THE ASSOCIATION BETWEEN PERIODONTITIS
AND END-STAGE RENAL DISEASE

A mini-thesis submitted in partial fulfilment of the requirements
for the degree of Master of Science in Dental Sciences in
Periodontics at the Faculty of Dentistry
University of the Western Cape

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Keywords

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End stage renal disease (ESRD)
Inflammation
Cytokines
Acute phase response (APR)
C-reactive proteins (CRP)
Haemodialysis (HD)
Peritoneal dialysis (PD)
Oral hygiene
SUMMARY

Patients who are in end-stage renal disease (ESRD) experience a significantly increased rate of atherosclerotic complications. Inflammation plays a central role in the pathogenesis of these complications. The major acute phase protein, C-reactive protein (CRP) has been found to predict all-cause and cardiovascular mortality in ESRD patients. Many patients in ESRD experience elevated CRP levels without an overt infection. Periodontal diseases in the general population have been associated with both an increased prevalence of atherosclerotic complications and an elevation in serum CRP values. **Aim:** The aim of the present study was to investigate whether periodontal disease is associated with increased systemic inflammation reflected by CRP values, in patients with ESRD on maintenance haemodialysis (HD) or peritoneal dialysis (PD). **Methods:** Eighty patients who were in ESRD were included in the study. Demographic information, medical history and CRP levels were recorded by the attending physician in the renal unit. Periodontal examination was carried out by a single examiner which included plaque index (PI) gingival index (GI), bleeding on probing (BoP), probing depths (PD) and clinical attachment loss (CAL). These measurements were recorded on Ramfjord teeth. The presence of any one sextant showing PD of ≥ 4 mm or clinical loss of attachment of ≥ 3 mm was diagnosed as periodontitis. **Results:** Mean age of subjects was 50.3±9.06 years with a median time on dialysis therapy of 24 months. 42.5% subjects were male and 62% subjects reported their race as Coloured. 57.5% (n=46) subjects were diagnosed to have periodontal disease, of these 52.2% had elevated CRP levels (>10mg/L). On the other hand, of the thirty four subjects with a healthy periodontium, only 29.4% (n=10) had elevated CRP levels. There was a statistically significant difference in serum CRP levels between these two groups (p=0.035). **Conclusion:** The results of the study showed significantly elevated levels of CRP in ESRD patients with periodontitis. Therefore, within the limitations of the study, it can be concluded that periodontal diseases may be an overlooked source of inflammation in ESRD patients.
DECLARATION

I hereby declare that “The association between periodontitis and end-stage renal disease” is my own work, that it has not been submitted before for any degree or examination in any university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Muhammad Nadeem

October 2006

Signed:........................................
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DEDICATION

To my father who passed away whilst I was working on this dissertation

To my mother for her constant support, prayers and sacrifice

To my supervisor whose guidance, encouragement, help and support made this project possible
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CHAPTER 1
INTRODUCTION

The incidence of chronic renal failure (CRF) and end-stage renal disease (ESRD) is rising constantly because the repair of the damaged parenchymal tissues is rare. Chronic renal failure is a progressive disease caused by the damage of the functional unit of the kidney, the nephron. Diseases which cause the destruction of nephrons are diabetes mellitus, pyelonephritis, glomerulonephritis, nephrosclerosis, polycystic kidney disease and vascular collagen disease. Chronic Kidney disease (CKD) is characterized by a reduction in glomerular filtration rate (GFR). The normal adult GFR is approximate 100 to 120 ml/min/1.73m² body surface area. The end-result of a reduced renal function is uraemia, which is a metabolic state of toxicity, affecting many organs. The morbidity and mortality of cardiovascular diseases are significantly higher in uremic patients.

In the last three to four decades, improvements in dialysis and transplantation have reduced morbidity and mortality among patients with ESRD. Because of increased life span, increasing number of such patients will present for dental treatment. The incidence of a variety of soft and hard tissue conditions such as gingival inflammation, gingival overgrowth, periodontal disease, enamel hypoplasia, pulp obliteration and osseous changes of the jaw seems greater among chronic renal failure patients.

Chronic renal failure is often compounded by multiple infections. The increased susceptibility to infection may be due to impairment in both specific and non-specific immune responses. Periodontal diseases are common among renal failure patients; however this relationship is not clearly understood.

In the last half century, it was assumed that periodontal infections are localized to the marginal periodontium and that, as such, they rarely have systemic implications. Recent studies have suggested a bi-directional adverse relationship between periodontal infections and systemic disease.
Epidemiological studies have reported an association between periodontal disease and several systemic conditions including diabetes, cardiovascular disease, and low weight preterm birth.\textsuperscript{9, 10}

Periodontal disease is a group of infectious diseases predominantly caused by Gram-negative, anaerobic and microaerophilic bacteria that colonize in supra- and subgingival plaque. These organisms affect local and systemic immune and inflammatory responses. The local inflammatory response to these bacteria or their products is the infiltration of periodontal tissue by the inflammatory cells such as polymorphonuclear neutrophils, lymphocytes, macrophages and plasma cells. Activated macrophages release cytokines which can result in local and systemic elevation of pro-inflammatory mediators such as interleukin-1 (IL1), interleukin-6 (IL6), prostaglandin E2 (PGE2) and tumour Necrosis factor alpha (TNF-\(\alpha\)).\textsuperscript{8, 11, 12, 13, 14}

Elevated levels of these cytokines can also initiate a systemic acute phase response (APR).\textsuperscript{12} The acute phase response (APR) represents an early and highly complex reaction of an organism to a variety of injuries such as bacterial, viral or parasitic infection; mechanical or thermal trauma, ischemic necrosis or malignant growth. There is also a concurrent increase hepatic synthesis and intravascular secretion of many plasma proteins with a wide range of actions. The components of the acute phase response are largely non-specific as compared to cellular or humoral immunity and include fever, neutrophilia, altered lipid metabolism, hypoferroemia, gluconeogenesis, activation of complement coagulation pathways and induction of acute phase proteins.\textsuperscript{10, 15, 16}

The major acute phase proteins includes C-reactive proteins (CRP), serum amyloid A, fibrinogen and hepatoglobin, whose concentrations increases with inflammation and albumin and transferrin whose concentrations decreases with inflammation.\textsuperscript{15} C-reactive proteins is the most sensitive marker used to evaluate the inflammatory status of the individual.\textsuperscript{16} Elevated levels of C-reactive protein are associated with systemic inflammation.\textsuperscript{13} Recent studies of the general population have suggested that oral infection, particularly those
associated with destructive periodontal disease may induce elevated serum levels of C-reactive proteins.\textsuperscript{17, 18, 19, 20}

C-reactive proteins are synthesized by hepatocytes at a relatively slow rate and then mainly retained by endoplasmic reticulum (ER) until secretion. During the acute phase response there is a rapid increase in CRP synthesis and sudden release from the ER.\textsuperscript{21, 22}

Many studies have shown elevated levels of inflammatory cytokines in gingival crevicular fluid from the gingiva with periodontal disease as compared to the healthy gingiva. However, it is still not clear how much the local inflammatory response in the periodontium can influence the systemic levels of inflammatory mediators.\textsuperscript{11, 22, 23}

A number of recent studies have shown that periodontitis may be associated with coronary heart disease and cerebrovascular disease.\textsuperscript{12, 24, 25, 26} Atherosclerotic cardiovascular diseases (CVD) and chronic kidney disease (CKD) share many common risk factors including inflammation.

C-reactive protein is used as a marker of infection or as an activity parameter of ongoing inflammatory disease in end-stage renal disease (ESRD) patients and is known to increase with declining renal function, even before reaching ESRD.\textsuperscript{4, 21}

Increased CRP level is not only present in patients on haemodialysis but also observed in ESRD patients on either conservative treatment or peritoneal dialysis. Raised CRP levels in ESRD patients indicate the presence of a chronically activated acute phase response. CRP is a significant and independent predictor of death in ESRD patients.\textsuperscript{4}

It has been observed that the C-reactive protein (CRP) level is elevated in a significant proportion of ESRD patients without any apparent reason.\textsuperscript{4} Therefore, the role of occult or less apparent infections should not be ignored.\textsuperscript{3}
as a potential source of inflammation in such persons. Chronic infections with *Chlamydia species* and *Helicobacter pylori* have been postulated as being a source of inflammation. The presence of periodontitis in these patients may also increase CRP and other inflammatory markers.\textsuperscript{21}

Therefore, the aim of the study is to investigate whether periodontitis is associated with increased systemic inflammation reflected by CRP values, in patients with ESRD on maintenance haemodialysis or peritoneal dialysis.
2.1. INTRODUCTION

End-stage renal disease (ESRD) patients experience a significantly increased rate of atherosclerotic complications. Inflammation plays a central role in the pathogenesis of atherosclerotic complications. C-reactive protein (CRP), the major acute phase protein, has been found to predict all-cause and cardiovascular mortality in ESRD patients. Many ESRD patients experience elevated CRP levels without an overt infection. Periodontal disease in the general population has been associated with an increased prevalence of atherosclerotic complications and an elevation in serum CRP values. Since periodontal examination is normally not performed as a part of medical assessment, periodontal diseases may be an overlooked source of inflammation in ESRD patients.

In reviewing the literature attention is given to the definition and stages of chronic kidney disease (CKD), the association of inflammation and C-reactive protein in ESRD, pathogenesis of periodontal diseases and its impact on systemic health as well as the contribution of periodontal infections in elevated systemic C-reactive protein levels.
2.2. CHRONIC KIDNEY DISEASE

Chronic Kidney disease has been defined according to the following criteria:\textsuperscript{27, 28, 29}

1. Kidney damage for $\geq$ 3 months, as defined by structural or functional abnormalities of the kidney, with or without decreased Glomerular filtration rate (GFR), manifest by either,
   - Pathological abnormalities or
   - Markers of kidney damage, including abnormalities in composition of the blood or urine, or abnormalities in imaging tests
2. GFR $< 60$ ml/min/1.73 m$^2$ for $\geq$ 3 months, with or without kidney damage.

