comparing continuous periodontal parameters, mean PD and CAL emerged as statistically significant. The difference in the mean values of continuous periodontal parameters (CAL, PD) between CKD and healthy groups was explained by the significantly higher percentage of diabetics in the CKD group as compared to the control group that has been shown to positively affect severity of periodontitis[81]. A possible solution to controlling this variable could be to exclude diabetics in our study population, although this would limit the number of CKD subjects as diabetes is the leading cause of renal failure, accounting for 44 percent of new cases in 2005[82]. Chuang and co-workers[83] compared 43 diabetic with 85 non-diabetic ESRD patients on HD. Decreased salivary flow and pH was associated with increased caries rates in the diabetic group but no difference was reported for gingival inflammation or periodontitis.

While the CKD group had a higher prevalence of periodontitis, BOP was similar among the groups. This is likely due to the similar plaque scores between CKD and control patients, as presence of plaque has been associated with gingival inflammation, and mechanical removal of plaque is followed by clinical changes associated with healthy gingiva[84]. In spite of similar plaque scores, CKD patients had more periodontal destruction than healthy controls. This can be explained by the significantly higher percentage of diabetics in the CKD group, as diabetes is an established risk factor for gingivitis and periodontitis[48, 85]. It is unknown whether the dental plaque of CKD patients differs in composition from that of healthy controls, but studies comparing plaque pH of children with chronic renal failure compared to renal transplant or healthy children have shown the plaque from children with ESRD to be significantly more alkaline. It has been postulated that this difference in plaque pH may account for the low prevalence of caries in the CKD population[86], but it is unknown if this affects periodontal status. Also, CKD patients on HD are characterized by uremia, which has been associated with defects in lymphocyte and monocyte function[87]. Lymphocytes and monocytes play important roles in immune response, and defects in the function of these cells have been associated with aggressive forms of periodontal disease[88] and therefore may play a role in the increased amount of periodontal destruction seen in these patients.

The data on the prevalence of periodontitis in CKD populations are conflicting. Direct comparison of results to other studies is difficult owing to the different

definitions of periodontitis used by different research groups. The periodontitis definition in this project was selected based on the current classification system used by the American Academy of Periodontology[79] that defines severe periodontitis as the presence of a single site with CAL \geq 5mm. BOP was added to this definition because a recent classification system [80] that identified new clinical and distinct biologic phenotypes at the biofilm-gingival interface (BGI) based upon DNA checkerboard analyses of plaque bacteria, serum immunoglobulin G (IgG) titers to bacteria, and gingival crevicular fluid (GCF) levels of inflammatory mediators includes BOP as one defining parameter. For example, a BGI-deep lesion/moderate bleeding was defined as one or more sites with PD \geq 4mm and BOP extent scores between 10 and 50 percent.

The prevalence of severe periodontitis did not differ significantly among control, hemodialysis, and pre-dialysis groups. Compared to HD patients, there were no significant differences with pre-dialysis subjects in regard to any of the periodontal parameters measured. Pre-dialysis patients had significantly higher mean CAL compared to controls. These results are different than those from Borawski[75], who found significantly higher mean CAL among hemodialysis patients compared to pre-dialysis patients. A direct comparison of prevalence of periodontitis to our results could not be performed owing to differences in the definition of periodontal disease, but pre-dialysis patients in Borawski's study had significantly higher community periodontal index and treatment needs (CPITN) scores than those patients on hemodialysis or controls, indicating an increased

prevalence and severity of periodontal disease in this population. The differences in sample size compared with our pilot study could also account for the conflicting results. Further large-scale studies with a standardized approach to evaluating the periodontal status of pre-dialysis populations are needed.

The cross-sectional design of this study prevents us from determining any cause-effect relationships between periodontal disease and chronic kidney disease. It is possible that this association may be due to a direct cellular effect on the nephron unit or its vasculature by periodontal pathogens. While a direct cellular invasion of periodontal pathogens in renal tissue has yet to be shown, periodontal pathogens have been shown to invade coronary and aortic endothelial cells[89, 90]. Furthermore, periodontitis patients have been shown to have chronic and recurrent episodes of low-level bacteremia[91, 92]. Some have hypothesized that bacteria may be filtered out of the blood at the glomerulus, where these organisms or their products may invade capillary endothelium or mesangial cells/matrix[93]. Future studies are needed to confirm the role of periodontal pathogens on renal tissue.

III. Serum Inflammatory Markers in CKD Patients

A. Objectives:

1. Hypothesis:

Inflammatory periodontal disease in CKD patients contributes to systemic inflammatory response, which may affect clinical outcomes.

2. Specific Objectives:

Using a population of stable CKD patients and healthy controls, we aimed to:

- a) Assess the levels of the systemic inflammatory markers, IL-6 and CRP, in the presence or absence of periodontal infection in HD and pre-dialysis CKD populations compared to healthy controls.

B. Introduction:

The role of inflammation in the pathogenesis of periodontal disease and other systemic diseases has been documented[94-96]. Although the exact pathogenic mechanism remains unclear, one hypothesis is that elevated amounts of inflammatory cytokines are released locally from the diseased periodontium into the bloodstream, leading to an increase in serum cytokines at the systemic level, thus contributing to disease outcomes. Diseased periodontal tissues have been shown to possess higher levels of IL-6 than healthy tissues[97, 98], and serum CRP has been shown to be elevated in patients suffering from periodontitis[99]. Additionally, evidence has shown that treatment of periodontal disease may be associated with reduced serum levels of inflammatory markers and corresponding improvement in glycemic control[100] and endothelial function[63], although the evidence is conflicting.

The leading cause of mortality in CKD patients is cardiovascular disease, regardless of CKD stage [101, 102] [103]. Elevated levels of inflammatory markers such as CRP and IL-6 are known predictors of cardiovascular outcomes in the HD population[104], and are linked to hypoalbuminemia, malnutrition, erythropoietin resistance and increased mortality[105, 106].

It has been recognized that approximately 30-50% of CKD[107] [108] patients have evidence of a systemic inflammatory response which may be caused by

persistent infections[27]. Since periodontitis as a chronic persisting infection has been associated with systemic elevation of inflammatory markers [99], it may also predict poor outcomes in CKD populations. Therefore, we hypothesize that severe periodontitis may represent a “non-traditional” risk factor for elevated systemic inflammation in CKD populations.

