EXPLORING THE LINKS BETWEEN PERIODONTAL HEALTH AND CARDIO-RENAL HEALTH IN PATIENTS WITH CHRONIC KIDNEY DISEASE

By

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Abstract

Chronic kidney disease (CKD) is a highly prevalent, chronic, non-communicable disease (NCD). CKD is associated with high morbidity and mortality, particularly from cardio-vascular events. Recently, the role of systemic inflammatory/oxidative stress burden in the morbidity and mortality associated with CKD is being appreciated. Periodontitis, another highly prevalent NCD, is caused by bacteria accumulating around the gingival margin of teeth and leads to local inflammation and destruction of the supporting tissues of teeth. There is a growing appreciation of the role of periodontitis in adding to the systemic inflammatory/oxidative stress burden. Periodontitis may represent an occult, modifiable source of such burden in patients with CKD. The seven manuscripts in this thesis aim to shed light on the relationship between periodontitis and CKD with a view of elucidating the causal mechanisms underpinning this relationship. The first three manuscripts demonstrate an association between periodontitis and incident cardio-vascular disease, systemic health and wellbeing, respectively. The final four manuscripts demonstrate the associations between mortality and periodontitis in patients with CKD, the association between periodontitis and CKD, an outline of an on-going, pilot RCT investigating the effects of one treatment of periodontitis on the cardio-renal health of patients with CKD and finally the causal mechanisms underpinning the associations between periodontitis and CKD are explored using path analysis structural equation modelling.

Dedication

This thesis is dedicated to my wife, Ajit Tanday. Without Ajit's constant, unwavering support, this work would not be complete. This work is also dedicated to my daughter, Lara Sharma, without whom this work would have been completed a lot sooner. However, I would not. I love you both.

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Critical review

Introduction:

Non-Communicable Diseases

Non-communicable diseases (NCDs) have become a major focus for the World Health Organisation (WHO), as they are the leading cause of mortality in the 21st century. NCDs account for 38 million (70%) deaths each year, with 16 million being recorded as premature (in people under 70 years of age) [1]. NCDs include cardiovascular disease (CVD), type 2 diabetes (T2D), rheumatoid arthritis (RA), chronic obstructive pulmonary disease (COPD), periodontitis (PD) and chronic kidney disease (CKD), all of which share common risk factors.

There has been limited progress by governments across the globe in combatting NCDs and their risk factors, prompting the WHO to release an NCD Progress Monitor on 18th September, 2017 [1]. The reason for the high profile of NCDs relates to their high human and health economic cost, but also due to the world's ageing population and increasing prevalence of NCDs globally. It is estimated that in 2010 the global economic impact of oral/dental diseases, principally caries, periodontitis and tooth loss approximated US$442 billion, with direct treatment costs of $298B, and $144B being due to indirect costs expressed as disability adjusted life years (DALYs) in terms of productivity losses due to caries, periodontitis, and tooth loss [2]. Currently, oral diseases exhibit the greatest age-standardised prevalence of all human diseases according to *The Global Burden of Diseases, Injuries, and Risk Factors Study 2017*, a study of the incidence, prevalence, and years lived with disability

(YLDs) for 354 different human diseases in 195 countries between 1990-2017 [3].

NCDs share several common risk factors, such as smoking, obesity, sedentary lifestyles, diets high in refined carbohydrates, which alongside a genetic predisposition lead to exaggerated systemic inflammation, which associated pathological consequences. Given the latest data from the GBD 2017 study, periodontitis would appear to be a significant, non-traditional risk factor for several systemic NCDs, worthy of exploration.

Chronic Kidney Disease

Chronic kidney disease (CKD) arises from abnormalities in the structure and/or function of the kidneys, present for more than 3 months and leading to decreased renal function. Globally, CKD affects approximately 13% of the population [4]. The leading causes of CKD are diabetes and hypertension [5] which are implicated in two-thirds of all cases. Other cases of CKD can be attributed to either immune/ inherited conditions or remain of unknown aetiology. As with other NCDs, the prevalence of risk factors for CKD is increasing globally as is the age profile of the world's population [6]. Therefore, the prevalence of CKD is also likely to increase and concomitantly increase the global health economic, as well as human, burden of disease.

The "gold standard" method for measuring renal function is based on the renal clearance of inulin, a plant-based polysaccharide. Inulin is continuously infused and multiple, strictly timed measurements of blood and urine are collected. As

inulin is neither absorbed nor secreted by the renal tubules, the rate of excretion of inulin in urine is used to quantify the glomerular filtration rate (GFR) with a low rate of filtration indicating poorer renal function. As this process is very time-consuming and labour intensive, other estimations of glomerular filtration rate (eGFR) are more commonly employed in routine medical practice. These depend on surrogate markers of renal function, such as serum creatinine levels, and are adjusted for the patient's age, sex and ethnicity, all of which impact upon serum creatinine levels. There are several formulas used in the estimation of GFR and, currently, the commonly employed formulae derive from the either the *Modification of Diet in Renal Disease* (MDRD) or the *Chronic Kidney Disease Epidemiology Collaboration* (CKD-EPI) groups. As an example, the 1999 MDRD equation [7] for calculating eGFR is:

$$eGFR = 175 \times Creatinine^{-1.154} \times Age^{-0.203} \times 1.212(\text{if Afro-Caribbean}) \times 0.742(\text{if Female})$$

where creatinine is measured in mg/dL and age is measured in years.

Aside from eGFR, another method for quantifying renal function derives from the amount of protein that leaks into urine due to renal tubule damage. This is called "proteinuria" and is expressed as a urinary albumin-creatinine ratio (ACR). The greater the amount of protein leaking into urine, the higher the ACR and thus the more damaged the kidneys are thought to be.

Together, eGFR and ACR can be used to categorise patients into five stages of

CKD (Table 1) with patients in stage 5 or End Stage Renal Disease (ESRD) having the poorest kidney function. Patients with ESRD commonly require renal replacement therapy (RRT) in the form of either dialysis or a renal transplant. RRT significantly increases the morbidity associated with CKD for individual patients, as well as the financial cost to the patient and/or healthcare system. In 2009-10, the direct, annual cost to the National Health Service (NHS) England, for treatment of patients with stages 3-5 CKD was estimated at approximately £1.45 billion. This represented approximately 1.3% of the overall NHS budget in that period with more than half the spend being on patients requiring RRT [8]. Whilst ESRD brings a significant burden in terms of morbidity, mortality and finance, the majority of patients with CKD do not progress to ESRD, rather they experience premature mortality, principally as a result of adverse cardiovascular events.

Current management protocols for patients with CKD involve the control of traditional risk factors such as diabetes, hypertension and smoking, which are implicated in the initiation and progression of CKD. Therefore, patients with CKD have their hypertension and glycaemic control closely monitored and are counselled on the advantages of smoking cessation. These measures are aimed at arresting or slowing down the progression of disease, beyond which, there is very little by way of treatment for CKD. However, at least 50% of the increased mortality seen in patients with CKD is not associated with these traditional risk factors [9]. Therefore, the focus of recent research has been the identification of other, non-traditional risk factors associated with increased mortality, in patients with CKD.

Table 1: Prognosis of CKD categorised by GFR and albuminuria category [10]

			Persistent albuminuria (mg/g) categories		
			Normal to mildly increased	Moderately increased	Severely increased
			<30	30-300	>300
GFR (ml/min/ 1.73 m^2) categories	Normal or high	≥90			
	Mildly decreased (stage 1)	60-89			
	Mildly to moderately decreased (stage 2)	45-59			
	Moderately to severely decreased (stage 3)	30-44			
	Severely decreased (stage 4)	15-29			
	Kidney failure (stage 5)	<15			

Green: low risk of adverse clinical outcomes (if no other markers of kidney disease, no CKD); Yellow: moderately increased risk; Orange: high risk; Red, very high risk.

The systemic inflammatory/oxidative stress burden is widely reported to be one such risk factor. This is evidenced by the fact that cardiovascular mortality in patients with CKD is not only related to the severity of the kidney disease [11] but also to an increased systemic inflammatory / oxidative stress burden. Furthermore, biomarkers such as C-reactive protein (CRP) are reliable indicators of cardiovascular and all-cause mortality in patients with CKD [12].