2.3 STAGES OF CHRONIC KIDNEY DISEASE \textsuperscript{27, 28, 29, 30, 31}

(Normal adult GFR is approximate 100 to 120 ml/min/1.73 m$^2$ body surface area)

<table>
<thead>
<tr>
<th>STAGE</th>
<th>DESCRIPTION</th>
<th>GRF (ml/ min/ 1.73 m$^2$)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Kidney damage with normal or ↑ GFR</td>
<td>$\geq 90$</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage with mild ↓ GFR</td>
<td>60-89</td>
</tr>
<tr>
<td>3</td>
<td>Moderate ↓ GFR</td>
<td>30-59</td>
</tr>
<tr>
<td>4</td>
<td>Severe ↓ GFR</td>
<td>15-29</td>
</tr>
<tr>
<td>5</td>
<td>Kidney failure (ESRD)</td>
<td>$&lt; 15$ (or dialysis)</td>
</tr>
</tbody>
</table>

Table-1. Stages of kidney disease

2.4. END-STAGE RENAL DISEASE (CKD Stage-5) \textsuperscript{27}

By definition all patients on dialysis or renal transplantation, irrespective of the level of GFR are categorised as being in end-stage renal disease.
2.5. **RISK FACTORS FOR CHRONIC RENAL DISEASE**  

(Factors that increase the risk of kidney damage)

- Age
- Diabetes
- Hypertension
- Family history of renal disease

**Initiating factors**

(Factors that initiate the kidney damage)

- Diabetes
- Malignant Hypertension
- Autoimmune diseases
- Primary glomerulopathies
- Systemic infections
- Nephrotoxic agents

**Progression factors**

(Factors that cause progressive decline in renal function after the onset of kidney damage)

- Persistent activity of the underlying disease
- Persistent proteinuria
- Elevated blood pressure
- Elevated blood glucose
- Nephrotoxins, Urinary tract infections, Obstructions, hypovolaemia
- High protein / phosphate diet
- Hyperlipidaemia
- Anaemia
- Cardiovascular disease
- Smoking
- Other factors: elevated angiotensin II, hyper-aldosteronism, increased endothelin, decreased nitric oxide
2.6. INCIDENCE AND PREVALENCE OF END-STAGE RENAL DISEASE IN VARIOUS PARTS OF WORLD

There are over one million dialysis patients worldwide, with an incidence of about a quarter of a million new patients each year. The incidence of patients with end-stage renal disease (ESRD) being treated by renal replacement therapy varies enormously depending on the level of affluence of the country.\textsuperscript{32}

The highly developed countries such as North America, Europe and Japan have the highest incident rates of treated end-stage renal disease, whereas the emerging countries have very low incident rates.\textsuperscript{32} The huge disparity in the prevalence of ESRD between the more and less developed countries probably due to the inadequacy of health care system. About 90\% of treated ESRD patients come from more developed countries that can still afford the cost of renal replacement therapy.\textsuperscript{31}

Disparities in the incidence of ESRD within and between more developed countries reflect the racial and ethnic differences. For example, in the USA and Australia the annual incidence of ESRD is significantly lower in whites than in African-American or aboriginal people.\textsuperscript{31}

The number of patients with ESRD probably underestimates the entire burden of the chronic kidney disease (CKD) because the numbers with earlier stages of the disease (stage 1 to 4) are likely to exceed by as much as fifty times those reaching ESRD. In addition data from most of less developed countries such as India and Nigeria (biggest economies in Asia and Africa) is not available.\textsuperscript{31} Similarly, no data is available about the prevalence of ESRD in South Africa.
2.7. COMPARISON OF HAEMODIALYSIS (HD) AND PERITONEAL DIALYSIS (PD)

The introduction of peritoneal dialysis in 1976 by Popovich and Moncrief et al as reported by Collins et al 33 provided an alternative to traditional haemodialysis therapy. Although there is much debate over the advantages of one therapy over another, no randomized prospective study comparing the two modalities has been conducted. 34 Due to the complexities in designing such studies it is unlikely that such a study will be done. 34 A wide variation in mortality outcome has been reported between HD and PD. The conflicting data shows the following results: 35
1- No difference in mortality risk between HD and PD.
2- PD having a lower risk than HD.
3- PD having a higher risk than HD.
4- Diabetic PD patients having a lower risk.
5- Diabetic PD patients having a higher risk.
Inconsistency in the comparisons between PD and HD is seems to be due to other unmeasured factors, which may not be distributed evenly between the two modalities.
2.8. INFLAMMATION IN END-STAGE RENAL DISEASE (ESRD)

Despite improvements in dialysis technology during the last 20 years, the mortality rate is still very high in ESRD patients. The majority of dialysis patients die within a five year period. This death rate is theoretically comparable to that of cancer patients with metastases. The main cause of mortality in ESRD is the cardiovascular disease which accounts for premature death in more than 50% of dialysis patients. The high cardiovascular mortality rate indicates that ESRD patients are subject to a process of accelerated atherogenesis. The causes of atherosclerotic cardiovascular disease in ESRD are probably multifactorial. Several risk factors have been identified. The identifiable risk factors include age, sex, race, family history, diabetes mellitus, dyslipidemia, hypertension, left ventricular hypertrophy, obesity and cigarette smoking. However, previous studies have shown that the high prevalence of cardiovascular disease in ESRD patients is only partly explained by these known risk factors. It has been postulated that additional factors such as, oxidative stress and inflammation may be more important than the established risk factors.

Persistent activation of inflammatory response has now been clearly identified as an independent risk factor for the development of cardiovascular complications in both general population and patients with ESRD. Approximately 30-50% of pre-dialysis, haemodialysis and peritoneal dialysis patients have serologic evidence of activated inflammatory response. The origin of inflammation in ESRD patients has not been recognised. There are no major differences in serum cytokine levels between long term dialysis patients and who have not been on dialysis, indicating that ESRD per se may be a more important cause of elevated cytokine level than the dialysis procedure itself.

Pecoits-Fihlo et al. found that circulating levels of several inflammatory markers are strongly associated with residual renal function. Thus glomerular filtration rate is an important determinant of increased inflammatory activity in patients with advanced renal impairment. However, the half life of various
cytokines is short and local tissue degradation may be the most important pathway of cytokine degradation. Therefore, more research is needed to determine the relative importance of kidney function in cytokine clearance.\textsuperscript{39}

The observation that increased levels of pro-inflammatory cytokines are found primarily in dialysis patients suggests that dialysis procedure with extracorporeal circulation of blood and use of bioincompatible peritoneal dialysis solution may also contribute to inflammation.\textsuperscript{36, 39} Other factors that may contribute include the use of non-biocompatible membranes, non-sterile dialysate and back leak of dialysate across the dialysis membrane. These factors have been associated with an inflammatory reaction in haemodialysis patients.\textsuperscript{36, 37} The presence of elevated pro-inflammatory cytokine in pre-dialysis patients strongly suggests that factors unrelated to dialysis therapy are largely responsible for inflammation in both dialysis and pre-dialysis patients.\textsuperscript{36}

The association between cardiovascular disease and inflammation in ESRD is well established; however it is still not clear if the acute-phase response merely reflects an epiphenomenon accompanying the established atherosclerotic disease or whether different acute-phase reactants are involved in the initiation and/or progression of atherosclerosis.\textsuperscript{36, 39}

Chronic inflammation is a common feature in ESRD patients and may cause progressive atherosclerotic cardiovascular disease. The causes of inflammation in ESRD are probably multifactorial and origin of inflammation remains unclear. Thus, the role of persistent chronic infection, such as, \textit{Chlamydia pneumoniae}, \textit{Helicobacter pylori} and “periodontal infection” should not be underestimated.

\section*{2.9. C-REACTIVE PROTEIN (CRP)}

The existence of CRP was first reported in 1930 by Tillet and Frances, who identified a substance in the sera of patients acutely infected with pneumococcal pneumonia that formed a precipitate when combined with C-polysaccharide of the cell wall of streptococcus pneumoniae, thus earning its name as “C-reactive protein.”\textsuperscript{21, 42} Subsequently, it was found that this reaction
was not unique to pneumococcal pneumonia and was found to occur in many other acute infections.\textsuperscript{42} CRP belongs to highly conserved pentraxin family of calcium dependent ligand-binding plasma proteins.\textsuperscript{21, 40, 42, 43} Pentraxin proteins are highly preserved in evolution and precede the development of the adaptive immune response. \textsuperscript{40} CRP is synthesized by hepatocytes with an overall molecular weight of approximately 11,800 Dalton (Da).\textsuperscript{42} The human CRP molecule is composed of five identical nonglycosylated polypeptide subunits each containing 206 amino acid residues. The protomers are non-covalently associated with an annular configuration with cyclic pentameric symmetry. Each protomer has the characteristic “lectin fold” composed of two layered $\beta$ sheet with flattened jellyroll topology. The ligand binding site, composed of loops with two calcium ions bound 4 Å apart protein side chains, is located on the concave face. The other face carries a single $\alpha$ helix. (Fig-1) \textsuperscript{43}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Molecular structure and morphology of human CRP (Pepys & Hirschfield 2003) \textsuperscript{43}
\textbf{a)} Negatively stained electron micrograph showing the typical pentameric disc-like structure face on and side on (arrows). (b) Ribbon diagram of the crystal structure, showing the lectin fold and two calcium atoms in the ligand-binding site of each protomer. (c) Space filling model of CRP molecule, showing a single phosphocholine molecule located in the ligand-binding site of each protomer.}
\end{figure}

C-reactive protein is considered the prototype acute phase protein.\textsuperscript{40} Like many acute phase proteins, CRP is normally present in trace levels in serum. An increase in the concentration of CRP is non-specific and can occur in response to a variety of infectious or inflammatory conditions.\textsuperscript{21, 42} In severe responses the concentration may increase by 1000 or 2000 fold. Under normal circumstances, CRP is synthesized by hepatocytes at a relatively low rate and then largely stored by the endoplasmic reticulum until secretion. During the acute phase response, there is rapid increase in synthesis of CRP and a
sudden release from the endoplasmic reticulum. One major pathway for the production of CRP by hepatocytes is through stimulation by cytokines, particularly, IL-6, IL-1 and TNF-α. Therefore, the cytokine network allows for a complex interplay of various stimuli that both stimulate and provide feedback inhibition to modulate the acute phase response.