C. Materials & Methods:

1. Subject recruitment

Based on studies by Borawski et al.[75] and Rao et al.[104], our power analysis estimated it would be necessary to recruit 43 HD, 43 pre-dialysis, and 15 healthy controls for CRP assessment and 29 HD, pre-dialysis, and healthy controls for IL-6 assessment. For the purposes of this analysis in this pilot feasibility study, the same group of subjects described in Objective 1 was used. The subjects signed a consent form that was approved by the Institutional Review Board (IRB) as described before. Medical and biochemical data were retrieved from the medical records as described in Objective 1.

2. Blood sampling and analysis

In HD subjects, blood samples were drawn from the arterial end of the vascular access immediately before initiation of the HD and then stored at -70° until

assay. In pre-dialysis and control subjects, 15 ml venous blood was collected prior to the oral clinical examination by a certified phlebotomist.

Within 2 hours of blood collection, sera were separated after clotting for 30 minutes at 4°C, followed by centrifugation at 3,000 x g for 15 minutes. Aliquots were stored at -80°C until testing. Sera were coded and analyzed in duplicate by *ELISA*¹ without the knowledge of the periodontal disease or CKD status of the study participants. The IL-6 and CRP assay analytical sensitivities were 2.0 pg/ml and <0.3 mg/L, respectively, and the variation in protein values within runs was <1% for both assays.

3. Statistical analysis

Continuous variables were tested for normality and logarithmically transformed if skewed. IL-6 and CRP levels were compared among the groups by Student's t test, ANOVA, and post-hoc Bonferroni test. Pearson's correlation coefficients were calculated to test the associations between serum CRP, serum IL-6, and periodontal variables. Any variables that had an association with $r \geq 0.5$ were included at the multivariate regression analysis as confounders. A multivariate linear regression model was run, using serum CRP and IL-6 as dependent variables and testing periodontitis as a predictor variable and adjusting for confounders based on the significance of the correlation coefficient. A p value of ≤ 0.05 was considered statistically significant.

¹ Diagnostic Products, Los Angeles, CA.

D. **Results:**

1. Serum inflammatory markers and CKD status

Serum CRP and IL-6 levels for control and CKD patients are listed in Table 5 and shown in Figures 7 and 8. CKD patients had a higher mean CRP levels (median 3.87, range 0.30-78.35 μ g/ml) compared to controls (median 1.41, range 0.42-18.00 μ g/ml). This difference was statistically significant ($p=0.014$). Similarly, IL-6 levels were higher in CKD patients (median 8.72, range 0.60-9.92pg/ml) when compared to control subjects (median 4.00, range 2.00-5.82pg/ml). This difference was also statistically significant ($p=0.038$).

Table 6 and Figures 9 and 10 show CRP and IL-6 levels in subjects according to renal status (hemodialysis, pre-dialysis, and controls). There were significant differences between the groups for both CRP and IL-6. In regard to serum CRP levels, control subjects (median 1.41, range 0.42-18.00 μ g/ml) had lower values than hemodialysis (median 5.78, range 1.87-41.80 μ g/ml) and pre-dialysis (median 2.48, range 0.30-78.35 μ g/ml) patients. This difference reached statistical significance for HD patients only ($p<0.05$). There were no significant differences between hemodialysis and pre-dialysis groups ($p>0.05$). Serum IL-6 levels were significantly elevated in hemodialysis patients (median 10.98, range 6.01-29.35pg/ml) compared to pre-dialysis patients (median 4.00, range 0.60-9.92pg/ml, $p<0.05$) and control subjects (median 4.00, range 2.00-5.92pg/ml, $p<0.05$). There were no significant differences between pre-dialysis and control subjects ($p>0.05$).

2. Serum inflammatory markers and periodontal status

Table 7 and Figures 11 -12 show CRP and IL-6 levels in subjects stratified based on the periodontal status. Subjects with severe periodontitis had lower serum CRP levels (median 2.935, range 0.21-41.8 μ g/ml) than periodontally-healthy subjects (median 3.26, range 0.42-78.32 μ g/ml), but this difference was not significant ($p>0.05$). In contrast, IL-6 levels in subjects with severe periodontitis (median 6.175, range 2.00-29.35pg/ml) were significantly higher compared to subjects without periodontitis (median 4.63, range 2.00-11.35, $p<0.05$).

3. Association between inflammatory markers and periodontal parameters

Correlations between serum IL-6/CRP levels and periodontal parameters are presented in Table 8. Both serum IL-6 and CRP levels were significantly related to PS ($r=0.385$; $p=0.039$ and $r=0.372$; $p=0.047$, respectively). There was a trend toward significance for the associations between serum IL-6 levels and CAL ($r=0.281$, $p=0.140$). In contrast to IL-6, serum CRP levels were highly related to CAL ($r=0.500$, $p=0.006$) as well as $CAL\geq 5\text{mm}$ ($r=0.409$, $p=0.027$). There was also a trend toward significance for the relationships between serum CRP levels and BOP ($r=0.294$, $p=0.122$) and PD ($r=0.307$, $p=0.106$). Furthermore, there was a significant correlation between periodontal status and serum IL-6 ($r=0.441$, $p=0.045$) as well as CRP and IL-6 ($r=0.450$, $p=0.041$) among CKD patients.

Multivariable linear regression analysis models were constructed to assess the interaction between medical and periodontal parameters and serum IL-6 and CRP levels in the CKD patients. Using serum IL-6 as a dependent variable (Table 9), HD status was a significant predictor for elevated serum IL-6 levels ($p=0.029$), but periodontal status was not ($p=0.350$). The interaction between HD and periodontal status was also not significant ($p=0.161$). When serum CRP was used as the dependent variable (Table 10), there was a significant effect of gender ($p=0.028$) and BMI ($p=0.013$), and dialysis status was a significant predictor of elevated serum CRP ($p=0.012$), but periodontal status was not ($p=0.432$). The interaction between HD and periodontal status did not have a significant effect on serum CRP levels ($p=0.256$).

E. Discussion

In our study, both serum CRP levels and IL-6 levels were higher in CKD subjects compared to controls. CKD is characterized by a chronic state of inflammation[33], so these results were expected and are consistent with those reported by others[22-24]. The sources of inflammation in CKD patients are still under investigation, but clotted access grafts[29], atherosclerotic processes[109], and persistent infections[27, 28] have all been suggested to be contributory factors. Residual renal function may also have an important role in the inflammatory process. For example, serum IL-6, IL-8, and TNF-alpha levels have been shown to be elevated in HD patients compared to healthy

controls[25]. In addition, patients on peritoneal dialysis have been reported to have increased CRP levels during the first year of dialysis[110]. Animal studies also suggest a reduced clearance of pro-inflammatory cytokines when renal function is impaired[111, 112].