One ubiquitous, chronic infectious-inflammatory disease, that has been demonstrated to influence the systemic inflammatory or oxidative stress burden, is periodontitis [13]. Moreover, the systemic oxidative stress and inflammation created by periodontitis has been reported as “additive” to that created by systemic co-morbidities [14]. The role of inflammation in the patho-physiology of other, chronic non-communicable diseases, such as CVD, is more established. For example, there is debate amongst the medical community as to whether the beneficial effects of statin use in patients with CVD is solely due to their beneficial effects on dyslipidaemia or, at least partially, also due to their role in lowering systemic inflammation. This is because, in addition to regulating dyslipidaemia, statins are known to lower systemic levels of inflammation [15]. The hypothesis that an anti-inflammatory agent may be efficacious in CVD inspired the CANTOS (Canakinumab Antiinflammatory Thrombosis Outcome Study) trial, which recently published some of its results [16].CANTOS was a double-blind, randomised controlled trial (RCT) investigating the effect of canakinumab, a monoclonal antibody targeting interleukin-1β (IL-1β) on a composite primary outcome of nonfatal myocardial infarction (MI), nonfatal stroke, or cardiovascular death. 10,061 participants, with previous MI and a high-sensitivity C-reactive protein level ≥ 2 mg/l were included in this study. Three doses of canakinumab, 50mg, 150mg and 300mg, were compared with a placebo dose. The study had a median follow-up of 3.7 years. The study found that the hazard ratios (HR) of the primary outcome, compared with placebo, were reduced in all doses of canakinumab, with the HRs being 0.93 (95% CI: 0.80 to 1.07]; 0.85 (95% CI: 0.74 to 0.98); and 0.86 (95% CI: 0.75 to 0.99) for

the 50, 150 and 300 mg doses respectively. However, canakinumab was associated with a higher rate of fatal infections than placebo. Canakinumab was shown to have no effect on the lipid profile of participants and hence, the beneficial impact seen was ascribed solely to its anti-inflammatory effects. Interestingly, the effect of canakinumab on fatal infections highlights the fact that pharmacological treatments that dampen down the systemic inflammatory response en masse may be a double-edged sword. In this respect, a non-pharmacological agent/therapy, able to drive down a specific inflammatory process would be a more targeted approach. Periodontal therapy may have the potential of being that therapy, as discussed below. In regards to CKD in particular, anti-inflammatory therapies can be thought of as therapies treating co-morbidities causing inflammation, including periodontitis or as therapies limiting the inflammation seen in sub-optimal dialysis procedures or finally, anti-inflammatory treatment strategies including dietary interventions, physical exercise and pharmacological interventions [17]. Dietary interventions including high-fibre [18], pomegranate juice [19] and fish oils [20] are shown to improve outcomes and reduce inflammation in patients with CKD. The benefits of exercise on levels of physical fitness, muscle strength and quality of life as well as levels of inflammatory markers in patients with CKD may also warrant the prescription of exercise to improve outcomes in such patients [21]. In regards to pharmacological interventions to limit inflammation in patients with CKD, the potential role of statins, as explained earlier, is also seen in patients with CKD [22]. Pharmacological interventions to limit oxidative stress, particularly in dialysis patients, have also been suggested, including antioxidant

supplementation, as a means to limit the oxidative-stress induced damage [23]. This suggests a growing recognition of the role of inflammation and oxidative stress in the patho-physiology of CKD as well as the role of controlling inflammation and oxidative stress as a means of improving outcomes in patients with CKD.

Periodontitis

Periodontitis is a highly prevalent, chronic inflammatory condition [24], initiated by the accumulation of pathogenic bacteria at and below the gingival margin. In susceptible individuals, failure to regularly disrupt and remove the biofilm results in failure of initial acute inflammatory response to restore microbial symbiosis and dysbiosis ensues [25]. The dysbiosis further aggravates the hosts' immune-inflammatory response, which becomes dysregulated and chronic in nature and destroys the connective tissues supporting and surrounding the teeth [26]. Occult periodontal infection contributes to the systemic inflammatory burden, through acute-phase and oxidative stress pathways, triggered by oral bacteraemia [27]. This is evidenced by increases in CRP, interleukin-6 (IL-6) and biomarkers of oxidative stress damage in patients with periodontitis [14, 28]. Furthermore, successful periodontal therapy is associated with reductions in these inflammatory markers [29].

The association between periodontitis and other systemic diseases, particularly CVD is now well established [30-32]. The potential mechanisms by which periodontal health may negatively influence systemic health include:

1) Metastatic injury by periodontal pathogens or their products entering the

circulation and subsequently stimulating an acute-phase response [27], neutrophil-mediated oxidative stress [14, 33] and neutrophil cytokine hyper-reactivity [34].

2) Loss of immunological tolerance and generation of autoantibodies in the periodontal tissues to neutrophil extracellular traps and/or *p.gingivalis* modifications to proteins, with subsequent systemic immunological disease [35].

3) Metastatic inflammation from the local inflammatory-immune response, within the periodontium, to periodontal pathogens or their products spilling into the circulation.

Periodontitis may therefore act as a co-morbid chronic inflammatory disease in patients with CKD, contributing to the development of CVD, diabetes and other NCDs like CKD. This pathway may be amenable to treatment as reductions in systemic inflammatory markers are reported following periodontal therapy in patients with CVD [36] and CKD [37].

At this stage, it is important to distinguish between “association” and “causation” or causal relationships. A (positive) association between two conditions, such as periodontitis and diabetes, simply means that these conditions are commonly seen together. Detecting associations between two conditions, such as periodontitis and diabetes, can be relatively straightforward and may be achieved through epidemiological studies of patients with and without periodontitis and comparing the prevalence of diabetes in these two groups. For example, in the periodontitis-diabetes paradigm, diabetes is more common in

people with periodontitis than those without [38], however one cannot infer the directionality of such an association.

In the earlier example of surveying patients with and without periodontitis, there are several reasons why the group with periodontitis may have a higher prevalence of diabetes, without there necessarily being a causal relationship between periodontitis and diabetes. This may be due to an imbalance between the groups in certain factors such as age or ethnicity or an imbalance in certain lifestyle or other risk factors such as a poor diet, which may explain why patients in one group are simultaneously more prone to both periodontitis and diabetes. These factors may act as confounders of the relationship between periodontitis and diabetes. A confounder can be thought of as a variable that may explain an association between the exposure (in this case periodontitis) and outcome (in this case diabetes) that does not exist due to a causal relationship between exposure and outcome. There are numerous strategies devoted to the identification of confounders. The identification of confound*ers*, in and of themselves, are not as important as the identification of confound*ing* and methods to limit this, which may include identification of confound*ers*. Confounders have been identified purely by statistical properties such as stepwise selection or a change in the effect estimates before and after the inclusion of the confounder in the model. Most epidemiological textbooks will define confounders as variables that meet a certain set of criteria. Typically, and for the purposes of this thesis, confounders are defined as variables that are associated with both the exposure and the outcome and are not in the causal

pathway. Adjusting for such confounders is commonly carried out to provide an estimate of the association between the exposure and outcome, independent of the confounders. In the past decade or so, there has been a move to identify confounding via an understanding of the underlying causal mechanism in action, often represented in graphical manner, including directed, acyclic graphs (DAGs). DAGs allow for the identification of confounding and also provide insight into variables to control for to eliminate/minimise this. Such variables may be thought of as confounders [39]. Even if the groups were perfectly matched in all these known confounders, there may still be an imbalance in hitherto unknown confounders. For example, there is some evidence for certain individuals exhibiting a constitutionally hyperinflammatory phenotype [40]. Such individuals will therefore be at an increased risk of other NCDs, where inflammation is part of the causal pathway of the disease, including diabetes and periodontitis, without one contributing the cause of the other. On the other hand, variables that are associated with both exposure and outcome AND are in the causal pathway between exposure and outcome are termed mediators. For example, periodontitis is associated with systemic inflammation and systemic inflammation is associated with diabetes but systemic inflammation may be in the causal pathway between periodontitis and diabetes. Adjusting for systemic inflammation may block the association between periodontitis and diabetes, if such an association is mediated largely/exclusively by systemic inflammation. The challenges in delineating confounders from mediators are particularly acute in relation to tooth-loss and edentulism, an issue that is tackled more thoroughly in the critique of the fourth manuscript in this thesis. It is recommended that, for

the purposes of inferring causal relationships, such confounders/mediators should be identified based on existing knowledge of biology, as opposed to an arbitrary statistical method/threshold [41].