C-reactive protein has a normal concentration of less than 2mg/L in healthy individuals. In the presence of infections or illness such as sepsis or rheumatoid arthritis the level of CRP increases sharply in the first 6-8 hours and can reach peak levels approaching 300mg/L after approximately 48 hours. The plasma half life of CRP is quite short at about 19 hours. Plasma half life and fractional clearance rates of CRP are nearly constant in normal subjects, as well as in patients with infectious, inflammatory and neoplastic conditions. Under stable conditions, CRP does not exhibit significant circadian variability and its stability as a laboratory measure is comparable to that of serum cholesterol levels. It has been found that CRP concentrations are actually relatively constant in an individual, both with regard to time of day, and over days and months, even over months to years. These characteristics make CRP a reliable marker of inflammation with such favourable biological responsiveness and analytic properties. However, the accuracy of CRP levels may be affected by some other factors which can raise the CRP levels, such as, testosterone, progesterone, administration of hormonal replacement therapy, insulin resistance, periodontitis and smoking.

The exact function of CRP is unclear. However two functional properties of CRP have been demonstrated. Firstly, its ability to recognize foreign pathogens, as well as, phospholipid constituents of damaged cells resulting in elimination of the target cells by the interaction with both humoral and cellular effector systems, Secondly, its ability to modulate the function of phagocytic cells.

In conclusion, due to its unique characteristics CRP is considered the most sensitive marker to evaluate the inflammatory status of the individual.
2.10. IMPORTANCE OF C-REACTIVE PROTEIN (CRP) IN END-STAGE RENAL DISEASE (ESRD)

The significance of CRP in ESRD population has increased over time. Initially regarded as a simple marker, it is now apparent that CRP is an active participant in pro-atherosclerotic phenomena’s, including local pro-inflammatory and thrombotic events. Since the first report by Bergström et al. as cited by Stenvinkle of an association between elevated CRP and increased mortality in haemodialysis patient, several investigators have reported similar findings in both haemodialysis (HD) and peritoneal dialysis (PD) patients. Cross-sectional studies based on a single determination of CRP show that 30-50% of pre-dialysis, haemodialysis and peritoneal dialysis patients have serological evidence of an activated inflammatory response with elevated serum levels of CRP. CRP appears to be an independent predictor of survival even after adjusting for all other variables.

Several studies have shown the significance of high CRP levels in pre-dialysis, haemodialysis and peritoneal dialysis patients. In a recent study Ortega et al. have shown that high CRP levels in pre-dialysis patients predict a constant inflammatory state on follow up and as in dialysis patients high CRP levels in pre-dialysis patients predict lower serum albumin concentrations, poorer response to erythropoietin (Epo) therapy and higher hospitalization rate.

Only few studies have examined the role of CRP in predicting outcome in peritoneal dialysis patients, however, Wang et al. clearly confirmed the significance of a single random CRP value in predicting all-cause and cardiovascular mortality in peritoneal dialysis patients. Similarly, Herzig et al. concluded that abnormally elevated CRP levels were associated with an increased risk of future myocardial infarction for at least 3 years in their study population of peritoneal dialysis patients. The predictive value of CRP was independent of nutritional indices, small solute clearance, smoking status and other possible confounders. On the other hand, in his study Fine showed that many patients with known severe atherosclerotic disease have normal CRP
levels and routine CRP measurements in peritoneal dialysis patients could not be supported by the results of his study.

Many studies showed that CRP was an independent predictor of outcome in chronic haemodialysis patients.\textsuperscript{45, 50, 51} In a recent study Korevaar et al.\textsuperscript{52} demonstrated that an increase in CRP level during single haemodialysis session was associated with a higher mortality risk independent of CRP level before the dialysis session.

Although the association between cardiovascular disease and inflammation in dialysis patients is well documented, it is still not fully established if the acute phase response merely reflects an epiphenomenon accompanying established atherosclerotic disease or whether different acute phase reactants are involved in the initiation and/or progression of atherosclerosis.\textsuperscript{39} Few reports suggest that CRP actually may be directly involved in the pathogenesis of atherosclerotic lesions.\textsuperscript{39, 40}

CRP is postulated to play a role in multiple stages of atherosclerotic lesion evolution.\textsuperscript{21} First, CRP appears to be a direct mediator of endothelial dysfunction and inflammatory cell recruitment via the up-regulation of expression of endothelial cell adhesion molecules, by promoting the release of monocyte chemotactic protein (MCP-1) and/or endothelial derived contracting factors and by inhibiting the nitric oxide production. Second, CRP promotes the uptake of low density lipoproteins (LDL) by foam cells. Third, it exerts an effect on vascular smooth muscle cells by promoting vascular smooth muscle migration and proliferation. Fourth, it contributes to fibrous plaque formation and necrosis by inducing apoptosis of endothelial cells. Finally, CRP inhibits the compensatory mechanism of angiogenesis (Fig-2).\textsuperscript{21, 40}

All these reactions may contribute to the growth of atheromatous plaque and to atherothrombotic complications. Therefore, a direct role of the CRP in the pathogenesis of atherosclerosis cannot be excluded.\textsuperscript{40}
Fig-2. Potential pro-inflammatory and pro-atherogenic actions of CRP (Adapted from Rattazzi et al 2003)

Abbreviations: (MAC) membrane attack complex, (ICAM-1) intercellular adhesion molecule-1, (VCAM-1) vascular cell adhesion molecule-1 (MCP-1) monocyte chemotactic protein-1 (IL-1) interleukin-1 (TNF-α) tumour necrosis factor alpha (LDL) low density lipoprotein

1) Endothelial cell expression of adhesion molecules (ICAM-1, VCAM-1) and pro-inflammatory cytokines (MCP-1)
2) Release of pro-inflammatory molecules by macrophages (tissue factor, IL-1, TNF-α)
3) Phagocytosis of cellular debris and lipoproteins by phagocytic cells.
4) Complement activation

In summary, it can be concluded that CRP is not only an inflammatory marker but may also be a direct cause of cardiovascular diseases in ESRD patients. However, this needs to be investigated further before its impact can be assessed completely.
2.11. PERIODONTAL DISEASES

Periodontal diseases are a group of infectious diseases caused by predominantly gram-negative bacteria. In its strictest sense, periodontal disease refers to both gingivitis and periodontitis. Periodontitis involves the destruction of the supporting structures of the teeth including the periodontal ligaments, bone and soft tissue and is the most significant of these because it causes tooth loss.

The presence of bacteria in the oral cavity has been known since the time of Anton von Leeuwenhoek, as reported by Tataakis & Kumar (2005), who described the presence of “animalcules” in dental plaque. The bacterial aetiology of periodontal diseases has been explored for over 100 years. However, the identification of specific causative species has been hampered by some of the unique features of periodontal diseases. The foremost of these features is that disease occurs in a site already colonized by a bacterial population. Thus, disease might be caused by overgrowth of one or more species in the resident population or by colonization by exogenous pathogens.

The plaque biofilm consists largely of microbes and host proteins that adhere to the teeth within minutes of a dental prophylaxis. No one knows how many bacterial species coexist in the dental plaque, but the number is very large. Some of the most common organisms associated with periodontal diseases are Porphyromonas gingivalis, Prevotella intermedia, Bacteriodes forsythus, Campylobacter rectus, Actinobacillus actinomycetemcomitans and Treponema denticola.

Microbial biofilm is the aetiological factor of periodontal diseases. Supragingival plaque contains many bioactive end products, such as fermented organic acid, sulfur component, tissue digesting enzymes, peptidoglycans and lipopolysaccharides. These components are diffused from supra-gingival plaque to the surface of gingival epithelium and increase the flow of crevice fluid and inflammatory fluid from periodontal tissue. This new nutritional supply,
which is delivered from serum, changes the ecosystem of the plaque adjacent to the inflamed gingiva. In this new environment, proteolytic bacteria in the plaque expand their ecologic niche and produce proteases, which accelerate tissue damage.\textsuperscript{58}

The junctional epithelium acts as a gate keeper, selectively allowing the passage of antigens and cells as well as producing a range of defensive molecules. Once the epithelial barrier with its antimicrobial peptides is breached adaptive immune response comes into play. Cytokines are central to this response, such that production of “appropriate” cytokines results in development of protective immunity and production of “inappropriate” cytokines leads to tissue destruction. Exactly how the immune system chooses these cytokines is unclear, although the genetic factors are most likely involved.\textsuperscript{59} (Fig-3)

(Fig-3)

![Diagram of Susceptibility to Periodontal Disease](image)

**Fig-3.** Susceptibility to periodontal disease (adapted from Seymour & Taylor)\textsuperscript{59}

In a recent review, Loesche and Grossman \textsuperscript{57} (2001) concluded that most, if not all, forms of periodontal disease are specific, albeit chronic infections. Regardless of whether the host is genetically predisposed to periodontal disease, or if the host is compromised by leukocyte defects, or diabetes, or is a smoker, or has poor oral hygiene, the clinical symptoms are almost always associated with the overgrowth of a finite number of anaerobic species.
2.12. PERIODONTAL DISEASES AND SYSTEMIC HEALTH

The concept that oral infections, such as periodontitis, may contribute to various systemic diseases is not new. Indeed, the possibility that a localized or focal infection could have systemic effects was a popular idea at the turn of the last century. A theory of focal infection developed that proposed that local “foci” of infection were responsible for the initiation and progression of various inflammatory conditions, such as arthritis, appendicitis and peptic ulcers. A focal infection is a chronic, localized infection that can disseminate microorganisms or toxic microbial products to contiguous or distant tissue and can adversely affect the distant target organs.