Serum CRP levels were significantly higher in HD patients compared to controls. While pre-dialysis patients had higher CRP levels compared to controls, this difference did not reach statistical significance. Other studies have found CRP levels to be elevated in pre-dialysis patients[113], and several researchers[113-115] have found an inverse relationship between GFR and CRP levels in pre-dialysis patients. The exact reason why there was no significant difference in CRP levels between HD and pre-dialysis patients cannot be explained. However, the incidence of elevated serum CRP levels has been shown to be different among different geographical populations[116], indicating that causes other than dialysis-related factors such as nutrition and lifestyle can also play a role in serum CRP levels. Our limited sample size could also explain these trends as well as our inability to discern a statistically significant difference between the two CKD populations.

Serum IL-6 levels were significantly higher in HD patients compared to both pre-dialysis patients and healthy controls. Our findings of increased IL-6 levels in HD patients is in agreement with others who have found plasma IL-6 to be elevated in these patients[117, 118]. The lack of a statistically significant difference in IL-6

levels between pre-dialysis patients and controls in some of our analysis is in contrast to what has been reported by others[119].

IL-6 levels have been shown to predict mortality in both HD and pre-dialysis patients[42, 104, 118] and may be a better predictor of mortality than CRP[119]. This leads one to question whether a medical or pharmacological intervention can alter this cytokine imbalance to improve outcomes. Statins and ACE-inhibitor drugs administered to CKD patients can lower IL-6 levels in both non-renal and CKD patients[120-123], but it is unknown if this affects mortality outcomes.

As expected, serum IL-6 levels were higher in patients with severe periodontitis compared to subjects without periodontitis. This is consistent with the findings reported by others[97, 98]. While there were statistically significant differences in IL-6 levels between periodontitis and non-periodontitis groups, this was not the case for CRP levels. The finding that CRP levels were higher in non-periodontitis patients compared to periodontitis patients is unexpected. Other authors[99] have shown CRP to be elevated in patients suffering from periodontitis, and treatment of periodontitis has been shown to decrease CRP levels in systemically healthy patients[68], so our findings are inconsistent with previous reports. This could be accounted for by our limited sample size (n=33) coupled with the limited number of patients displaying severe periodontitis (n=12). The mechanism for the differences in serum inflammatory mediators in the presence

of diseased and healthy periodontal tissue is still under investigation. One possibility is that these mediators are produced from the periodontium itself. It has been shown that gingival fibroblasts from chronic periodontitis lesions produce greater amounts of IL-6 than healthy controls[97]. IL-6 has also been found to be elevated in the gingival crevicular fluid[124] and serum[68] of chronic periodontitis patients. This may be due to polymorphisms resulting in an increased expression of the IL-6 (-174) gene in the presence of periodontal infections[125]. It has been hypothesized that this increase in pro-inflammatory mediators could theoretically spill over into the circulation, thus inducing systemic effects[126].

Both serum IL-6 and CRP levels were significantly correlated with plaque scores. It has been postulated that high-responder individuals produce high levels of inflammatory mediators and cytokines as part of their host inflammatory response to the presence of plaque[127, 128] and, consequently, these individuals are more susceptible to periodontitis. Based on this evidence, however, we would not expect a direct relationship between inflammatory mediator level and plaque score. Other authors[74] have found no difference in gingival index scores between chronic renal failure patients on HD and systemically healthy control patients with similar levels of plaque, suggesting that HD patients may exhibit a similar inflammatory response to dental plaque as healthy patients.

The significance of HD status as an independent predictor of serum IL-6 and CRP levels in the multivariate analysis was expected, as hemodialysis patients have been shown to have elevated serum inflammatory markers compared to non-hemodialysis patients[21-24]. In our study, periodontal status was not a predictor of serum IL-6 or CRP. However, multiple studies performed on non-CKD populations have shown that serum IL-6 and CRP levels are elevated in periodontal patients when compared to periodontally-healthy controls[65-67], and that periodontal treatment can reduce levels of these markers[65]. Further studies on CKD populations are needed to verify our findings. The interaction between the CKD and periodontal status was not significant for either inflammatory marker. The limited sample size of our CKD (n=21) and periodontitis (n=12) subjects in this pilot study could account for these findings.

A limitation of our study was the sample size and the cross-sectional design of our study does not allow us to make any conclusions regarding a cause and effect relationship between periodontal disease and systemic inflammation. Further large-scale, intervention studies are needed.

IV. Concluding Remarks

1. Severe periodontitis was found to be significantly more prevalent in a stable CKD population compared to healthy controls. There was no statistically significant difference in prevalence of severe periodontitis between hemodialysis, pre-dialysis, and control subjects.
2. Serum IL-6 and CRP levels were statistically significantly higher in CKD patients compared to healthy controls. For serum IL-6, hemodialysis patients had significantly higher levels compared to pre-dialysis and control patients. Serum CRP levels were significantly higher in hemodialysis and pre-dialysis patients compared to controls. There were no significant differences in CRP or IL-6 levels between pre-dialysis and control subjects.
3. Subjects with severe periodontitis had higher serum levels of IL-6 compared to periodontally-healthy subjects. There were no significant differences with regards to serum CRP levels.
4. HD status was a significant independent predictor of both serum IL-6 and CRP levels in CKD patients.

V. Index (Tables and Figures)

Table 1: Stages of chronic kidney disease

Stage	Description	Glomerular Filtration Rate (GFR)
At increased risk	Risk factors for kidney disease (e.g. , diabetes, high blood pressure, family history, older age, ethnic group)	More than 90
1	Kidney damage (protein in the urine) and normal GFR	More than 90
2	Kidney damage and mild decrease in GFR	60 to 89
3	Moderate decrease in GFR	30 to 59
4	Severe decrease in GFR	15 to 29
5	Kidney failure (dialysis or kidney transplant needed)	Less than 15

Table 2: Population characteristics of CKD and control groups

Variable	CKD Subjects (n=21)	Control Subjects (n=13)	P-Value
Age (years)	55±9.7	54±12	0.46
Gender (% female)	42.5	46.7	0.86
Diabetics (%)	35.0	7.6	0.0001