In this respect, the criteria defined by Austin Bradford Hill in his essay in 1965 [42], may prove useful in guiding from association to causation. Bradford Hill set-out some “view-points” which should be considered “before deciding that the most likely interpretation of [the association] is causation” [42]. Even though, using modern epidemiological techniques, the Bradford Hill criteria are less critical [43], they may still provide a useful basis for establishing associations and aid researchers in transitioning from associations to causal relationships. These “view-points” included:

1) *The strength of the association*. If the magnitude of the association is large, for example as expressed by an odds ratio or a relative risk, it is more likely that the association will not be attenuated by some unmeasured or imperfectly measured confounder. This makes the association more likely to be non-artefactual. For example, the fifth manuscript in this thesis [44], [page number 101], shows that participants with CKD have a 4-times higher odds (OR 4.0, 95% CI: 2.7 to 5.9) of having periodontitis, compared with a local, community dwelling control population. This association may be further attenuated if all known and unknown confounding was accounted for but it is unlikely to attenuate to be clinically/statistically insignificant.
2) *Consistency*. If an association has only been observed by one group at

one point in time, it may just be artefactual. Reproducibility of the association from different populations at different times and or locations lends credence to the association.

3) *Specificity*. This property relates to the association being seen between two, very specific conditions with an underlying, biologically plausible mechanism established or suspected. If this is the case, it strengthens the causal assumption. If, however, this criterion is not met, it does not imply a lack of causation. The example given in Bradford Hill's essay stems from a lack of specificity between smoking and deaths from lung cancer as smokers have an increased risk of mortality from other conditions, some of which, at the time, could not be causally linked to smoking. Here, a lack of specificity is not detrimental to the assumption of a causal relationship between smoking and deaths from lung cancer. However, the presence of this specificity would have strengthened the causal assumption.
4) *Temporality*. This criterion implies that the "cause" of a disease must precede the development of the disease itself. Temporality is, in theory, an indisputable criterion in the establishment of causality and it is easy to imagine it being fulfilled in an infective model where exposure to a single pathogen causes a specific disease. However, practically, this becomes more challenging, especially as the exposures may often be subclinical for a period of time and are very often not the sole cause, but rather a contributory factor, in the disease process. For example, in the case of periodontitis and CKD, it is practically impossible to establish precisely

when either of these diseases starts and hence establishing a temporal relationship between these diseases is challenging.

5) *Biological gradient*. This criterion states that if exposure to a risk factor or pathogen or condition causes or contributes to another disease process, greater exposure should be linked to worse outcomes in that disease, barring a plateau effect.
6) *Plausibility*. Fundamental to any step from association to causation is the ability to postulate the underlying mechanism by which the causal relationship is expressed. In the absence of such an explanation, implying causality becomes challenging.
7) *Coherence*. This criterion is an extension of the plausibility criterion above in that the plausible explanation should fit with what is currently known of the biology of the disease. With the evolution of the knowledge base, not meeting this criterion, or the previous, allied criterion, may not be a barrier to determining causality.
8) *Experiment*. Intervening, in an experimental fashion, to alter the exposure to an agent suspected of contributing to a condition and then monitoring changes in the development or progression of that condition may further strengthen the causal hypothesis. However, such experiments may not be feasible for ethical or practical reasons.
9) *Analogy*. If the biological mechanism from one established causal relationship is accepted, other associations, employing the same or similar biological mechanisms should require a lesser burden of proof before they are thought of as causal.

The evidence gathered as part of this doctoral research thesis, aims to shed some light on the potential causal relationship between periodontitis (or periodontal inflammation) and CKD prevalence and sequelae including progression and early mortality. This evidence is divided into two parts. Part 1, comprising of manuscripts 1 [page no 59], 2 [page no 75] and 3 [page no 81] will provide insight into the associations between periodontal health and general health and well-being. Following from this, manuscripts in Part 2, comprising of manuscripts 4 [page no 89], 5 [page no 101], 6 [page no 111] and 7 [page no 128], will focus on the links between periodontitis and chronic kidney disease and associated outcomes.

Part 1: Evidence linking periodontitis and systemic diseases

Manuscript Number 1 of 7

"The epidemiological evidence behind the association between periodontitis and incident atherosclerotic cardiovascular disease" [31], [page number 59]

This study aimed to systematically review the literature to ascertain the evidence for the association between periodontitis and incident atherosclerotic cardiovascular disease (ACVD). For inclusion in this review, periodontitis was defined based on clinical and/or radiographic examination. ACVD was defined as coronary heart disease/CHD (angina, myocardial infarction, death from coronary heart disease), cerebrovascular disease (transient ischaemic attack, stoke) or peripheral arterial disease. Incident primary or secondary ACVD events were included in the study. Further details of the search strategy and review methods are included in the full manuscript [31].

The electronic search yielded 1,395 records. Screening of titles and, if available, abstracts, limited the full-text review to 62 articles. Following full-text review, a further 50 articles were excluded, yielding 12 articles that fulfilled the selection criteria for inclusion in this review. Three cohort studies and three case-control studies focussed on CHD alone, one cohort study and two case-control studies focussed on cerebrovascular disease alone and one cohort study focussed on peripheral arterial disease alone. Two cohort studies focussed on mortality from ACVD, including both CHD and cerebrovascular disease. Overall, all studies, bar one, reported higher incidences of ACVD in participants with periodontitis, compared to those with less severe or no periodontitis. Where the analyses

were conducted, the effect was more pronounced in younger patients, under 60 or 65 years of age, with some suggestion that the effect was stronger in males than females.

This systematic review had several strengths, one of which was the strict inclusion criteria, focussing on incident ACVD and studies with objectively measured periodontitis. By only employing clinically or radiographically assessed periodontitis, studies using surrogate measures of periodontitis, such as tooth loss, or studies using composite measures where periodontal health was only one component, or studies using self-reported periodontitis were excluded. This made the findings more robust by limiting the degree of misclassification of the exposure (periodontitis).

This review highlighted several sources of heterogeneity, rendering meta-analyses inappropriate. The lack of use of standardized protocols, such as those provided by the European Federation of Periodontology (EFP) [45] or the Centre for Disease Control and Prevention/American Academy of Periodontology (CDC/AAP) [46], contributed to this. In addition, as noted by other researchers [47, 48], the choice of measure of periodontitis may have an effect on the magnitude of the association detected between periodontitis and the systemic disease of interest. This may speak to the underlying mechanisms linking periodontitis to varying systemic diseases as some measures of periodontitis, such as tooth loss or mean attachment loss, only provide insight into the historical disease burden, whereas measures like cumulative probing

depth or periodontal inflamed surface area (PISA) [49], capture current disease/inflammatory burden. A further limitation of this review is the lack of use of a standardized tool to determine the risk of bias.

Notwithstanding these limitations, this systematic review confirmed that the incidence of ACVD is higher in patients with periodontitis or worse periodontal health compared with patients without periodontitis or with better periodontal health.

Manuscript Number 2 of 7

"The relationship between general health and lifestyle factors and oral health outcomes" [50], [page number 75]

This study aimed to assess the association between oral health and general health in the preceding year, as well as high-risk lifestyle factors.
Data from 37,330 patients attending private, non-specialist dental practices throughout the UK, examined by 493 dentists, were analysed. Oral health was measured using a composite oral health score (OHS) based on patient self-reported oral pain, function (eating ability) and dental appearance as well as findings from a dental examination (Table 2)**.**

The OHS employed in this study has been previously reported and evaluated [51]. The individual patient score ranges from 0-100 with 100 representing perfect oral health. Patients' general health in the preceding year was assessed by responses (yes/no) to the question "have you experienced a major health

problem in the last year for example a stroke, heart attack or cancer?" High-risk lifestyle factors were assessed by patients' self-reporting of diabetes (yes/no), tobacco use (ever smoked cigarettes, cigars or pipe or used smokeless tobacco) and alcohol consumption (none, <1 drink/day, 2 drinks/day, 3 or more drink/day).