There is substantial evidence that the relationship between periodontal diseases and systemic diseases may be bi-directional. That is, not only the systemic conditions have oral manifestations, but periodontal diseases can also affect certain systemic conditions. It has been found that people with periodontal diseases have higher levels of circulating bacterial pro-inflammatory components, compared to people with healthy gums. The local inflammatory response to these bacterial products is the infiltration of periodontal tissue by the inflammatory cells including polymorphonuclear neutrophils, lymphocytes, macrophages and plasma cells. Activated macrophages release cytokines which can result in local and systemic elevation of pro-inflammatory mediators such as IL1, IL6, PGE2 and TNF-α. Elevated levels of these cytokines can also initiate a systemic acute phase response (APR). The acute phase response (APR) represents an early and highly complex reaction of an organism to a variety of injuries such as bacterial, viral or parasitic infection; mechanical or thermal trauma, ischemic necrosis or malignant growth. The acute phase refers to physiological and metabolic alterations that ensue immediately after the onset of infection or tissue injury. The hepatic synthesis and intravascular secretion of many plasma proteins with a wide range of actions is also increased. The components of the acute phase
response are largely non-specific as compared to cellular or humoral immunity and purpose of these responses is to restore homeostasis and to remove the cause of disturbance. Characteristic features of systemic acute phase response include fever, neutrophilia, altered lipid metabolism, hypoferroemia, increased gluconeogenesis, increased (muscle) protein catabolism and transfer of amino acids from muscles to liver, activation of complement and coagulation pathways and induction of acute phase proteins (Fig-4).

**Fig-4.** Schematic of the cascade of stimulation & response activities during acute phase response (Adapted from Ebersole & Cappelli 2000)
The major acute phase proteins includes C-reactive proteins (CRP), serum amyloid A, fibrinogen and hepatoglobin, whose concentrations increases with inflammation, and albumin and transferrin whose concentrations decreases with inflammation. The serum C-reactive protein concentrations closely follow the course of the acute phase response to inflammation, therefore, its measurement can provide a valuable and timely barometer for many disease processes.

Pro-inflammatory cytokines and mediators are significantly elevated with gingival inflammation during destructive periodontitis. Cytokines appears to play a major role in the clinical symptoms and tissue destruction with progressive periodontitis. There is also strong evidence for cytokines eliciting the systemic acute phase response in various inflammatory conditions.

From many studies on the systemic effects of serum pro-inflammatory cytokine levels potentially elevated by periodontitis, researchers have hypothesized that periodontitis-induced elevation of IL-1 and TNF-α may play a major role in the development of a variety of systemic conditions and diseases. In fact some studies have shown that in advance periodontitis, the levels of IL-1 and TNF-α are sufficiently elevated in gingival crevicular fluid to be detectable systemically by biological serum assay. Thus, patients with advanced periodontitis could be considered systemically compromised even in the absence of overt clinical symptoms of disease.

2.12-1. Periodontal infections and cardiovascular disease

Coronary heart disease is the leading cause of adult mortality and morbidity throughout the world. The major contributing factor in the majority of the cases of cardiovascular disease and cerebrovascular disease (stroke) is atherosclerosis. Hypercholesterolemia, dyslipidemia, obesity, hypertension, diabetes mellitus and smoking are well established risk factors for atherosclerosis and its complications. One of the outcomes of atherosclerotic process is the narrowing of the arteries resulting from subendothelial deposition of cholesterol, cholesterol esters and calcium within the vessel wall. The
cause of acute coronary syndrome is the destabilization of pre-existing atherosclerotic plaque resulting in clot formation on the surface of plaque. Inflammation within the atheromatous plaque is a factor which may lead to its destabilization.\textsuperscript{68}

Chronic infections, including dental infections have been linked to increased risk for cardiovascular diseases.\textsuperscript{69} The research documenting the association between periodontal diseases and cardiovascular diseases is recent, with the first study published in 1989.\textsuperscript{70} Several mechanisms have been proposed to explain such an association. One of these is based on the potential for the inflammatory phenomenon of periodontitis to have effects by the systemic dissemination of locally produced mediators such as C-reactive protein (CRP), interleukin 1 beta (IL-1$\beta$) and IL-6 and tumour necrosis factor alpha (TNF-$\alpha$).\textsuperscript{71} Elevated levels of CRP have recently gained special attention as a risk factor for cardiac and cerebrovascular events. Elevated levels of IL-6 have also been associated with unstable angina.\textsuperscript{72}

Majority of the studies published have suggested that periodontitis may be associated with cardiovascular events.\textsuperscript{70} In a recent case control study, Geerts et al\textsuperscript{67} (2004) concluded that periodontitis is a significant risk factor for cardiovascular disease after adjusting for other confounding factors. Similarly, Czerniue at al\textsuperscript{68} (2004) found that the periodontal status of patients admitted to the coronary care unit due to acute coronary syndrome was unacceptable.

Beck and Offenbacher\textsuperscript{70} in their review article published in 2001 suggested that at that time there is not enough evidence to state that periodontal infection is a cause of cardiovascular disease. On the other hand, in a recent systematic review, Scannapieco et al\textsuperscript{66} (2003) concluded that periodontal disease appears to be moderately associated with atherosclerosis-induced diseases such as coronary artery disease, stroke and peripheral vascular disease. Although the extent to which the initiation and/or progression of atherosclerosis is influenced by periodontal infection is presently unknown.
Diabetes mellitus is a metabolic derangement characterized by impairment in glucose use. Diabetes occurs in two major forms; type 1 diabetes is the result of a reduction in or the elimination of insulin production by beta cells in the pancreas.\textsuperscript{60} While type 2 diabetes results from defects in the insulin molecules or from altered insulin cell receptors and represent the impaired insulin function.\textsuperscript{73}

The association between diabetes and periodontitis has long been discussed. Most of the early studies tended to consider the relationship as unidirectional, noting a higher incidence of periodontitis in diabetes patients. More recent work has reported the converse relationship.\textsuperscript{62} Recent evidence suggests that chronic subclinical inflammation play an intermediary role in the pathogenesis of type 2 diabetes. Elevated levels of the inflammatory markers, such as C-reactive protein (CRP), interleukin-6 (IL-6) and tumour Necrosis factor-alpha (TNF-\(\alpha\)) are reported to be significant risk indicators of type 2 diabetes.\textsuperscript{74, 75}

Several recent studies have suggested that oral infection, particularly those associated with destructive periodontal disease may induce elevated serum levels of C-reactive proteins and other pro-inflammatory mediators.\textsuperscript{17, 18, 19, 20} Engebretson et al.\textsuperscript{76} (2004) reported that poor glycemic control is associated with elevated levels of IL-1\(\beta\) in gingival crevicular fluid.

Many studies have been conducted to see the effect of periodontal therapy on glycemic control in type 2 diabetes mellitus patients. In a recent study, Kiran et al.\textsuperscript{77} (2005) concluded that non-surgical periodontal therapy is associated with improved glycemic control in type 2 diabetes mellitus. Similarly Rodrigues et al.\textsuperscript{73} (2003) showed in their study that effective periodontal treatment resulted in lower glycemic levels. Lwamoto et al.\textsuperscript{75} (2001) evaluated the effect of antimicrobial periodontal treatment on circulating TNF-\(\alpha\) and glycated haemoglobin levels in patients with type 2 diabetes and found that anti-infectious treatment is effective in improving the metabolic control in diabetics possibly through reduced serum TNF-\(\alpha\) and improved insulin resistance.
2.12-3. Periodontal infections and adverse pregnancy outcomes

Preterm delivery and low birth weight is one of the major social and economic public health problem not fully explained by the currently established risk factors. Preterm delivery is the most significant cause of neonatal mortality and long term health problems including respiratory distress syndrome, cerebral palsy, pathologic heart conditions, epilepsy and severe learning problems. Despite the significant improvement in medical care and vigorous attempts to reduce preterm birth by wide spread use of drugs and public health interventions, the preterm birth rate have not declined but instead have increased over the last two decades.

Offenbacher et al. were the first to report that periodontitis was a possible risk factor for preterm low birth weight. After adjusting for other known risk factors they suggested that pregnant women with periodontitis were at a higher risk of delivering a preterm low birth weight infant as compared to women without periodontitis.

Medianos et al. (2002) hypothesized that translocation of periodontal pathogens to foetal-placental unit induce a maternal and/or foetal response that results in preterm birth. The bacteria involved in chronic periodontal inflammation are similar to those found in women with bacterial vaginosis. The potential of C. rectus and P. gingivalis in mediating the adverse pregnancy outcomes was recently studied in mouse model. In a most recent study by Yeo et al. (2005) on pregnant mice, conclude that remote subcutaneous maternal infections with C. rectus increase foetal resorption and foetal growth restrictions.

Maternal serum levels of some cytokines have been reported to be associated with preterm low birth weight. Such cytokines include interleukin 6 (IL-6) interleukin 1β( IL-1β) interleukin-8 (IL-8) and tumour necrosis factors. It had been suggested that women who were diagnosed of threatened premature labour showed poorer periodontal condition and elevated serum levels of interleukin-8 and interleukin-1β as compared to controls. Lopez et al. in one
of their study conducted in 2002, suggested that inflamed periodontal tissues produce significant amounts of pro-inflammatory cytokines including interleukin \( \text{IL-1}\beta \) (IL-1\( \beta \)) interleukin-6 (IL-6), prostaglandin \( \text{E}_2 \) (PGE\( \text{E}_2 \)) and tumour necrosis factor alpha (TNF-\( \alpha \)). In the most recent study by Dortbudak et al.\(^{86} \) (2005) concluded that pregnant women with elevated levels of PGE2, IL-6, and IL-8 in their amniotic fluid in the 15-20 weeks of pregnancy and with periodontitis are at higher risk for preterm birth.

Most of the recent studies on the possible relationship between periodontal diseases and risk of preterm low birth weight infants suggest periodontal disease as a risk factor for preterm low birth weight deliveries.\(^{23, 81, 82, 86} \) On the other hand Noack et al.\(^{79} \) (2005) concluded that periodontitis was not a detectable risk factor for preterm low birth weight in pregnant women in the population they included in their study.