Table 3: Periodontal characteristics of CKD and control groups

Variable	CKD Subjects (n=21)	Control Subjects (n=13)	P-Value
Severe periodontitis (%)	47.6	15.4	0.02
PD (mm)	2.66±0.09	2.4±0.10	0.03
CAL (mm)	3.21±0.18	2.47±0.10	0.004
BOP (%)	16.06±3.4	15.47±3.82	0.45
PS (%)	53.35±8.05	43.79±7.07	0.19
Missing teeth (n)	8.09±3.23	4.62±0.56	0.01
Sites CAL≥5mm (%)	16.80±4.22	2.55±1.5	0.001

Figure 1: Percent of subjects with severe periodontitis

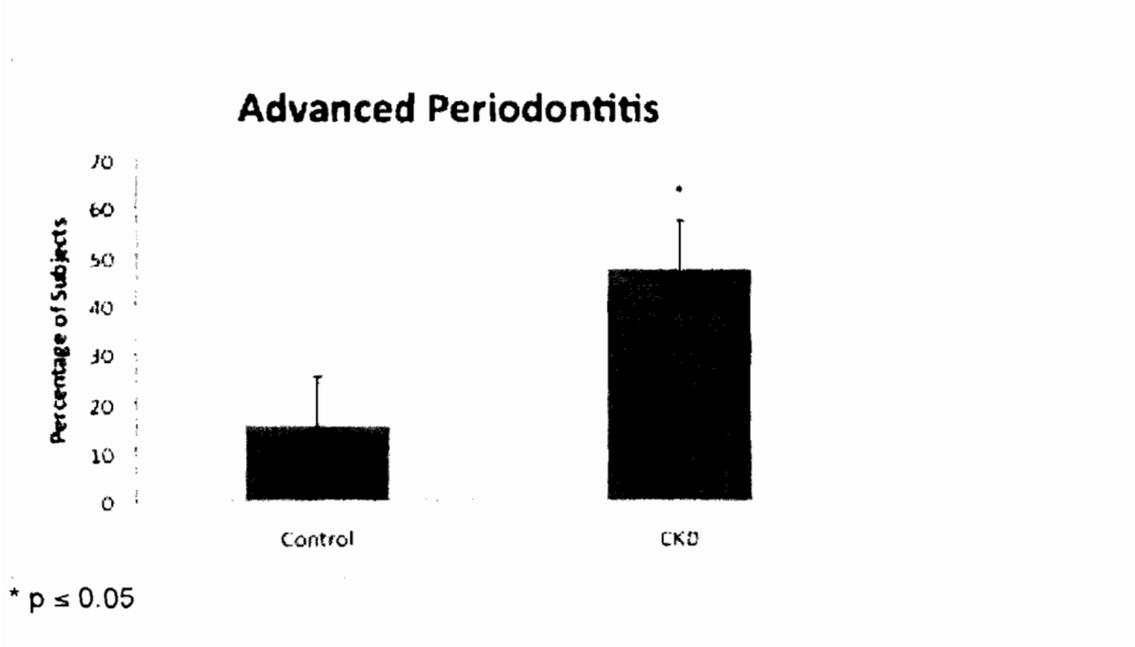


Figure 2: Comparison of periodontal parameters (PPD, CAL) between CKD and control groups

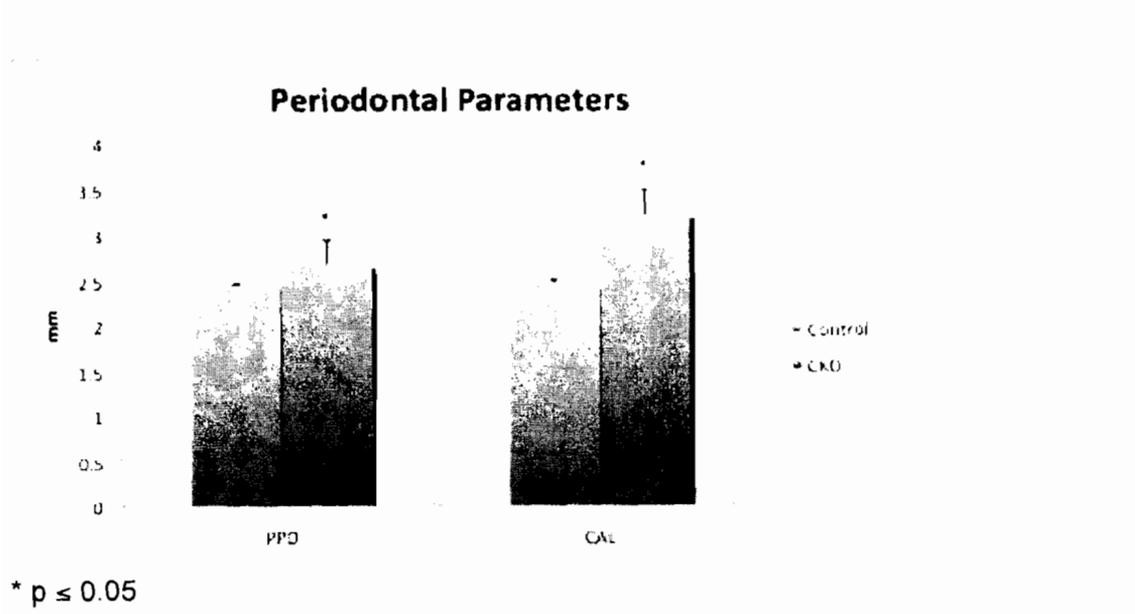


Figure 3: Comparison of periodontal parameters (BOP, PS, CAL \geq 5) between CKD and control groups