Table 2: Generation of the oral health score (OHS)

	Possible scores
Comfort	0 (pain) 4 (some pain) 8 (no pain)
Function	0 (problems) 4 (minor problems) 8 (no problems)
Appearance	0 (unhappy) 4 (some concern) 8 (happy)
Occlusion	0 (less than 10 teeth in each jaw opposed) 8 (at least 10 teeth in each jaw opposed)
Soft Tissues	0 (needs treatment or referral) 4 (needs observation) 8 (healthy)
Tooth health	0 (more than 30% of teeth need treatment) 6 (10-30% of teeth need treatment) 12 (less than 10% teeth need treatment) 18 (sound restorations, caries free) 24 (no restorations, caries free)
Tooth Wear	0 (much more wear than expected for age) 6 (more wear than expected for age) 12 (normal wear for age)
Gum Health	0 (severe periodontal disease) 6 (moderate periodontal disease) 12 (mild periodontal disease) 18 (gingivitis only) 24 (healthy)
TOTAL	Range 0-100

Linear regression analyses were carried out to assess the association between oral health and general health in the preceding year as well as high-risk lifestyle factors, adjusting for age, diabetes status, alcohol consumption, tobacco use, vomiting and reflux, salivary flow and dental attendance. The study found that diabetes, tobacco use, excessive alcohol consumption (three or more drinks per

day), and poor overall health in the preceding year were all negatively associated with the mean OHS of patients. With regards to high-risk lifestyle factors, having diabetes was associated with a 1.7 point (95% CI: 1.3 to 2.1; p<0.001) drop in OHS compared with not having diabetes, any tobacco use was associated with 2.7 point (95% CI: 2.5 to 2.9; p<0.001) drop in OHS compared with no tobacco use, and excessive alcohol consumption (3 or more drinks/day) was associated with a 1.8 point (95% CI: 1.3 to 2.4; p<0.001) drop in OHS compared with no alcohol consumption. Patients who reported a major health problem in the preceding year had a mean OHS 0.7 points (95% CI: 0.2 to 1.2; p=0.006) lower than that of patients who did not report such events.

The strengths of this study included the large sample size and wide geography of data collection, rendering the findings more generalizable, in some respects. The study confirmed, in a novel, non-specialist setting, some of the associations seen in other cohorts such as the association between diabetes, tobacco use, alcohol consumption, systemic health and periodontal health. However, there were also several limitations to this study which could not be addressed. These included the use of the OHS to infer the periodontal health of patients. The OHS is a composite score based on patient reported and clinically examined parameters, only some of which relate to periodontal health (Table 2). In addition, absence of some key covariates such as sex, ethnicity and socio-economic status, hindered more robust statistical analyses. The addition of these covariates in the model may have further attenuated the observed associations. The lack of granularity in this dataset with regard to the

periodontal component of the OHS as well as the unavailability of some key covariates was improved upon in a subsequent manuscript [52]. The use of self-reported major health-problems in the preceding year as a surrogate for overall systemic health was also problematic because what patients consider “major health problems” may vary between patients. In addition, deterioration in systemic health prior to the preceding year would not be captured by this question. The generalizability of the findings in this study are limited by the fact that the patients here pay privately for their dental care, which is not the norm in the UK. A further limitation stemmed from the heterogeneity that may arise from almost 500 dentists carrying out the data entry. This could lead to some misclassification, which deviates this study from the ideal but, conversely, makes it more representative of the “real-world” [53]. Finally, the clinical significance of the magnitude of these associations, ranging from 0.7 points to 2.7 points out of 100, is questionable and the highly statistically significant results are likely a feature of the large sample size.

In summary, this paper provided confirmation of the association between oral and systemic health and lifestyle factors, in a large cohort of patients from a novel, non-specialist dental setting.

Manuscript Number 3 of 7

“Association between periodontal health status and patient-reported outcomes in patients managed in a non-specialist, general dental practice” [52] , [page number 81]

“Health”, as defined by the WHO, incorporated patient-oriented outcomes and is a “state of complete physical, mental and social well-being and not merely the absence of disease or infirmity” [54]. Therefore, in assessing the associations between periodontal and systemic health, it is important to determine whether periodontitis is associated with a compromise in the general well-being of patients. The manuscript, titled “Association between periodontal health status and patient reported outcomes in patients managed in a non-specialist, general dental practice” [52], was conceived with this in mind. The aim of this study was to “explore the associations between clinical and radiographic periodontal parameters and patient reported experience of oral pain, dietary restrictions and dental appearance in a large, non-specialist dental practice patient cohort”.

As in the previous study [50], this study used routinely captured data from 14,620 dentate patients. This data was collected by 355 dentists in 233 non-specialist dental practices across the UK. Data were available on the patients’ periodontal health, as collected by the dentist, and based on categories of periodontal measures such as

1) Periodontal probing depth (PPD) with the deepest probing depth in each sextant categorised into <5mm, 5-7mm or >7mm
2) bleeding on probing (BoP) in each sextant, recorded dichotomously (yes/no)
3) maximum radiographic alveolar bone loss (ABL) per sextant, categorised as <2mm, 2-4mm or >4mm

Based on the worst sextant score for PPD, BoP and ABL, patients were assigned to one of eight different periodontal status (exposure) categories (Table 3).

Patients' general well-being was assessed using patient reported outcomes (PROs). These were documented using questions relating to

1) pain i.e. "are you experiencing any pain or discomfort in your mouth?" with responses being "yes" or "some" or "no"
2) dietary restriction i.e. "do your teeth allow you to eat an unrestricted diet?" with responses being "yes" or "mainly" or "no"
3) appearance i.e. "how do you feel about the appearance of your teeth?" with responses being "happy", "some concerns" or "unhappy"

Data were collected on a range of covariates that might confound the association between periodontal health and PROs including self-reported age, sex, smoking and diabetes status, tooth grinding habits and frequency of sugar intake. In addition, data collected by the dentist on numbers of teeth with or needing restorations, oral hygiene, dental attendance patterns, cervical abrasive tooth-wear and salivary flow were employed as covariates. After adjusting for covariates in a logistic regression model, an increase in the prevalence of PROs was found to be associated with worsening periodontal health. The adjusted probability of reporting pain in patients with the best periodontal health was 13.8% (95% CI: 12.4 to 15.2%) compared with 20.7%

(95% CI: 17.2 to 24.2%) in patients with the worst periodontal health. Similarly, the adjusted probability of reporting a restricted diet was 10.8% (95% CI: 9.5 to 12.0%) in patients with the best periodontal health compared with 19.2% (95% CI: 15.9 to 22.5%) in patients with the worst periodontal health. The adjusted probability of reporting unhappiness with appearance was 22.2% (95% CI: 20.6 to 23.8%) in patients with the best periodontal health compared with 34.3% (95% CI: 30.3 to 38.4%) in patients with the worst periodontal health. These associations exhibited a dose-dependent relationship, with worsening periodontal health being associated with an increased probability of reporting pain, restrictions in diet or unhappiness with dental appearance (Figures 1-3).

This study had several strengths. Similarly to the previous study [50], findings were derived from populations that frequently go unreported in the literature, as data here are collected from a large number of non-specialist dental practices. The study also benefits from a large sample size, allowing for the generation of precise estimates. Compared with the previous study, access to data such as the sex of patients, along with other key covariates, as well as more granular periodontal data, allowed for more robust statistical analyses.

However, this study also shares some of the limitations of the previous study [50] in that it represents secondary data analysis, rather than analysis of data collected for the purposes of this research. Such real-world data suffers from several shortcomings such as omission of certain covariates, such as patient ethnicity, in data collection and lack of training and calibration of dentists which

may lead to misclassification of periodontitis. The results of this study are also not generalizable to the entire population as the patients in this cohort were part of a dental payment capitation scheme and are likely to differ in a number of ways from the general population of the UK. These differences could include differences in socio-economic status, access to dental care and attitudes to healthcare.

In conclusion, this study demonstrated the association between periodontal health and general well-being in a large, non-specialist, general practice-based population resident in the UK.