In conclusion, it can be summarized that although a number of studies suggest that periodontal infections are associated with systemic diseases but the exact mechanism by which the periodontal diseases have systemic effects still remains unclear and speculative. Therefore, such periodontal-systemic associations must be investigated through large, prospective randomized clinical studies as well as interventional studies.
PERIODONTAL – SYSTEMIC CONNECTION

PERIODONTAL INFECTIONS

Bacteria

Bacterial products (LPS, Fimbriae)

Inflammatory mediators (IL-1, INF-γ, IL-6, IL-8)

BLOOD STREAM

Systemic inflammatory response

Heart & Blood Vessels

Endothelial injury
Lipid deposition
Monocyte migration
Muscle proliferation

ACUTE PHASE PROTEINS

CRP, SSA, IL-6, TNF-α, IL-1

PLACENTA / UTERUS

Contraction of uterine smooth muscles,
Pre-term rupture of membrane

LIVER & PANCREAS

Insulin resistance
Glucose intolerance

DIABETES

ATHEROSCLEROSIS
CARDIOVASCULAR DISEASE

PRETERM LOW BIRTH WEIGHT

Fig-5 Immune responses and putative mechanism proposed to link periodontal disease and systemic disease. (Adapted from Amar & Han 2003) 62

Abbreviation: (LPS) lipopolysaccharides (IL-1) interleukin-1 (INF-γ) interferon gamma (IL-6) interleukin-6 (IL-8) interleukin-8 (CRP) C-reactive protein (TNF-α) tumour necrosis factor alpha
2.13 PERIODONTAL INFECTIONS AND C-REACTIVE PROTEIN (CRP)

As discussed earlier, CRP is an acute phase protein produced by liver in response to inflammatory stimuli. Established risk factors for “high normal” values of CRP within the general population include older age, smoking, chronic bacterial infections and chronic bronchial inflammation. However, raised CRP levels have been observed among individuals with no apparent established risk factor, suggesting the role of other pathological conditions.\(^\text{87}\)

As mentioned earlier, periodontal disease is a chronic inflammatory process that occurs in response to a predominantly Gram-negative bacterial infection originating from dental plaque. Recent studies of the general population have suggested that oral infection, particularly those associated with destructive periodontal disease may induce elevated serum levels of C-reactive proteins.\(^\text{17, 18, 19, 20}\)

Noak et al.\(^\text{12}\) (2001) in their case control study compared the levels of CRP in patients with different severity of periodontal disease to healthy controls and found significantly elevated CRP levels in subjects with more than 3mm mean attachment loss.

Similarly, Craig et al.\(^\text{15}\) (2003) on the basis of the results of their study suggested that destructive periodontal diseases were associated with changes in serum components that are consistent with an acute phase response. Furthermore, they found that elevated levels of serum IgG antibody to P. gingivalis could increase the relative risk of high CRP levels.

Aiuto et al.\(^\text{8}\) (2004) in a preliminary intervention study showed that treatment of periodontitis was associated with a significant reduction in serum CRP and IL-6 in otherwise healthy individuals affected with severe, generalized periodontitis.

Similarly in a more recent study, on short term effects of intensive periodontal therapy on serum inflammatory markers Auito et al.\(^\text{88}\) (2005) concluded that periodontal therapy (either standard or intensive) resulted in an additional
reduction in serum CRP of at least 0.5mg/L compared with the untreated controls.

Lwamoto et al.\textsuperscript{89} (2003) in their study on the effects of antimicrobial and non-surgical periodontal treatment on serum levels of CRP and TNF-\(\alpha\) found that periodontal treatment was effective in reducing the CRP and TNF-\(\alpha\) concentration in patients with chronic periodontitis.

In conclusion, there is substantial evidence that periodontal infections may contribute to elevated systemic CRP levels in otherwise healthy individuals and periodontal therapy results in a significant reduction in serum CRP.

2.14. PERIODONTAL INFECTIONS AND END-STAGE RENAL DISEASE (ESRD)

As described earlier the ESRD patients experience a greatly increased rate of atherosclerotic complications. In both ESRD patients and general population it has become evident that inflammation plays a central role in the pathogenesis of atherosclerotic complications.\textsuperscript{36, 38}

C-reactive protein (CRP) the major acute phase protein has been found to predict all-cause and cardiovascular mortality in ESRD patients. Hepatic synthesis of CRP is up-regulated by pro-inflammatory cytokines released locally at the site of inflammation, although many ESRD patients experience elevated CRP values in the absence of overt infection or inflammation.\textsuperscript{4}

Oral infection, particularly those associated with destructive periodontal disease in the general population have been associated with both an increased prevalence of atherosclerotic complications as well as an elevation in serum CRP levels.\textsuperscript{17, 18, 19, 20} In view of the prevalence of periodontal diseases in general population, and since the periodontal examination is not normally performed as part of medical assessment, periodontal diseases may be an overlooked source of inflammation in ESRD patients.
Only a couple of studies have been conducted on this issue. Kshirsagar et al.\textsuperscript{24} (2005) in their cross sectional study of 5,537 individuals, concluded that there was a significant association between periodontal disease and renal insufficiency after adjustment for numerous demographic and medical covariates. The population for this study was drawn from the Atherosclerosis Risk in Communities (ARIC) study. The exposure variable, periodontal disease and outcome renal insufficiency were determined concurrently from the participants at ARIC study visit 4. Periodontitis was categorized as healthy/gingivitis, initial and severe. Renal insufficiency was defined as glomerular filtration rate (GFR) less than 60 ml/min/1.73 m\textsuperscript{2}. This study was a cross sectional exploratory analysis. Thus, periodontal disease may be a true risk factor, a risk indicator or a factor merely associated at a statistical level. Therefore, this issue needs to be investigated further to establish the exact nature of relationship between periodontal disease and kidney disease.

Rahmati et al.\textsuperscript{18} (2002) examined the sera of 86 haemodialysis (HD) patients and measured the CRP and IgG antibody titres against six different periodontal pathogens. The results suggested that elevated levels of IgG antibody to bacterial species associated with destructive periodontal diseases were associated with elevated CRP levels in HD patients.
3.1. **Aim**

The aim of the study is to investigate whether periodontitis is associated with increased systemic inflammation reflected by CRP values, in patients with ESRD on maintenance haemodialysis or peritoneal dialysis.

3.2. **Objectives of this study are:**

- Determine the prevalence of periodontitis in ESRD patients.
- Determine the CRP levels in ESRD patients.
- Compare the CRP levels in ESRD patients with and without periodontitis.

3.3. **Null Hypotheses**

There is no significant difference in the CRP level in ESRD patients with or without periodontitis.
CHAPTER 4
MATERIALS AND METHODS

4.1. STUDY DESIGN:

The study was a cross sectional analytic observer blind study. The part of the data capture sheet (Appendix 1) containing information regarding medical history and CRP levels was completed by a physician in the renal unit. Periodontal examination was carried out by a single examiner (no calibration or standardization was performed) without any knowledge of medical history and CRP levels of patient to avoid observer bias.

4.2. STUDY POPULATION:

Patients with ESRD attending the renal unit at Tygerberg hospital for treatment of ESRD disease, and either on haemodialysis or peritoneal dialysis as a part of their treatment.

4.3. SAMPLE SIZE:

Eighty patients with ESRD on haemodialysis or peritoneal dialysis were included in the study, because it was a convenient sample size and only comparable study available used the same sample size and found statistically significant results.¹⁸

4.4. INCLUSION CRITERIA:

Patients with end-stage renal disease either on haemodialysis or peritoneal dialysis
Patients on dialysis for not less than three months
4.5. EXCLUSION CRITERIA:

Patients on dialysis less than three months
Patients that underwent periodontal treatment during the previous three months
Patients who took antibiotics during the previous three months
Patients with any known infection
Edentulous patients

4.6. INFORMED CONSENT:

Study was approved by the Ethical and Research Committee of the University of the Western Cape.

Patients were first approached by a staff member of the renal unit to ascertain the patient interest, followed by a verbal explanation of the study. Each volunteer also received a “consent form” (Appendix-2) describing the essential features of the study and had the opportunity to question the investigator. If the patient was willing to participate in the study, informed consent for participation was obtained and each volunteer was asked to sign the consent form.

4.7. DATA COLLECTION:

Patient demographics, smoking status, vascular access mode, type of dialysis, adequacy of dialysis and CRP levels were obtained from the patients file. Patients were also asked to complete a questionnaire about their oral hygiene habits. (See Appendices 1 & 3)

4.8. DATA ANALYSIS

The information obtained was entered into a Microsoft Excel format and statistically analysed by Chi-square test for each variable using a commercially available statistical software package (SPSS 13.0, SPSS Inc.). For the few
continuous variables descriptive statistics, such as means, standard deviation and medians were calculated.

4.9. **CLINICAL EXAMINATION:**

The periodontal examination included Plaque index (PI), Gingival index (GI), Bleeding on probing (PoB), Probing depths (PD) and Clinical attachment loss (CAL).

Clinical measurement was recorded by a single examiner and intra examiner calibration was done by repeating 10% of the sample. (Intra-examiner reproducibility [within +/- 1.0 mm] was 98.2%).

The plaque index (PI) and gingival index (GI) were measured at four sites per tooth (mesial, buccal, distal and lingual/palatal) on six teeth using the Ramfjord teeth and were given a score from 0-3 according to Silness & Loe 1964. (Appendix 3)

Bleeding on probing (BoP) was recorded at two sites per tooth (Buccal & lingual/palatal) using Ramfjord teeth and will be given score 0- if no bleeding and 1- if bleeding occurs within 10 seconds of probing.

Probing depth (PD) was measured from gingival margin to the base of pocket at 6 sites per tooth (mesio-buccal, buccal, disto-buccal, disto-lingual, lingual and mesio-lingual) on six teeth using the Ramfjord teeth.

Clinical attachment loss (CAL) was measured from cementoenamel junction (CEJ) to base of the pocket, six sites per tooth (mesio-buccal, buccal, disto-buccal, disto-lingual, lingual and mesio-lingual) on six teeth using the Ramfjord teeth.

Mean values were calculated for all variables.
Sextants were examined and the most severe index value in each sextant was recorded. A sextant was examined only if two or more teeth were present. If no index teeth were present, all the remaining teeth in that sextant were examined and the highest score was recorded. Clinical measurements were recorded to the nearest millimetre using a manual calibrated periodontal probe (Fig-6).