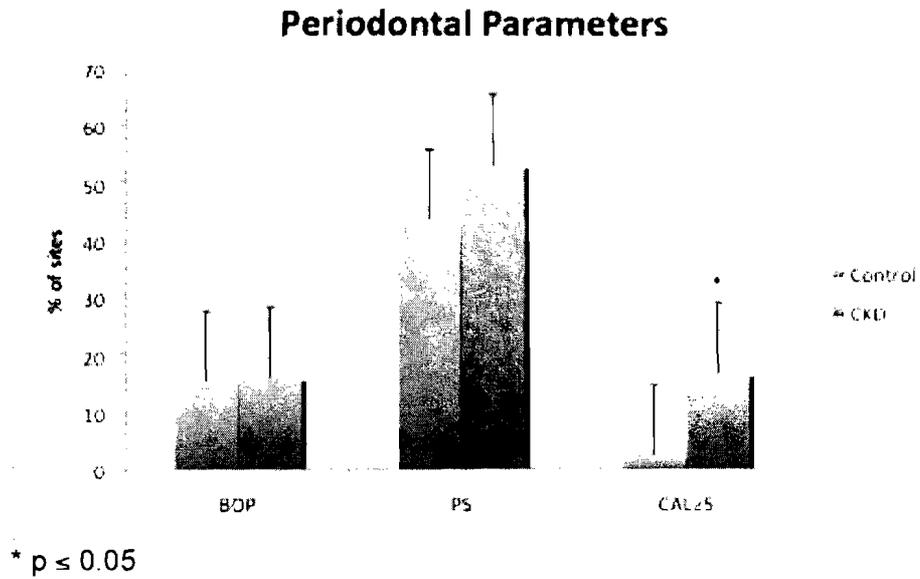


Table 4: Periodontal characteristics of subjects according to renal status

Variable	Hemodialysis Subjects (n=12)	Pre-dialysis Subjects (n=9)	Control Subjects (n=13)	P-Value
Severe periodontitis (%)	45.4	44.4	15.4	0.13
PD (mm)	2.80±0.11 ^a	2.57±0.12	2.4±0.10	0.049
CAL (mm)	3.32±0.19 ^a	3.06±0.24 ^b	2.47±0.10	0.002
BOP (%)	21.74±4.86	9.91±3.59	15.47±3.82	0.174
PS (%)	59.42±9.88	40.07±12.30	43.79±7.07	0.300
Missing teeth (n)	6.42±1.03	10.62±2.65 ^b	4.62±0.56	0.030
Sites CAL≥5mm (%)	22.01±6.49 ^a	13.57±5.81	2.55±1.5	0.016

a - $p \leq 0.05$ between HD and control subjects

b - $p \leq 0.05$ between pre-dialysis and control subjects

c - $p \leq 0.05$ between HD and pre-dialysis subjects

Figure 4: Percent of subjects with severe periodontitis according to renal status

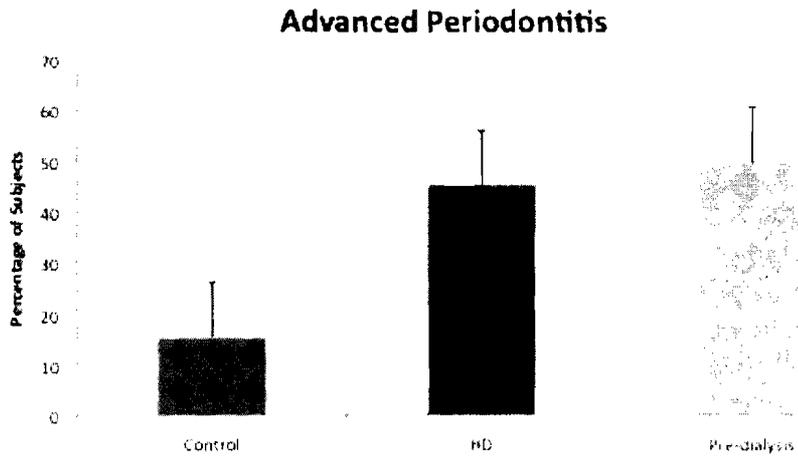
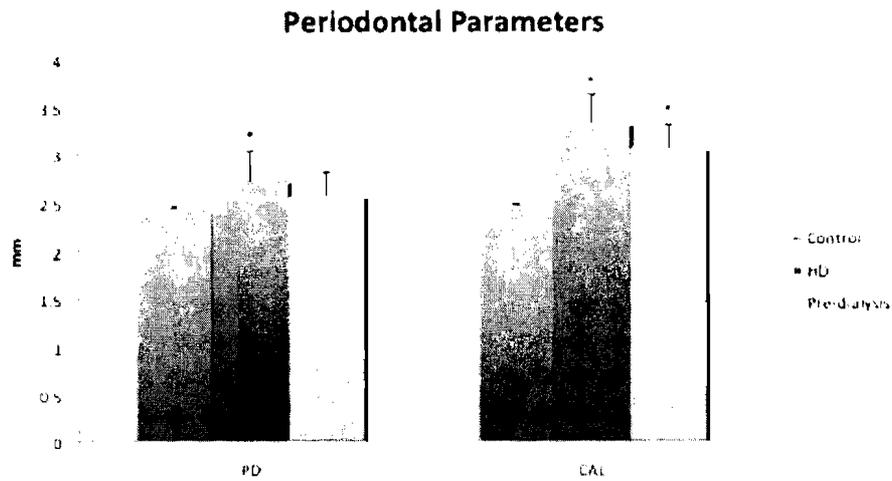
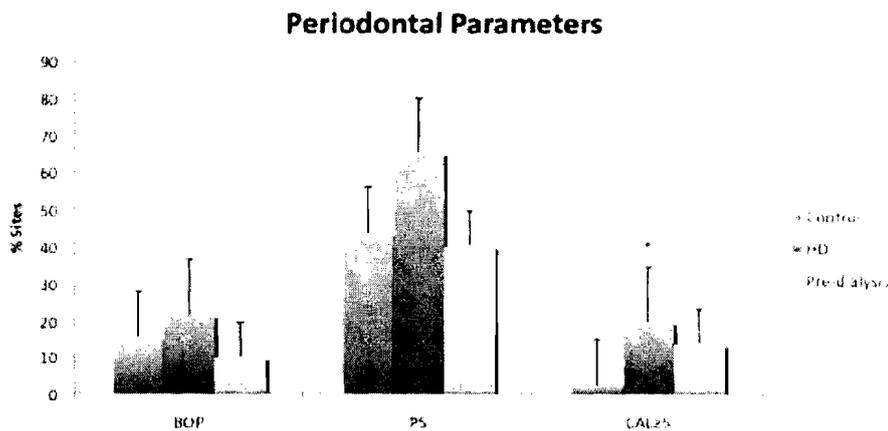


Figure 5: Comparison of periodontal parameters (PPD, CAL) according to renal status



* $p \leq 0.05$ compared to control group

Figure 6: Comparison of periodontal parameters (BOP, PS, CAL \geq 5) according to renal status