Table 3: Cohort demographics. Data are unadjusted and are expressed mean (SD) unless otherwise stated

	Whole cohort	**Cohort categorised by periodontal parameters**							
		I PPD<5mm ABL<2mm BoP-	**II PPD<5mm ABL<2mm BoP+**	**III PPD<5mm ABL 2-4mm BoP-**	**IV PPD<5mm ABL 2-4mm BoP+**	**V PPD 5-7mm ABL 2-4mm BoP+/-**	**VI PPD<5mm ABL>4mm BoP+/-**	**VII PPD 5-7mm ABL>4mm BoP+/-**	**VIII PPD>7mm ABL>4mm BoP+/-**
N (%)	14,568 (100%)	2,693(18.5%)	3,081(21.2%)	2,330(18.0%)	2,898(9.9%)	1,225(8.6%)	911(6.3%)	868(6.0%)	532(3.7%)
Age (years)	55 (16)	48 (16)	46 (16)	62 (12)	59 (13)	59 (13)	66 (11)	64 (11)	63 (12)
Male (%)	43	40	43	43	43	48	44	46	49
Diabetic (%)	5.7	3.8	3.4	5.5	6.3	6.6	9.1	9.6	11.5
Never smoker (%)	63	69	70	62	62	58	51	48	48
Teeth present (not including wisdom)	25 (4)	26 (4)	26 (3)	25 (4)	25 (4)	25 (4)	23 (5)	23 (5)	23 (5)
Restored teeth	11 (6)	9 (6)	9 (6)	13 (5)	13 (5)	12 (5)	12 (5)	12 (5)	11 (5)
Teeth needing restoration	0.3 (1.0)	0.2 (0.9)	0.3 (1.1)	0.3 (0.7)	0.4 (1.0)	0.3 (1.0)	0.4 (1.1)	0.4 (1.1)	0.4 (1.1)
Frequency of dental visits less than recommended (%)	5	3	6	4	4	7	3	7	8
Improvement in oral hygiene needed (%)	44	18	54	27	58	52	58	56	64
High frequency of sugar intake (%)	11	12	14	10	10	11	11	10	14
Patient reported outcomes (%)									
Pain	15	14	15	14	15	17	14	18	21
Diet restrictions	11	11	9	10	11	13	13	16	20
Unhappiness with appearance	26	23	26	26	26	27	30	31	33

ABL: Alveolar bone loss; Bleeding on Probing: BoP, either present (+) or absent (-); PPD: periodontal probing depth

Figure 1) Probability of reporting oral pain Vs Periodontal parameters
ABL: Alveolar bone loss; Bleeding on Probing: BoP, either present (+) or absent (-); PPD: periodontal probing depth

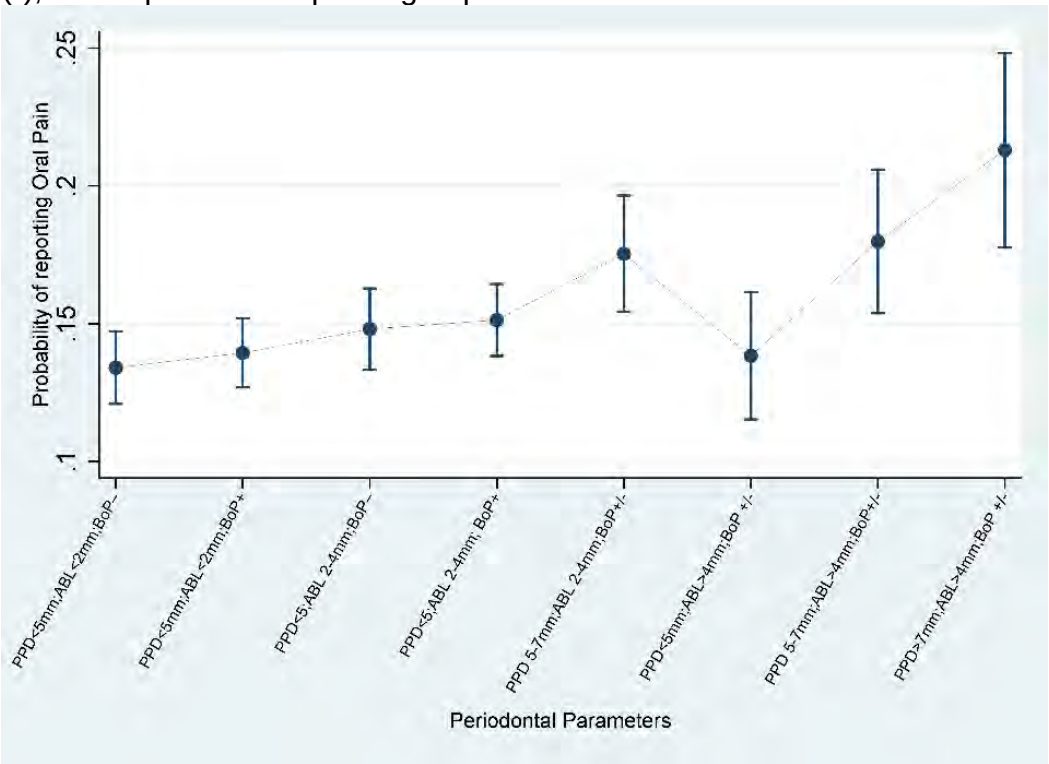


Figure 2) Probability of reporting restricted diet Vs Periodontal parameters
ABL: Alveolar bone loss; Bleeding on Probing: BoP, either present (+) or absent (-); PPD: periodontal probing depth

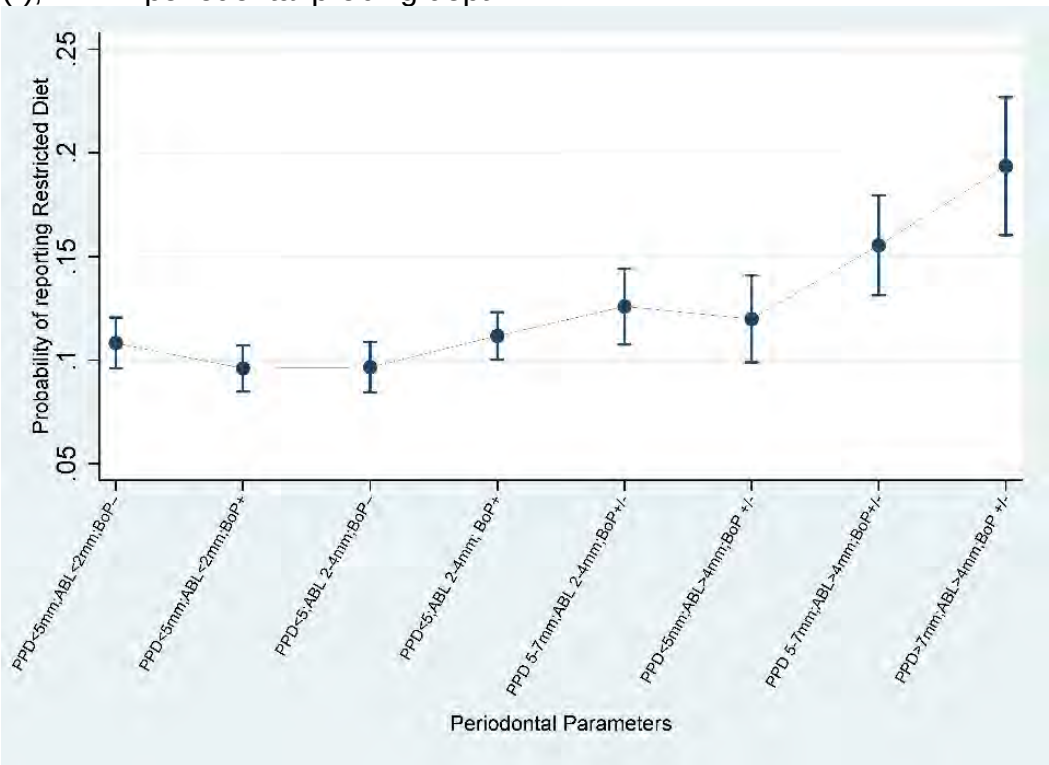
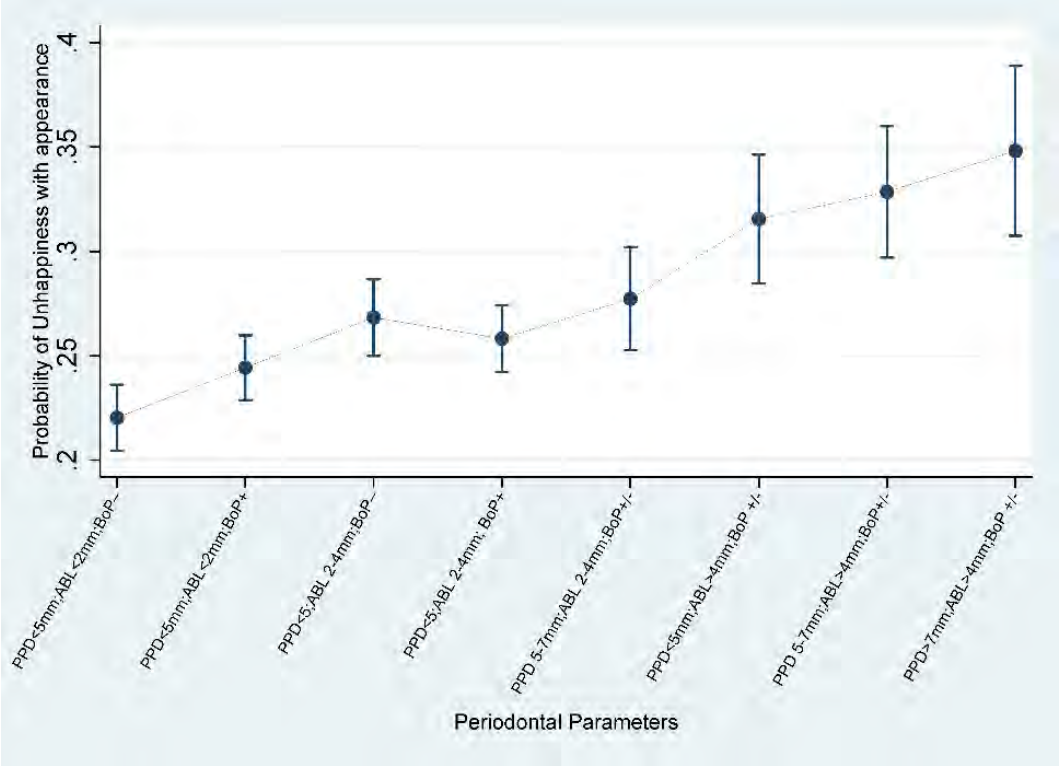