**Fig. 6** Periodontal probe

### 4.10. PERIODONTAL DISEASE ASSESSMENT

The presence of one sextant showing PD of $\geq 4$ mm or clinical loss of attachment of $\geq 3$ mm was diagnosed as periodontitis. \(^{79,80}\)

### 4.11. C-REACTIVE PROTEIN (NORMAL AND ELEVATED)

CRP was considered acceptable (normal) for ESRD patients if serum CRP level was $\leq 10$ mg/L and elevated if CRP level was $> 10$mg/L. \(^{18}\)
CHAPTER 5
RESULTS

5.1. DEMOGRAPHIC CHARACTERISTICS

Eighty end-stage renal disease patients (ESRD) were included in the study. Mean age of the subjects was 50.3±9.06 years; with a median time on dialysis therapy of 24 months (range 3-156 months)

Thirty four (42.5%) subjects were male; fifty (62%) subjects reported their race as Coloured, 20 (25%) subjects as Blacks and 10 subjects (13%) as others.

Only six (7.5%) subjects had diabetes and 14 (17.5%) were current smokers.

Fifty two (65%) subjects were on peritoneal dialysis and 35% (n=28) were on haemodialysis. Arteriovenous fistulae (AVF) were used in 20 (25%) haemodialysis patients as vascular access while temporary catheter (TC) was used in 8 (10.0%) subjects.

Dialysis was assessed as adequate in 48 (60%) subjects calculated by urea clearance (Kt / V) and urea reduction ratio (URR) for haemodialysis patients, and by urea levels in peritoneal dialysis patients.
5.2. PERIODONTAL DISEASE

Forty six (57.5%) subjects were diagnosed to have periodontal disease with an overall mean plaque index (PI) 1.56 ±0.55, gingival index (GI) 1.11± 0.47, bleeding on probing (BoP) 0.31±0.27, probing depths 2.66 ±0.45 and attachment loss (AL) 1.09 ± 1.00.

5.3. C-REACTIVE PROTEIN (CRP)

Mean CRP value for patients with periodontal disease was 20.01 ± 20.21mg/L and for those with healthy periodontium was 9.14 ± 7.17mg/L. Overall, 42.5 % (n=34) subjects showed elevated levels of CRP.

- CRP was considered acceptable for ESRD patients if serum CRP level was ≤10 mg/L.\(^\text{18}\)
- Normal CRP value for healthy individual < 2 mg/L.\(^\text{42}\)

Distribution pattern of CRP in ESRD patients with or without periodontal disease was as follows (Fig-7).

![Graphic presentation of distribution pattern of CRP in ESRD patients with and without Periodontal Disease](image-url)
The affect of various variables such as age, gender, race, smoking, dialysis type, adequacy of dialysis and periodontal disease on CRP levels was statistically analysed using the chi square test. Although diabetes has been reported to have a direct effect\(^{30, 31}\) it was not included in the test because only 6 subjects were diabetic. The number was too small to give any statistically significant result.

Periodontal disease showed a statistically significant difference (p<0.05) while no other variables was found to have any statistically significant difference.

*Of the forty six subjects diagnosed having periodontal disease, 52.2% had elevated CRP values. On the other hand, of the thirty four subjects with a healthy periodontium, only 29.4% (n=10) had elevated CRP levels. There was a statistically significant difference in serum CRP levels between these two groups (p=0.035). Therefore, the results of the study suggest that periodontal diseases are associated with elevated CRP values in ESRD population.* (Table-2)

There were only 14 active smokers among the participants of the present study. Noteworthy is that of the 14 smokers 57.1% (n=8) showed a high CRP level.

Dialysis was assessed adequate in 60 % subjects. Of the 48 participants in which dialysis was assessed adequate, only 33.3% (n=16) showed elevated levels of CRP. While of the 30 subjects in which dialysis was assessed inadequate 53.3% (n=16) showed elevated CRP levels. These clinical trends were found to be statistically not significant when tested by Chi-square test, however, the p value was very close to significance level(p<0.05).

(Table-2)
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<th>Significance</th>
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<td>&gt; 40</td>
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<td>24 (70.6%)</td>
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</table>

*1 CRP was considered acceptable for ESRD patients if serum CRP level was ≤10 mg/L.
(Normal CRP value for healthy individual < 2 mg/L)

*2 For the purpose of statistical analysis subjects who reported their race other than coloured or black were excluded because number was too small to give any significant results

*3 Adequacy of two participants was not available
5.4. DRUGS

Some drugs such as aspirin, non-steroidal anti-inflammatory drugs (NSAID), 3-hydroxy-3-methylglutaryl co-enzyme A reductase inhibitors (statins) and angiotensin-converting enzyme inhibitors (ACE) tend to lower the CRP values in ESRD patients. The affects of these drugs were also statistically analysed using the chi square test (Table 3).

Statins were being used by only two subjects and no one was on NSAID.

There was no statistically significant difference in CRP values when analysed for other two drugs.

Aspirin was prescribed to 27.5% (n=22) participants. Of the twenty two subjects using aspirin, 72.7% (n=16) showed normal CRP levels. Although the result was not statistically significant in terms of the use of aspirin but yet it showed a trend towards low CRP levels (p=0.073) in subjects using aspirin (Table-3).

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<td>(51.7%)</td>
</tr>
<tr>
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<td>Yes</td>
</tr>
<tr>
<td></td>
<td>(53.8%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>(59.3%)</td>
</tr>
</tbody>
</table>

* CRP was considered acceptable for ESRD patients if serum CRP level was ≤10 mg/L. (Normal CRP value for healthy individual < 2 mg/L)
5.5. ORAL HYGIENE HABITS

Forty subjects (50%) reported brushing twice a day (BD) and six (7.5%) three times a day (TDS). Most of them (85%) never used dental floss. Only 22.5% used mouthwash in their daily routine and majority of them (70%) visited their dentist only in case of some dental problem (Fig-8).

![Oral Hygiene habits](image)

**Fig. 8** Oral hygiene practice of ESRD patients

The possible impact of the oral hygiene habits of the ESRD patients on their periodontal status was analysed using the chi square test (Table 5).
For the purpose of analysis variables were divided into following groups: (Table 4)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brushing</td>
<td>Group -1</td>
<td>Once a day</td>
</tr>
<tr>
<td></td>
<td>Group -2</td>
<td>More than once a day</td>
</tr>
<tr>
<td>Dental floss</td>
<td>Group -1</td>
<td>Used dental floss</td>
</tr>
<tr>
<td></td>
<td>Group -2</td>
<td>Never used dental floss</td>
</tr>
<tr>
<td>Visit to dentist</td>
<td>Group -1</td>
<td>Visited dentist at least once a year</td>
</tr>
<tr>
<td></td>
<td>Group -2</td>
<td>Visited dentist only in case of problem</td>
</tr>
</tbody>
</table>

There was statistically significant difference in the periodontal status with regards to brushing frequency, use of mouth wash and visit to dentist. However, no significant difference was found for the use of floss (Table-5).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Periodontal disease</th>
<th>Total</th>
<th>Significance (p&lt;.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Brushing</td>
<td>Once a day</td>
<td>28 (82.4%)</td>
<td>6 (17.6%)</td>
</tr>
<tr>
<td></td>
<td>More than once</td>
<td>18 (39.1%)</td>
<td>28 (60.9%)</td>
</tr>
<tr>
<td>Floss</td>
<td>Yes</td>
<td>6 (50.0%)</td>
<td>6 (50.0%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>40 (58.8%)</td>
<td>28 (41.2%)</td>
</tr>
<tr>
<td>Mouth wash</td>
<td>Yes</td>
<td>4 (22.2%)</td>
<td>14 (77.8%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>42 (67.7%)</td>
<td>20 (32.3%)</td>
</tr>
<tr>
<td>Visit to dentist</td>
<td>Once a year</td>
<td>4 (16.7%)</td>
<td>20 (83.3%)</td>
</tr>
<tr>
<td></td>
<td>Infrequently</td>
<td>42 (75.0%)</td>
<td>14 (25.4%)</td>
</tr>
</tbody>
</table>
Of the thirty four participants who brushed their teeth once a day, 82.4% (n=28) showed the signs of periodontal disease while of the 46 subjects who brushed their teeth more than once a day only 39.1% (n=18) had periodontal disease.

Similarly, of the twenty four participants who visited their dentist at least once a year only four (16.7%) had periodontal disease, on the other hand, of the fifty six subjects who visited their dentist only in response to some dental problem, 75.0% (n=42) had periodontal disease (Table-5).
Despite a rapid improvement in dialysis technology during the last 20 years, the mortality rate is still very high in ESRD patients. The majority of maintenance dialysis patients die within a five year period. In fact the death rate is comparable to that of cancer patients with metastases.\textsuperscript{36,37} The main cause of mortality in ESRD is the cardiovascular disease which accounts for premature death in more than 50\% of dialysis patients.\textsuperscript{36, 37, 38, 39} The high cardiovascular mortality rate indicates that ESRD patients are subject to a process of accelerated atherogenesis.\textsuperscript{36}

Several studies have shown that the high prevalence of cardiovascular disease in ESRD patients is only partly explained by the traditional risk factors.\textsuperscript{39, 40} Therefore, non-traditional factors, such as, oxidative stress and inflammation may be more important.\textsuperscript{38, 39}

It has now been recognized that about 30-50\% of pre-dialysis, haemodialysis and peritoneal dialysis patients have serologic evidence of activated inflammatory response.\textsuperscript{36,39} The origin of inflammation in ESRD patients remains unclear.\textsuperscript{36, 37, 39, 41}

The significance of CRP in ESRD population has increased over time. From a simple marker, it now appears that CRP is an active participant in proatherosclerotic phenomena’s.\textsuperscript{21} Elevated C-reactive protein (CRP) levels have been observed in a significant proportion of ESRD patients without any apparent reason.\textsuperscript{4} Therefore, the role of occult or unapparent infections could not be ignored as a potential source of inflammation in these patients. Chronic infections with \textit{Chlamydia species} and \textit{Helicobacter pylori} have been postulated as a source of inflammation. The presence of periodontitis in such patients may also increase CRP and other inflammatory markers.\textsuperscript{21}
The concept that oral infections, such as periodontitis, may contribute to various systemic diseases is not new. Oral infection, particularly those associated with destructive periodontal disease in the general population have been associated with an increased prevalence of atherosclerotic complications as well as an elevation in serum CRP levels.