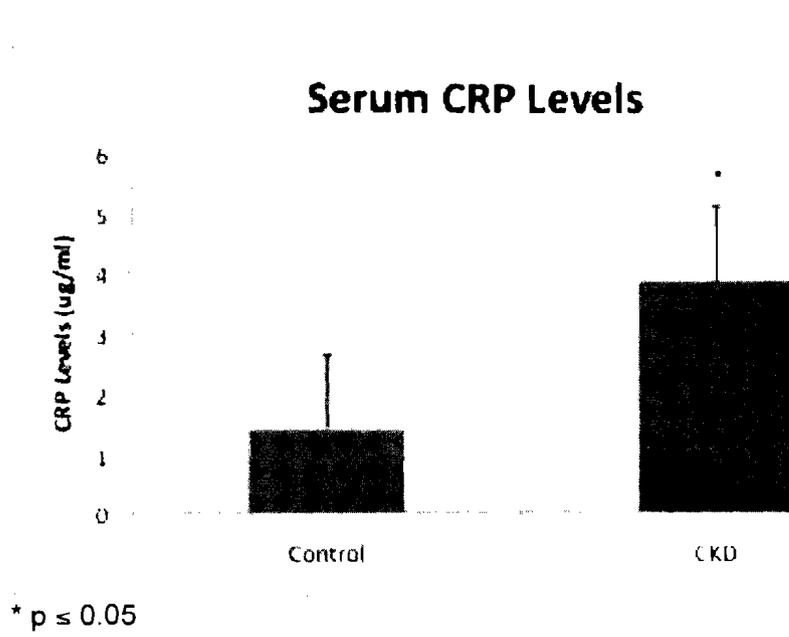


* $p \leq 0.05$ compared to control group

Table 5: Serum inflammatory markers in CKD and control subjects

Variable	CKD Subjects (n=21)		Control Subjects (n=13)		P-Value
	Median	Range	Median	Range	
CRP (μ g/ml)	3.87	0.30-78.35	1.41	0.42-18.00	0.014
IL-6 (pg/ml)	8.72	0.60-9.92	4.00	2.00-5.92	0.038

Figure 7: Comparison of serum CRP levels between CKD and control subjects



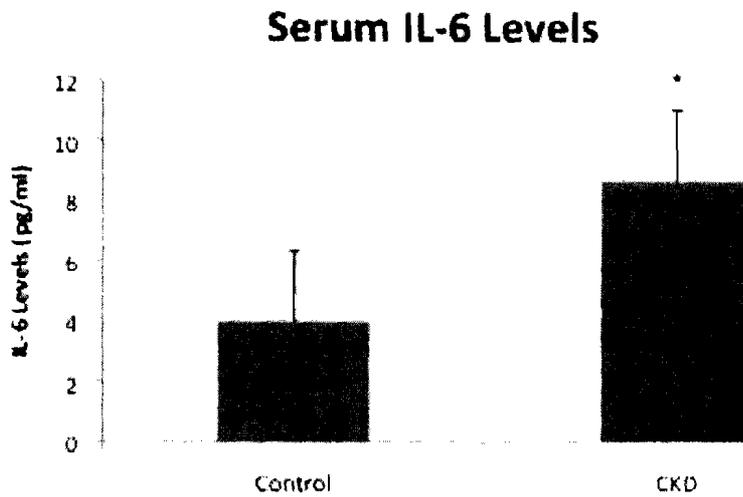


Figure 8: Comparison of serum IL-6 levels between CKD and control subjects

* $p \leq 0.05$

Table 6: Serum inflammatory markers in subjects according to renal status

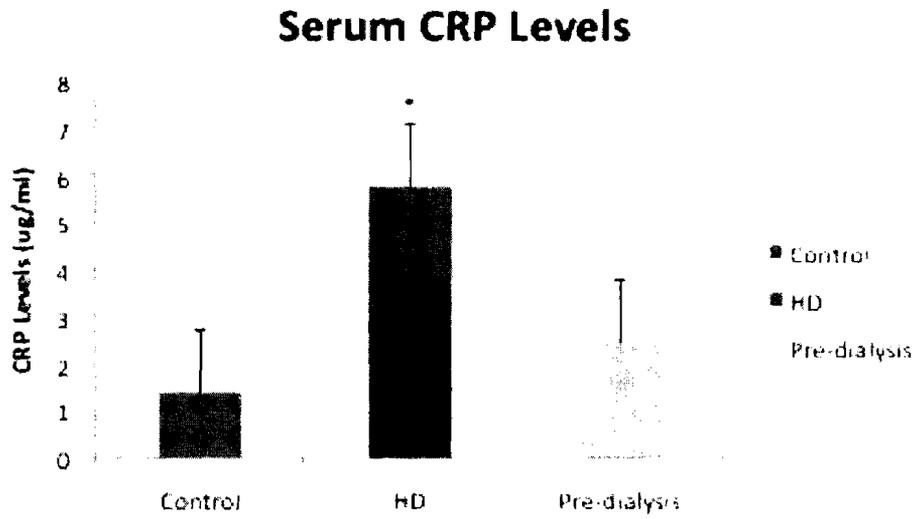
Variable	HD Subjects (n=12)		Pre-dialysis Subjects (n=9)		Control Subjects (n=13)		P-Value
	Median	Range	Median	Range	Median	Range	
CRP (µg/ml)	5.78 ^a	1.87-41.80	2.48	0.30-78.35	1.41	0.42-18.00	0.031
IL-6 (pg/ml)	10.98 ^{a,c}	6.01-29.35	4.00	0.60-9.92	4.00	2.00-5.92	0.019

a - $p \leq 0.05$ between HD and control subjects

b - $p \leq 0.05$ between pre-dialysis and control subjects

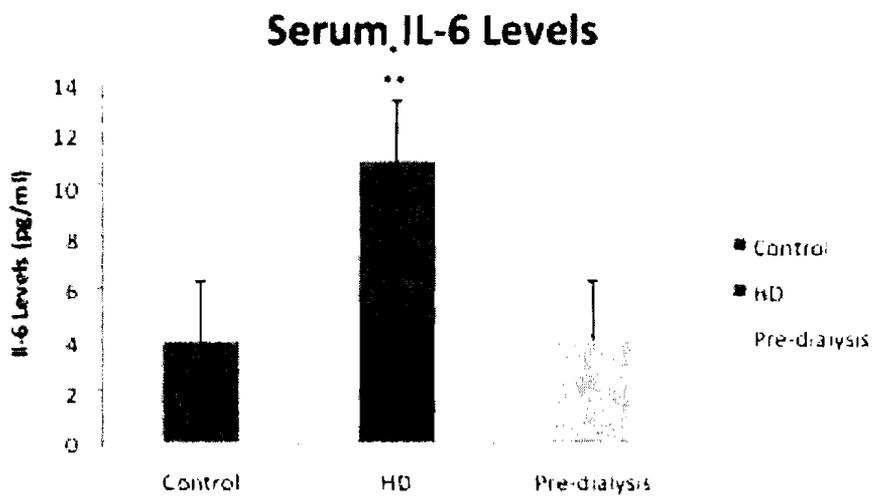
c - $p \leq 0.05$ between HD and pre-dialysis subjects