Figure 3) Probability of reporting unhappiness with appearance Vs Periodontal parameters

ABL: Alveolar bone loss; Bleeding on Probing: BoP, either present (+) or absent (-); PPD: periodontal probing depth



Part 2: Evidence linking periodontal disease to CKD

Given the evidence accumulated in this thesis, on the associations between poorer periodontal or oral health and atherosclerotic cardiovascular disease, general systemic health as well as general well-being, the following studies focus on the links between periodontal health and CKD in particular. The rationale for studying CKD was based upon the fact that it is a common, but largely underexplored NCD where systemic inflammation impacts upon its onset and progression. In addition, patients with CKD experience multi-morbidity with diabetes and CVD.

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"Association between Periodontitis and mortality in stages 3-5 Chronic Kidney Disease: NHANES III and linked mortality study" [55] , [page number 89]

The aim if this study was to assess, using data from a robust, US population-based survey with linked mortality data, the association between periodontitis and all-cause and cardiovascular mortality in individuals with stage 3-5 CKD. A further aim was to contextualise the magnitude of this association by comparing it to the magnitude of the associations between other, traditional risk factors (diabetes, hypertension and tobacco smoking) and all-cause and CVD mortality in individuals with stage 3-5 CKD.

For this study, data were derived from the Third National Health and Nutrition Examination Survey (NHANES III, 1988-1994), which provided a representative sample of the civilian, non-institutionalised population in the US. In addition, linked mortality data was available for NHANES III participants up to 31st

December 2006. CVD mortality was assessed by the coding used to indicate the cause of death. These codes derived from the ninth and tenth revisions of the International Classification of Diseases (ICD-9 and ICD-10). For this study, CVD mortality was limited to cases where the underlying cause of death was coded between 53 and 75 (inclusive) which relates to mortality resulting from CVD.

A case of periodontitis was defined using the 2007 CDC/AAP classification [46]. The CKD-EPI equation was used to calculate eGFR [56]. Individuals with an eGFR <60 ml/min/1.73m^2 were classified as having stage 3-5 CKD. Albuminuria was classified as

- “none” if the albumin-creatinine ratio (ACR) was 30mg/g or less,
- “micro-albuminuria” if the ACR was between 30 and 300mg/g, and
- “macro-albuminuria” if the ACR was 300mg/g or more.

In addition, data was collected on a range of covariates that might confound the associations under investigation. These included self-reported age, sex, ethnicity, alcohol consumption, socio-economic status, level of physical activity and history of cardiovascular events. In addition, pulse pressure, the difference between systolic and diastolic blood pressures, body-mass index (BMI) and serum cholesterol levels were measured and included as covariates.

The statistical analyses in this study were challenging and considered the complex survey design and sampling weights in order to yield estimates generalizable to the US population. Cox proportional hazards models were fit to

assess the association between periodontitis, diabetes, hypertension and smoking and all-cause and CVD mortality in individuals with CKD.
From the NHANES III survey, 13,784 individuals aged 20 and over with periodontal health, serum creatinine and linked mortality data were included in the analyses. Of these, 861 (6%) had CKD. Individuals in this survey had a median follow-up time of 14.3 years.

The fully-adjusted, 10 year all-cause and CVD mortality rates for individuals with CKD, with and without periodontitis, diabetes, hypertension and smoking habits are summarised (Table 4). Individuals with periodontitis and CKD had an increased 10-year mortality rate compared with individuals with CKD who were periodontally healthy. The magnitude of this increase in mortality was similar to that seen individuals with CKD and diabetes (instead of periodontitis) compared with individuals with CKD alone.

Edentulous patients were included in this study but formed a cohort distinct from periodontitis. This is because even though tooth-loss can result if periodontitis progresses, tooth-loss is not solely due to periodontitis. The challenges of accounting for tooth-loss, including complete tooth-loss or edentulism, continue to vex researchers in the field of periodontal-systemic disease links. On the one hand, as not all tooth-loss is due to periodontitis, it can be thought of as a surrogate measure/confounder for something akin to healthcare- behaviour. In that, if someone loses teeth due to dental decay, arising from a poor diet and poor oral hygiene, they are less likely to look after their general health and more

likely to have periodontitis. In this, non-periodontitis related tooth-loss, tooth-loss is a confounder as it is not in the causal pathway between periodontitis and systemic health but is associated with both. On the other hand, as periodontitis can lead to tooth-loss, tooth-loss due to periodontitis may be in the causal pathway between periodontitis and systemic health. This is because periodontitis can lead to tooth-loss, which may lead to changes in diet, which has an effect on general health and longevity. Without knowing the cause of tooth-loss in a patient, it is impossible to decide if tooth-loss is in the causal pathway, and therefore should be left out of the model, or is not in the causal pathway and may be contributing to confounding and needs to be adjusted for. This thought extends to edentulousness. Is edentulousness a marker of previous, severe periodontitis susceptibility or does it represent the ultimate treatment of periodontitis? Is edentulousness a surrogate for poor healthcare behaviour/access or is it a surrogate for socio-economic status? Edentulous patients in a study may also represent survivor-bias as they have survived long enough to be included in the study, despite having the risk-factors (poor oral hygiene, susceptibility to periodontal disease, poor healthcare behaviour/access etc) which rendered them edentulous in the first place. This may explain why this study found that younger edentulous participants (under 65) had a significantly increased rate of all-cause mortality, HR 1.85 (1.41 to 2.44), compared to edentulous individuals 65 years and older, HR 1.18 (1.04 to 1.33) respectively. Similarly, in regards to CVD mortality, edentulous participants under the age of 65 had an increased rate, HR 2.03 (1.31 to 3.13), compared to edentulous participants 65 years and older, HR 0.89 (0.71 to 1.10). This survival

bias, a type of selection bias, is also seen in the apparent protective effect of smoking on Alzheimer's disease in elderly patients [57].

The strengths of this study lie in the sampling techniques employed in the NHANES survey coupled with the large sample size, detailed covariate measurements and the length of follow-up, with hard outcomes allowing for fairly accurate point estimates, generalizable to the entire US population. This study does have some important limitations that are acknowledged in the manuscript. These stem partly from the probing protocol of the NHANES III survey that employed a part-mouth probing protocol that is known to underestimate the prevalence of periodontitis compared with a full-mouth probing protocol [58]. As a result, the associations noted in this study are likely to underestimate the true magnitude of the association between periodontitis and all-cause or CVD mortality in patients with CKD. A second important criticism of this study is that the periodontal and renal health of patients was only measured at one time point and assumed not to change throughout the median follow-up time of nearly 15 years. The study is forced to make this assumption as there is only a "virtual" follow-up of these individuals, hence changes in periodontal, renal or other health/lifestyle aspects could not be captured.

In summary, the reported study demonstrated an association between periodontitis and increased all-cause and CVD mortality in patients with CKD, in a sample generalizable to the US population.