The primary aim of the present study was to determine whether periodontal diseases contribute to increased systemic inflammation, reflected by CRP levels in patients with ESRD. Elevated CRP level is known to be a robust risk factor for both cardiovascular and all-cause mortality in this population.

The null hypothesis tested stated that there is no significant difference in the CRP level in ESRD patients with or without periodontitis. Data from the present study indicate that patients with periodontitis showed significantly elevated CRP levels (> 10 mg/L) compared to those with healthy periodontium. This finding supports observations in the general population of associations between elevated CRP level and periodontal disease status.

Elevation of CRP values in both general and ESRD population is multi-factorial in cause, and individual patients may have many potential sources that could contribute to the inflammatory state. Therefore, determining the contribution to elevated CRP levels from a specific source may be confounded by many other factors. Possible impact of other variables such as such as age, gender, race, smoking, dialysis type, and adequacy of dialysis on CRP levels was also statistically analysed.

Age is a known risk factor for both chronic kidney diseases, as well as periodontal diseases. The mean age of the sample in the present study was 50.3±9.06 years which is slightly less than that of other comparable study done by Rahmati et al. (2002) with a mean age of 53±16.1 years. No significant difference was found in CRP levels among the subjects > 40 years of age compared to those < 40 years of age. These results do not support the observations in other studies of association with elevated CRP levels with
increased age.47,92 This may be due to the fact that the sample size was small and most of the subjects (72.5%) were older than 40 years age.

Smoking has also been recognized as a progression factor 29, 30 for chronic kidney diseases and a risk factor for periodontal diseases.54, 93 However, there was no statistically significant difference in CRP levels between smokers and non-smokers. Only 14 (17.5%) participants in the present study were current smokers. This low number might due to the false reporting by the participants or there may be a reduction in smoking habit especially in the light of the awareness of the risks associated with cigarette smoking. Nevertheless, small numbers do not allow any meaningful conclusion.

Comparison was also done between different dialysis modalities. No significant difference was found in CRP levels between haemodialysis (HD) population compared to peritoneal dialysis (PD). Both modalities showed the same pattern of CRP levels. Although there is much debate over the advantages of one therapy over another, no randomized prospective study comparing the two modalities has been conducted. Due to the complexities in designing such studies it is unlikely that such a study will be done.34

In the present study, periodontitis was diagnosed using the criterion of presence of one sextant showing PD of $\geq 4$ mm or clinical loss of attachment of $\geq 3$ mm. The periodontitis appears to be a site specific disease; it is desirable to record as many sites as possible to increase the probability of finding the disease activity. However, it is not practiced routinely due to cost, patient burden and examiner reliability issues. To reduce the chances of systematic error, sites that have a higher probability of attachment loss are usually included. The levels of underestimation associated with the number of the sites used are not fully known and also no correction methods are available. The most well known index is the “Periodontal Disease Index” which use measures on 6 specific teeth (Ramfjord teeth): the maxillary right first molar, the maxillary left central incisor and maxillary left first bicuspid, the mandibular first left molar, the mandibular right central incisor and the mandibular first right bicuspid. Index
teeth are chosen because they represent all the teeth in the mouth or because they are the most susceptible.\textsuperscript{94}

The partial mouth recordings may be accurate and efficient in estimating the mean periodontal measures but may severely under and/or over-estimate the prevalence of certain periodontal conditions.\textsuperscript{95, 96} Another important consideration with regards to estimation of periodontal disease using partial recording protocol is that it shows only the prevalence of disease and do not give an account of the extent and the severity of the disease.

Nevertheless, in a recent study on the usefulness of using Ramfjord teeth in predicting periodontal status, Mumghamba and co-workers\textsuperscript{97} (2004) concluded that overall high agreement between Ramfjord teeth and full-mouth periodontal pocket situation suggest that periodontal assessment using Ramfjord teeth may be useful alternative to full mouth measurements.

A significant association was observed between periodontitis and high serum CRP values in ESRD population. In majority of the subjects (70.6\%) without periodontitis the serum CRP levels were normal. However, normal CRP levels were also observed in participants with periodontitis. The possible explanation for this observation is that in most cases the parameters that are measured for the estimation of periodontal disease, such as, probing depths and attachment loss, are the measurement of past disease and do not give an indication as to whether the disease is active or progressing. Moreover, there are important differences in the host response to the bacterial challenges.\textsuperscript{99, 70} In summary the results of present study suggest that periodontitis may be an overlooked source of inflammation in ESRD patients.

Another potential source of confounding is the effect of medications on CRP values. Some drugs such as aspirin, 3-hydroxy-3-methylglutaryl co-enzyme A reductase inhibitors (statins) and angiotensin-converting enzyme inhibitors (ACE) tend to lower the CRP values in ESRD patients.\textsuperscript{90, 91} These drugs are commonly administered to patients with ESRD and could have confounding effect.
However, the results failed to show any significant difference in CRP levels with regards to the use of these drugs. These finding are in contrast to other studies that suggest a decrease in serum CRP value with the use of these drugs.\textsuperscript{90, 91} The possible reason for these results may be the fact that only two subjects in the sample population were on statin therapy. This number is too small to give any statistically meaningful results.

Aspirin was prescribed to 27.5\% (n=22) participants. Takeda et al.\textsuperscript{98} (2003) observed that statin but not aspirin used in general doses have an anti-inflammatory action assessed by measurement of CRP levels. Although the results was not statistically significant in terms of the use of aspirin but yet they showed a trend towards low CRP levels (p=0.073) in subjects using aspirin.

Periodontal diseases are common among renal failure patients, however it is not fully established whether it is related to host alterations or to the carelessness of oral hygiene.\textsuperscript{7} Davidovich et al.\textsuperscript{1} (2005) in their study on four renal failure groups provide evidence that the progression of the periodontal disease in chronic renal failure patients was correlated with bad oral hygiene.

Marakoglu et al.\textsuperscript{2} (2003) in a case control study observed that there was no significant difference in the clinical parameters of periodontal disease between the two groups. On the basis of their findings they concluded that renal failure does not seem to be a traditional risk factor for more severe periodontal destruction.

Kitsou et al.\textsuperscript{7} (2000) in their case control study on experimental gingivitis, among chronic renal failure patients and healthy controls observed that there was no significant difference in the development of gingivitis between two groups.

Participants of the present study were asked to complete a questionnaire regarding their oral hygiene habits. The data showed highly significant difference in the periodontal status of the subjects in terms of brushing
frequency, use of mouth wash and visit to dentist. However, there was no significant different with regards to the use of dental floss.

These results support the findings of the other studies which suggest that high prevalence of the periodontal diseases in renal failure patients compared to healthy individuals is due to negligence of the oral hygiene.\textsuperscript{1,2}

The results failed to show any statistically significant difference in the prevalence of periodontal disease with regards to the use of dental floss. This finding may be due to fact that majority of the participants (85%) never used dental floss.

Therefore, it can be suggested that worsening of the periodontal status in renal failure patients is mainly due to the negligence of the oral hygiene rather than of chronic uraemia in the ESRD population.

Since the results of the current study suggest that periodontitis may be an overlooked source of inflammation in ESRD patients, it is very important that efforts should be made to motivate the renal patients to improve their oral hygiene practices to reduce the incidence of periodontal diseases among this population.

Data from the present study showed that patients who brushed their teeth more than once a day and used mouth wash had a significantly less prevalence of periodontal disease as compared to those who brush their teeth only once a day and never used mouth wash (Table 5). Therefore, it can be recommended that the patients with ESRD must be educated on regular basis about the importance of maintaining the oral health because it could directly effect the long term survival of the ESRD patients.

Results also indicated that there is highly significant difference in the expression of periodontal disease among patients who visited their dentist at least once a year compared to those who visited their dentist infrequently (Table 5). Therefore, dental check up must be an integral part of the medical
evaluation of the ESRD patients and they should be referred to the dentist for dental check up preferably every six months.

Several studies have shown that periodontal therapy resulted in a decrease in serum CRP and other inflammatory markers in healthy individuals with periodontal disease.\(^8,88,89\) Therefore, dentist could play a key role in maintaining the CRP levels of ESRD patients within acceptable limits by providing them with periodontal therapy, thus improving their quality of life because increased levels of CRP predict all-cause and cardiovascular mortality in these patients.
There are some important limitations to the present study:

Firstly, the analysis was cross sectional, which prevents the determination of causation.

Secondly, only the physical measurements of periodontal disease was made and not the effect of periodontitis on biochemical markers. There may be important differences in the host response to bacterial challenges.

Thirdly, partial mouth recordings protocol was used for the estimation of periodontal diseases which may be accurate and efficient in estimating the mean periodontal measures but could severely under and/or over-estimate the prevalence of periodontal disease.

Finally, the sample size was not large enough to represent the ESRD population.
CHAPTER 8
CONCLUSIONS

This study was conducted to investigate whether periodontitis is associated with increased systemic inflammation reflected by CRP values, in patients with ESRD on maintenance haemodialysis or peritoneal dialysis.

The results of the study showed significantly elevated levels of CRP in ESRD patients with periodontal disease as compared to those with healthy periodontium. Therefore, within the limitations of the study, it can be concluded that periodontal diseases may be an overlooked source of inflammation in ESRD patients.

The results also suggested that higher prevalence of the periodontal diseases in ESRD patients is mainly due to the negligence of the oral hygiene rather than of chronic uraemia in the ESRD population.
CHAPTER 9
RECOMMENDATIONS

Since the results of the current study suggest that periodontitis may be an overlooked source of inflammation in ESRD patients. Therefore, it is recommended that efforts should be made to motivate the renal patients to improve their oral hygiene practices to reduce the incidence of periodontal diseases among this population.