Figure 9: Comparison of serum CRP levels according to renal status



* $p \leq 0.05$ compared to control group

Figure 10: Comparison of serum IL-6 levels according to renal status



* $p \leq 0.05$ compared to control group

** $p \leq 0.05$ compared to pre-dialysis group

Table 7: Serum inflammatory markers in subjects according to periodontal status

Variable	Non-Periodontitis Subjects (n=22)		Periodontitis Subjects (n=12)		P-Value
	Median	Range	Median	Range	
CRP ($\mu\text{g/ml}$)	3.26	0.42-78.32	2.935	0.21-41.8	0.286
IL-6 (pg/ml)	4.63	2.00-11.35	6.175	2.00-29.35	0.015

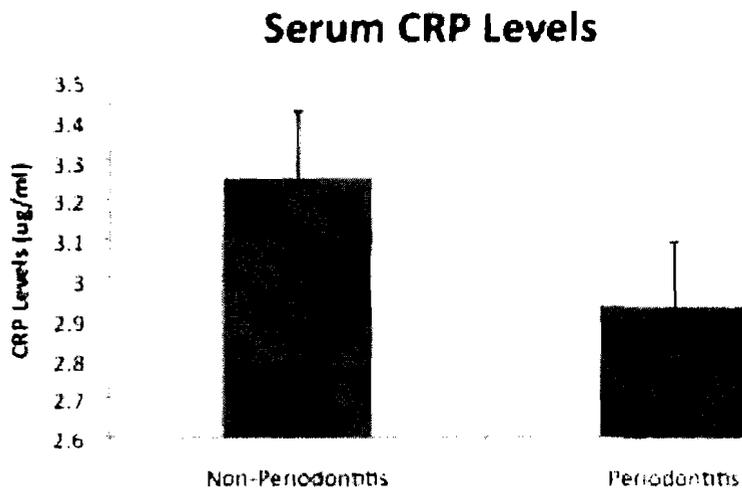
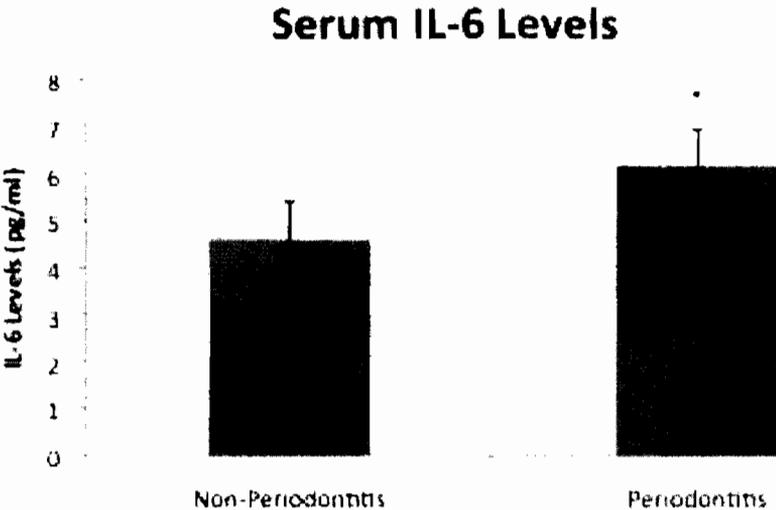


Figure 11: Comparison of serum CRP levels according to periodontal status

Figure 12: Comparison of serum IL-6 levels according to periodontal status



* p ≤ 0.05

Table 8: Correlations between inflammatory markers and patient characteristics and periodontal variables

Variable		Serum IL-6	Serum CRP
Age	R	0.101	0.223
	P	0.603	0.245
Gender	R	0.072	0.032
	P	0.710	0.868
BOP	R	0.166	0.294
	P	0.388	0.122
PS	R	0.385	0.372
	P	0.039	0.047
PD	R	0.205	0.307
	P	0.287	0.106
CAL	R	0.281	0.500
	P	0.140	0.006
CAL \geq 5mm	R	0.189	0.409
	P	0.326	0.027

Table 9: Multivariable linear regression analysis in CKD patients with serum IL-6 as the dependent variable

Variable	P-Value
Intercept	0.144
Age (years)	0.938
Gender	0.436
BMI	0.780
Glucose (mg/dl)	0.662
Dialysis status (yes/no)	0.029
Periodontitis status (yes/no)	0.350
Dialysis status*Periodontal status	0.161

Table 10: Multivariable linear regression analysis in CKD patients with serum CRP as the dependent variable

Variable	P-Value
Intercept	0.397
Age (years)	0.165
Gender	0.028
BMI	0.013
Glucose (mg/dl)	0.741
Dialysis status (yes/no)	0.012
Periodontitis status (yes/no)	0.432
Dialysis status*Periodontal status	0.256

VI. Appendix

1) Medical History Questionnaire for Control Subjects

University of Connecticut Health Center
 School of Dental Medicine, University Dentists
MEDICAL HISTORY QUESTIONNAIRE
 (Parent or Guardian to answer for child under 18 years of age.)

Patient ID Number _____

Name _____ M _____ F _____ Date of Birth _____

Address _____

Telephone: (Home) _____ (Work) _____ Height _____ Weight _____

Today's Date _____ Occupation _____

Answer all questions by circling either YES or NO. Fill in blank spaces when indicated. ANSWERS ARE FOR OUR RECORDS ONLY AND ARE CONFIDENTIAL.