Table 4: Fully adjusted 10-year all-cause and CVD mortality for individuals with CKD by risk factor combinations

Risk Factor	10-year all-cause mortality (95%CI)		10-year CVD mortality (95%CI)	
	Without periodontitis	With periodontitis	Without periodontitis	With periodontitis
CKD	32% (29 to 35%)	41% (36 to 47%)	16% (14 to 19%)	22% (19 to 27%)
CKD + Diabetes	43% (38 to 49%)	55% (47 to 63%)	24% (19 to 30%)	32% (27 to 39%)
CKD + Hypertension	34% (29 to 39%)	44% (37 to 52%)	21% (16 to 28%)	29% (22 to 37%)
CKD + Smoking	58% (51 to 65%)	71% (62 to 79%)	33% (24 to 44%)	43% (32 to 56%)

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"The periodontal health component of the Renal Impairment In Secondary Care (RIISC) cohort study: a description of the rationale, methodology and initial baseline results" [44] , [page number 101]

The association between periodontitis and CKD has been the subject of a number of studies and systematic reviews. In 2013, Chambrone et al. [59], conducted the first systematic review and meta-analysis to assess the association between periodontitis and CKD. Nine full-texts were included in the review, four of which were included in the meta-analysis. The meta-analysis showed that individuals with periodontitis had increased odds of having CKD compared with periodontally healthy individuals (OR 1.65; 95% CI: 1.35 to 2.01). The latest systematic review and meta-analysis, conducted by Zhao et al. in 2018 [60], included 7 case-control and 38 cross-sectional studies, four of which, with the highest quality, were included in a meta-analysis. This study also found that individuals with periodontitis had increased odds of having CKD

compared with periodontally healthy individuals (OR 3.54; 95% CI: 2.17 to 5.77).

This implies an association between periodontitis and CKD which is present even after adjustment of some confounding factors that may explain the association. The present study was included in the latest systematic review [60]. This study derives from the RIISC cohort, the details of which are available elsewhere [61]. Briefly, the cohort was established in 2010, with the aim of identifying risk factors associated with the progression of CKD and adverse outcomes in patients with CKD. RIISC is an ongoing, prospective, bio-clinical cohort which aimed to recruit 1000 participants with stage 3-5 (pre-dialysis) CKD, who were at a high-risk of progression of CKD, as defined by the inclusion/exclusion criteria (Table 5), and follow these participants for 10 years. The current manuscript aimed to report the periodontal health, at baseline, of the first 500 participants in this cohort. The comparator group for this cohort was derived from a representative, regionally matched population of the UK, derived from the 2009 Adult Dental Health Survey (ADHS-2009) [62]. The ADHS is conducted in the UK, every 10 years, with the aim of monitoring the oral health of the nation over time. For this study, the comparator group was limited to the West Midlands region of the UK, yielding a sample size of 876 individuals.

The periodontal health of the RIISC cohort was measured at baseline using PPD, recession, BOP at all interproximal sites i.e. four sites per tooth, for all teeth present. The probing protocol differed in the ADHS-2009, which employed

a protocol similar to the basic periodontal examination (BPE) where the maximum PPD per sextant was recorded, categorised into 0-3.5mm, 4-5.5mm, 6-8.5mm and 9mm or more. In addition, PPDs were recorded at two interproximal sites per tooth, on the lingual surface for lower dentition and buccal surface of upper dentition.

Table 5: Inclusion and exclusion criteria for the RIISC study

Inclusion Criteria	Exclusion Criteria
1. Patient aged 18 years or older 2. Able to provide informed consent 3. Secondary care renal clinic follow-up for at least 1 year prior to recruitment 4. High-risk CKD defined as: I. A decline of eGFR of 5 ml/min/year or 10 ml/ min/5 years; and/or II. urinary ACR > 70 mg/mmol on three occasions; and/or III. CKD stage 4 or 5 (not on dialysis)	1. ESRD requiring treatment with RRT 2. Receiving immunosuppression

Due to the limitations in the probing protocol of the ADHS-2009, in this study, periodontitis was defined as any sextant with a PPD ≥ 4mm, and severe periodontitis was defined as any sextant with a PPD ≥ 6mm. Periodontal measurements in the RIISC cohort were adapted to mimic the ADHS-2009 protocol to facilitate comparisons.

After adjusting for age, sex, ethnicity, smoking and socio-economic status, both

cohorts had a similar prevalence of edentulism. However, participants in the RIISC cohort had 4-times higher odds (OR 4.0, 95% CI: 2.7 to 5.9) of having periodontitis and 3.8-times higher odds (OR 3.8, 95% CI: 2.5 to 5.7) of having severe periodontitis, compared with the ADHS-2009 cohort.

The strengths of this study lay in the novelty of the cohort, being the first longitudinal cohort of pre-dialysis CKD patients at a high-risk of progression. The study highlighted one potential short-coming of this cohort, which lay in its high prevalence of periodontitis. In the RIISC cohort, of the 500 patients included in this study, 469 underwent a dental examination. Of these, 80 patients (17%) were edentulous. The remaining 389 dentate patients, classified according to the 2007 CDC/AAP classification of periodontitis [46], revealed only 17 (4%) to be periodontally healthy, 171 (44%) to have moderate periodontitis and 201 (52%) to have severe periodontitis. The lack of periodontally healthy individuals meant that, for this and future studies in this cohort, using periodontitis, as defined by the 2007 CDC/AAP classification, as the exposure or outcome, the findings may be biased by a lack of a periodontally healthy comparator group. The challenges in choosing case definitions or measures of periodontitis, when investigating the links between periodontitis and systemic diseases have also been highlighted by other researchers [47, 48].

In summary, this study confirmed an association between periodontitis and CKD, as observed by other researchers in other populations, thereby

strengthening the argument that this association is non-artefactual.

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Periodontal Inflammation Influences Renal Function in Patients With Chronic Kidney Disease" [63] , [page number 111]

Having demonstrated the association between periodontitis and CKD as well as the association between periodontitis and increased all-cause and CVD mortality in patients with CKD, the biological mechanisms underpinning these associations were investigated. Given that the association between periodontal health and systemic health is not limited to CKD, it is likely that the underlying mechanism, if any, is not entirely specific to CKD. One factor driving the "*metastatic injury by periodontal pathogens"* hypothesis is the body of research by our group investigating peripheral blood neutrophil (PBNs) hyper-reactivity and hyper-activity in patients with periodontitis [33]. Studies have repeatedly demonstrated excessive release of reactive oxygen species (ROS) from PBNs in patients with periodontitis under various conditions of priming and stimulation, both pre- and post-periodontal therapy [40, 64-67]. In addition, a type-1 interferon gene expression signature was identified in PBNs from periodontitis patients implicating microbial stimulation of toll-like receptors in the hyper-responsive neutrophil phenotype [68]. Similar data has emerged for cytokine hyper-reactivity [34] and studies on priming and stimulatory factors within patient's plasma have demonstrated both constitutive and bacterially-induced causes for the reported hyper-responsiveness of PBNs in periodontitis patients [64]. The common underpinning condition is one of oxidative stress, which has

been shown to manifest in the circulation of periodontitis patients who suffer from co-morbid NCDs such as type-2 diabetes [14]. Therefore, we hypothesised that intact bacteria, pro-inflammatory bacterial products or inflammatory cytokines disseminate via the ulcerated sulcular epithelium [69], seen in periodontitis and trigger a systemic inflammatory or oxidative stress response which may adversely affect renal function by inducing structural damage to the kidneys or their vascular supply.

An alternative hypothesis is that the association between periodontitis and CKD may be the result of the influence of renal function on periodontal health, mediated via a similar inflammatory or oxidative stress pathway. In addition, declining renal function may lead to altered blood chemistry, resulting in saliva with a high inorganic content, which may encourage increased calculus formation and therefore a predisposition to periodontitis. Finally, compromised renal function may directly or indirectly, via medication in patients with renal transplants, alter immune function which may also predispose to periodontitis. The latter two mechanisms are more relevant in patients with ESRD with renal transplants or undergoing dialysis [70].

Alternatively, periodontitis and CKD may have a bi-directional relationship, as has been suggested by some investigators [71], or the association between the two may be artefactual, resulting from unknown, unquantifiable or imperfectly measured confounders, without any causal link.