Results also indicate that there is highly significant difference in the expression of periodontal disease among patients who visited their dentist at least once a year compared to those who visited their dentist infrequently. Therefore, it is recommended that dental check up must be an integral part of the medical evaluation of the ESRD patients.

Because of study limitations and the potential of residual confounding, these preliminary findings must be interpreted with caution.

It is recommended that such an association must be investigated through large, prospective randomized clinical studies as well as interventional studies.

It is also recommended that multi-centre studies should be done with larger sample size to represent the ESRD populations.

Periodontal examination should be done with a full mouth recording protocol and the effect of periodontitis on biochemical markers should also be evaluated to overcome the differences in host response to bacterial challenges.
REFERENCES


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probing depths and attachment level estimate using random partial mouth
recording. *Community Dentistry and Oral Epidemiology* 34:1-10

The usefulness of using Ramfjord teeth in predicting periodontal status of a
Tanzanian adult population. *Journal of Clinical Periodontology* 31:16-18

Relationship between effects of Statins, aspirin and angiotensin II
modulators on high-sensitive C-reactive protein levels. *Atherosclerosis*
169:155-158
# APPENDIX 1

## DATA CAPTURE SHEET

### DEMOGRAPHIC DETAIL

<table>
<thead>
<tr>
<th>Patients name</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>File number</td>
<td>Gender</td>
</tr>
<tr>
<td>Race</td>
<td>Level of education</td>
</tr>
</tbody>
</table>

### CLINICAL ASSESSMENT

<table>
<thead>
<tr>
<th>Primary kidney disease</th>
<th>Previous transplant dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td>Smoking</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>Peripheral vascular disease</td>
</tr>
<tr>
<td>Dry weight and BMI</td>
<td>Infection, fever, or antibiotic Rx past three months</td>
</tr>
</tbody>
</table>

### DIALYSIS

<table>
<thead>
<tr>
<th>Type of dialysis</th>
<th>Time on dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular access</td>
<td>Adequacy of dialysis</td>
</tr>
</tbody>
</table>

### MEDICATION AND CURRENT DOSES (include last three months)

<table>
<thead>
<tr>
<th>Nifedipine</th>
<th>ACE-I</th>
<th>Statins</th>
<th>NSAIDS</th>
<th>Aspirin</th>
<th>EPO dose (unit per week)</th>
</tr>
</thead>
</table>

### BIOCHEMICAL

<table>
<thead>
<tr>
<th>CRP</th>
<th>Ferritin</th>
<th>Haemoglobin</th>
<th>Cholesterol</th>
<th>Albumin</th>
</tr>
</thead>
</table>
APPENDIX 1
PERIODONTAL ASSESSMENT

Ref. No ____________    File No __________

Plaque Index:            
(Ramfjord teeth)        

<table>
<thead>
<tr>
<th>16</th>
<th>21</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>41</td>
<td>36</td>
</tr>
</tbody>
</table>

Gingival Index:         
(Ramfjord teeth)        

<table>
<thead>
<tr>
<th>16</th>
<th>21</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>41</td>
<td>36</td>
</tr>
</tbody>
</table>

Bleeding on probing:    
(Ramfjord teeth)        

<table>
<thead>
<tr>
<th>16</th>
<th>21</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>41</td>
<td>36</td>
</tr>
</tbody>
</table>

Probing Depths:         
(Ramfjord teeth)        

<table>
<thead>
<tr>
<th>16</th>
<th>21</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>41</td>
<td>36</td>
</tr>
</tbody>
</table>

Clinical Attachment Loss: 
(Ramfjord teeth)        

<table>
<thead>
<tr>
<th>16</th>
<th>21</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>41</td>
<td>36</td>
</tr>
</tbody>
</table>

Periodontal Disease:    

Y
N

Additional information:                               

____________________________________________________

____________________________________________________

Date of examination: ________________    Hospital: ________________
ADEQUACY OF DIALYSIS:

Dialysis was considered adequate if the following criteria were met:
1. It was done three times a week for duration of four hours in each session
2. Urea reduction rate (URR) was 65% or more
3. Urea clearance (KT/V) was 1.2

PLAQUE INDEX (PI): (Silness & Loe 1964)

0=No plaque in the gingival area.
1=A film of plaque adhering to the free gingival margin and the adjacent area of the tooth. Plaque may only be recognized by running a probe across the tooth surface.
2=Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen by naked eye.
3=Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface.

GINGIVAL INDEX (GI): (Silness & Loe 1964)

0= Normal gingiva
1= Mild inflammation – slight change in colour, slight oedema. No bleeding on probing
2= Moderate inflammation – redness, oedema and glazing. Bleeding on probing
3= Severe inflammation – marked redness, oedema and ulceration. Tendency to spontaneous bleeding
RAMFJORD TEETH:

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>44</td>
<td>41</td>
<td>36</td>
</tr>
</tbody>
</table>

BMI  BODY MASS INDEX

MEDICATION

ACE-1:  ANGIOTENSIN-CONVERTING ENZYME INHIBITORS

STATINS  3-HYDROXY-3-METHYLGLUTARYL CO-ENZYME A REDUCTASE INHIBITORS

NSAIDS  NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

EPO  ERYTHROPOIETIN
APPENDIX -2

CONSENT FORM

Association between periodontitis and end-stage renal disease

Dr. M Nadeem¹ Prof. L Stephen¹ Dr. C Schubert² Prof. MR Davids²
¹ Faculty of Dentistry, University of Western Cape
² Renal Unit Tygerberg Hospital

The purpose of this study is to investigate whether periodontitis (Gum disease) is associated with increased systemic inflammation reflected by C-reactive protein (CRP) values, in patients with ESRD on maintenance hemodialysis or peritoneal dialysis.

If you participate in the study you will undergo an examination of the mouth and your medical history will be obtained from your hospital folder. A history and examination will be performed by one of the attending renal doctors to search for any active infection. CRP values, a blood test which reflects inflammation, will also be taken from your routine laboratory reports.

Should we find any symptoms and signs of infection; the appropriate therapy will be started. You will get free professional oral examination, advice and referral for any dental treatment needed.

Participation in the study is on a voluntary basis. There will be no harmful interventions and if you wish to withdraw from the study at any stage; you are free to do so. The information obtained in the study will be treated with utmost confidentiality and the names of the participants will not be divulged for any other purpose.

For any further information, please contact the undersigned:

Dr. M. Nadeem (Dentistry Department- cell no 073 1899 499) or
Dr. C. Schubert (Renal Unit- 021-9385666)
CONSENT

I have read the information, asked questions concerning areas that were unclear, and received satisfactory answers. I am willing and agree to participate in this study. I may withdraw at any time without any treatment being withheld from me. I will not have waived any of my legal rights by signing this consent form. Upon signing this form, I will receive a complete copy of the entire consent form.

...........................................  ...........................................  ...........................................
Participant Name     Signature     Date

...........................................  ...........................................  ...........................................
Witness Name         Signature     Date
APPENDIX -2
TOESTEMMINGSVORM

Verhouding tussen periodontitis en eindstadium niersiekte

Dr. M Nadeem ¹ Prof. L Stephen ¹ Dr. C Schubert ² Prof. MR Davids ²
¹ Fakulteit Tandheelkunde, Universiteit van die Weskaap en
² Niereenheid Tygerberg Hospitaal

Die doel van die studie is om te bepaal of periodontitis (tandvleis siekte) bydra tot verhoogde sistemiese inflammasie in pasiënte wat dialise ontvang.

As u deelneem in die studie sal u ’n mond ondersoek ondergaan en u mediese geskiedemis sal geneem word vanuit die hospitaal lêer. ’n Geskiedenis en ondersoek sal uitgevoer word deur een van die niereenheid dokters om se soek vir enige aktiewe infeksie. CRP wa ardes, ’n bloed toets wat inflammasie reflekteer, sal geneem word van u roetine laboratorium uitslae.

Indien daar enige simptome of tekens van infeksie is, sal die toepaslike terapie begin word. U sal ’n gratis professionele tand skoonmaak, mond ondersoek, advice en verwysing vir verdere tanheelkundige behandeling onvang indien nodig.

Deelname aan die studie is vrywillig. Daar is geen nadelige ingrepe nie. Indien u op enige stadium wil ontkerk van die studie, mag u so doen. Die inligting verkry vanuit die studie sal met uiterse vertroulikheid hanteer word en die name van die deelnemers sal nie bekend gemaak word vir enige ander doel nie.

Vir enige verdere informasie kontak asblief die ondergenoemde:

Dr. M Nadeem (Tandheelkunde Departement- sel no 073 1899 499)
Dr. C Schubert (Niereenheid- 021-9385666)
TOESTEMMING

ek het die inligting gelees, vrae gevra in verband met dele wat onduidelik was en bevredigende antwoorde ontvang. Ek neem vrywillig deel aan die studie. Ek mag ter enige tyd onttrek sonder dat behandeling vanaf my weeehou sal word. Ek word nie ontneem van enige wetlike rete deur die ondertekening van hierdie toestemmingsvorm nie. Na ondertekening van die vorm sal ek 'n kopie van die toestemmingsvorm ontvang.

……………………………..   ………………  ……………
Deelnemer Naam    Handtekening   Datum

……………………………..   ………………  ……………
Getuie Naam     Handtekening   Datum
APPENDIX-3
QUESTIONNAIRE

Ref. No _______________ Folder no _______________ 

Brushing habits:

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once a day</td>
<td>Twice a day</td>
</tr>
<tr>
<td>Once a week</td>
<td>Three times a day</td>
</tr>
<tr>
<td>Never</td>
<td></td>
</tr>
</tbody>
</table>

Use of floss:

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Every day</td>
<td>Once a week</td>
</tr>
<tr>
<td></td>
<td>Never</td>
</tr>
</tbody>
</table>

Use of mouth wash:

<table>
<thead>
<tr>
<th>Options</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

Visit to dentist:

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>6 months</td>
</tr>
<tr>
<td>Infrequently</td>
<td>once a year</td>
</tr>
</tbody>
</table>