1. My last medical physical examination was on (approx.) _____
2. The name & address of my personal physician is _____
3. Are you now under the care of a physician YES NO
 If yes, what is the condition being treated _____
4. Have you had any serious illness or operation..... YES NO
 If yes, what was the illness or operation _____
5. Have you been hospitalized within the past 5 years..... YES NO
 If yes, what was the problem _____

6. Do you have or have you had any of the following diseases or problems:
- | | | |
|--|-----|----|
| Rheumatic fever or rheumatic heart disease..... | YES | NO |
| Heart abnormalities present since birth..... | YES | NO |
| Cardiovascular disease (heart trouble, heart attach, angina, stroke, high blood pressure, heart murmur)..... | YES | NO |
| Do you have discomfort or pressure in the chest upon exertion..... | YES | NO |
| Are you ever short of breath after mild exercise..... | YES | NO |
| Do your ankles swell..... | YES | NO |
| Do you get short of breath when you lie down, or do you require extra pillows when you sleep..... | YES | NO |
| Have you been told you have a heart murmur..... | YES | NO |
| Asthma or hay fever..... | YES | NO |
| Hives or a skin rash..... | YES | NO |
| Fainting spells or seizures..... | YES | NO |
| Diabetes..... | YES | NO |
| Jaundice or liver disease..... | YES | NO |
| Hepatitis..... | YES | NO |
| Arthritis or other joint problems..... | YES | NO |
| Stomach ulcers..... | YES | NO |
| Kidney trouble..... | YES | NO |
| Dialysis patient..... | YES | NO |
| Tuberculosis..... | YES | NO |
| Do you have a persistent cough or cough up blood..... | YES | NO |
| Venereal disease..... | YES | NO |
| AIDS/ARC or HIV+..... | YES | NO |
| Other (list) _____ | | |

7. Have you had surgery or radiation treatment for a tumor, cancer or other condition of your head or neck..... YES NO
8. Have you had abnormal bleeding associated with previous extractions, surgery or trauma. YES NO

9. Do you have any blood disorder such as anemia, including sickle cell anemia YES NO
 10. Are you a hemophiliac YES NO
 11. Are you a present or past IV drug user YES NO
 12. Are you at increased risk for the HIV virus or AIDS YES NO
 13. Are you taking any drug or medicine YES NO

14. **Are you taking any of the following:**

Antibiotics or sulfa drugs.....	YES	NO	Drugs for heart trouble ie		
			digitalis.....	YES	NO
Anticoagulants (blood thinners).....	YES	NO	Nitroglycerin.....	YES	NO
Medicine for high blood pressure.....	YES	NO	Antihistamine.....	YES	NO
Steroids (Prednisone, Cortisone).....	YES	NO	Oral contraceptive or other hormonal		
Drugs that change your mood.....	YES	NO	therapy.....	YES	NO
Aspirin.....	YES	NO	Anticonvulsant drugs.....	YES	NO
Insulin, tolbutamide (Orinase) or			Sedatives or sleeping pills.....	YES	NO
similar drug.....	YES	NO	Other drugs _____		

15. Do you suffer from any birth defect or other disability..... YES NO

16. **Are you allergic or have you reacted adversely to:**

Local anesthetics.....	YES	NO	Iodine or X-ray dyes.....	YES	NO
Penicillin or other antibiotics.....	YES	NO	Codeine or other narcotics.....	YES	NO
Sulfa drugs.....	YES	NO	Other _____		
Aspirin.....	YES	NO			

17. Have you had any serious trouble associated with any previous dental treatment YES NO

If yes, explain _____

18. Do you have any disease, condition, or problem not listed above..... YES NO

If so, please explain _____

19. Does your employment expose you regularly to x-rays or other ionizing radiation YES NO

20. Are you wearing contact lenses..... YES NO

21. Are you pregnant or have you recently missed a menstrual period..... YES NO

22. Are you presently breast feeding..... YES NO

Chief dental complaint (why did you come to the dentist today?) _____

TO THE BEST OF MY KNOWLEDGE, THE ABOVE INFORMATION IS COMPLETE AND ACCURATE

Signature of Patient or Legal Guardian

Signature of Dentist

HEALTH HISTORY UPDATED:

Date: _____

2) Medical Data Extraction Form for CKD Subjects

Date: ___ / ___ / ___

Reviewer: _____

**Hemodialysis and Pre-dialysis Patient
Chart Review**

SECTION 1: PARTICIPANT DEMOGRAPHICS

1. Pt ID: _____ (3 digit #)
2. DOB: ___ / ___ / ___
3. Ethnicity:
1-Asian 2-Black 3-White 4-Native Hawaiian/Pacific Islander
5-Am Ind/Alaska Native 6-Hispanic 7-Unknown
4. Gender: male female

SECTION 2: MEDICAL HISTORY

5. Does the patient have history of diabetes? Y N
If yes: Type I Type II Insulin-requiring
6. Is the patient a smoker? Current Former Never smoked
7. Weight: _____ Height: _____ BMI: _____
8. Menopause Y N
9. Hyperlipidemia Y N
10. Infection within the last 2 months (vascular access/fistula, UTI, bronchitis, peritonitis, pneumonia, abdominal abscess etc)

Date _____

- *1. Does patient have a history of cardiovascular disease? Y N
if yes check appropriate diagnosis
Heart failure Coronary heart disease Post MI
Coronary artery bypass grafting (CABG)
Angina pectoris Cerebrovascular disease (stroke or ischemic attack)

12. Does the patient have history of hypertension?

Y N

13. What is the patient's pre-dialysis kidney disease diagnosis? (Please specify etiology of underlying disease)

SECTION 3: Biochemical data (Most recent)

14. Serum albumin _____

15. Blood urea nitrogen (BUN): _____

16. Creatinine: _____

17. Ferritin level: _____

18. Total Iron Binding Capacity (TIBC) _____

19. Total cholesterol: _____

20. HDL-cholesterol: _____

21. LDL-cholesterol: _____

22. Triglycerides: _____

23. Dialysis adequacy (Kt/V): _____ (only for hemodialysis subjects)

24. Dialysis vintage (years on dialysis) _____ (only for hemodialysis subjects)

SECTION 4: MEDICATION

25. Has the patient been on non-steroidal anti-inflammatory medication within the last month?

Y N

Dose _____ Duration: _____

26. Has the patient ever been on anti-hyperlipidemic meds? Y N

Dose: _____ Duration: _____

27. Please attach medication list:

28. Please attach SF-36 questionnaire

Page 3 of 3

3) Abbreviations used

ANOVA – Analysis of variance

AV – Arteriovenous

BGI – Biofilm-gingival interface

BMI – Body mass index

BOP – Bleeding on probing

BUN – Blood urea nitrogen

CAL – Clinical attachment loss

CKD – Chronic kidney disease

CRP – C reactive protein

CVD – Cardiovascular disease

ELISA – Enzyme-linked immunosorbent assay

ESRD – End stage renal disease

GFR – Glomerular filtration rate

HD – Hemodialysis

HDL – High density lipoprotein

Ig - Immunoglobulin

IL – Interleukin

IRB – Institutional Review Board

LDL – Low density lipoprotein

PPD – Pocket probing depth

TIBC – Total iron binding capacity

UCHC – University of Connecticut Health Center

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