Therefore, the study reported here was conducted with the following three aims:

1. To confirm the association between periodontal inflammation and renal function
2. To assess the association between periodontal inflammation and measures of systemic inflammation and oxidative stress, independent of renal function, in patients with CKD
3. To test theoretical causal pathways linking periodontal inflammation and renal function by employing path analysis using structural equation modelling (SEM).

The study utilised baseline data from the RIISC cohort, described earlier. In December 2015, the RIISC cohort closed recruitment at 770 patients due to a lack of continued funding. The periodontal and renal measurements in the RIISC cohort have also been described earlier. For this analysis, the inflamed area of periodontal soft tissue, exposed to the sub-gingival biofilm was approximated using the periodontal inflamed surface area (PISA) score [49]. PISA uses BOP and PPD data to quantify the surface area of inflamed periodontal tissues in mm^2. Systemic oxidative stress was quantified by measuring serum levels of oxidised lipids in the form of F2-α-isoprostanes, and oxidized proteins in the form of protein carbonyls. Systemic inflammation was quantified by serum CRP concentrations as a measure of the acute-phase response, and acquired immunity was measured using a marker of plasma cell activation in the form of serum free light chain (FLC) concentrations.

To address the first two aims of this study, linear regression analysis was performed with PISA as the exposure or independent variable and eGFR as the outcome or dependent variable, adjusting for age, sex, ethnicity, diabetes, smoking and socio-economic status, BMI and blood pressure. In the models investigating the association between periodontal inflammation and measures of systemic inflammation and oxidative stress burden, eGFR was included as a covariate, as the aim was to quantify this association, independently of renal function. To address the third aim of this paper, theoretical directed acyclic graphs (DAGs) were constructed in both the PISA-eGFR and eGRF-PISA directions to explore biologically plausible mechanisms that might explain the associations between periodontal inflammation and renal function (Figures 4&5).

The current study confirmed the association between periodontal inflammation and renal function. After adjusting for the covariates mentioned earlier, a 1 S.D. increase in PISA score was associated with a 5.4% (95% CI: 1.4 to 9.4%; p=0.009) decrease in eGFR. In the models with periodontal inflammation as the exposure and measures of systemic oxidative stress as the outcome, a 1 S.D. increase in PISA score was associated with a 12.0% (95% CI: 3.2 to 21.6%; p=0.007) increase in F2-α-isoprostanes and an 8.8% (95% CI: 1.4 to 16.6%; p=0.018) increase in protein carbonyls. Finally, in models with periodontal inflammation as the exposure and measures of systemic inflammation as the outcome, a 1 S.D. increase in PISA score was associated with a 4.8% (95% CI: -7.0 to 18.0; p=0.443) increase in serum CRP and a 0.5% (95% CI: -4.0 to 5.3;

p=0.814) increase in total serum FLC concentration. Results from the path analysis using SEM confirmed the hypothesis that periodontal inflammation has an indirect effect on renal function, mediated via oxidative stress, such that a 10% increase in PISA score resulted in a 3.0% decrease in eGFR (95% CI: 0.6 to 5.3%; p=0.014). There was no significant direct or indirect effect of renal function on periodontal inflammation via the pathways depicted in the DAG (Figure 5).

This study was the largest of its kind to employ detailed periodontal phenotyping of patients with high-risk CKD. The detailed bio-clinical and demographic phenotyping of these patients allowed adjustment for a variety of potential confounders in the regression models and accounting for these in the DAGs constructed for the SEM. The study also had the strength of employing the PISA score to quantify periodontal inflammation. This measure is devoid of some of the criticism levelled at the use of case definitions of periodontitis, designed for epidemiological purposes, in investigating the association between periodontitis and systemic diseases [47, 48].

This study does have several limitations, which are highlighted briefly here and in more detail in the manuscript. The main limitation stems from the assumptions made in the DAGs prepared for the SEMs. The addition or omission of variables, as well as the addition, omission and direction of the arrows in the model are assumptions made by the authors, based on biological plausibility and available knowledge. As comprehensive as these models are,

and models of increasing complexity were used, they do not come close to approximating the true complexity of this system. Hence, these findings will require corroboration from other cohorts, as well as longitudinal observational and interventional studies.

In conclusion, this study confirms the association between periodontal inflammation and renal function, seen in an earlier, smaller cohort. In addition, this study confirms the hypothesis that periodontal inflammation influences renal function via changes in systemic oxidative stress levels.

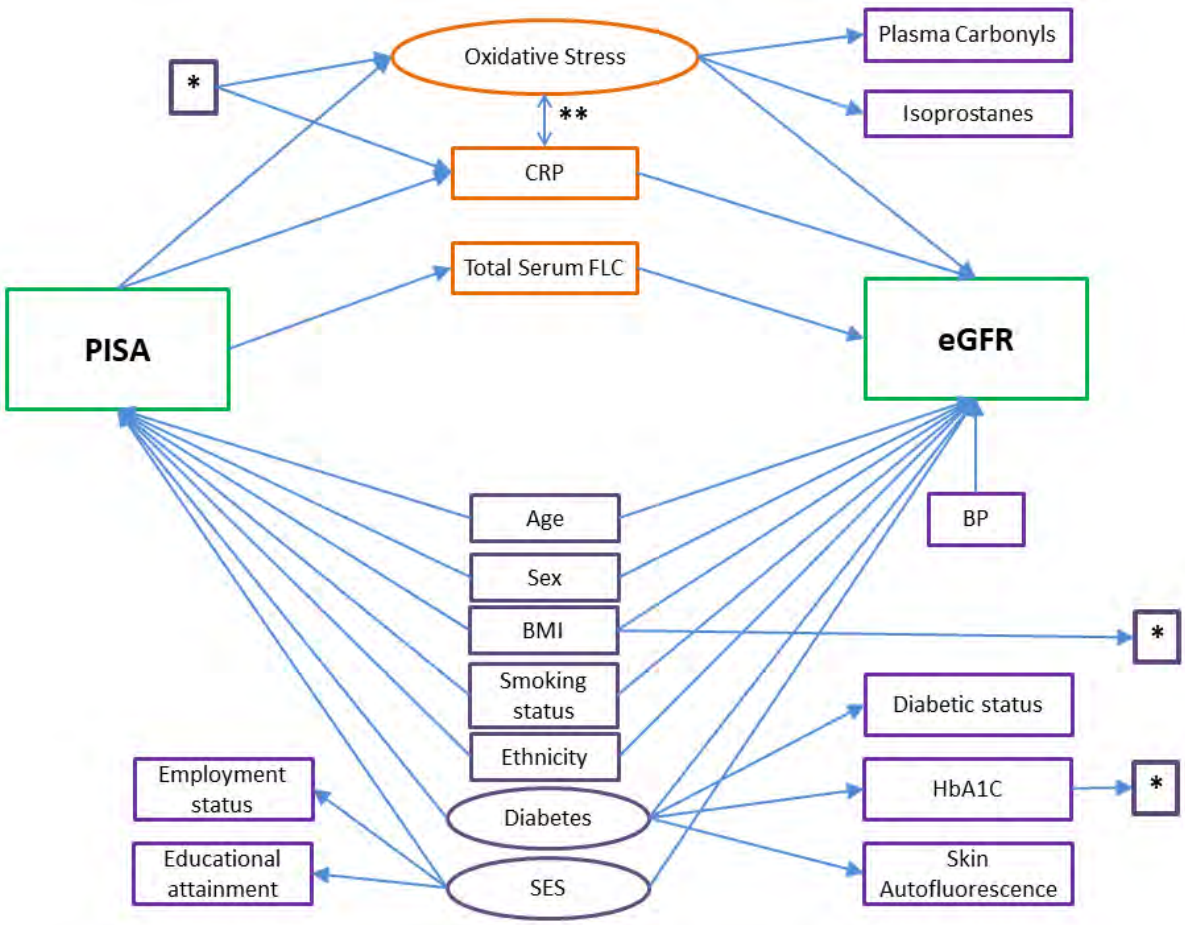

Figure 4: Visual representation of structural equation model with renal function, eGFR, as the outcome.

Rectangles: Observed variables; Ovals: Latent variable
Green: Exposure and outcomes of interest; Purple: confounders; Orange: Mediators

*- paths included in Model 2, in addition to the base model. **- paths included in Model 3, in addition to Model 2.

BMI- body mass index; BP- blood pressure; CRP- c-reactive protein; eGFR- estimated glomerular filtration rate; FLC- free light chain; HbA1C- glycated haemoglobin; PISA- periodontal inflamed surface area; SES- socio-economic status

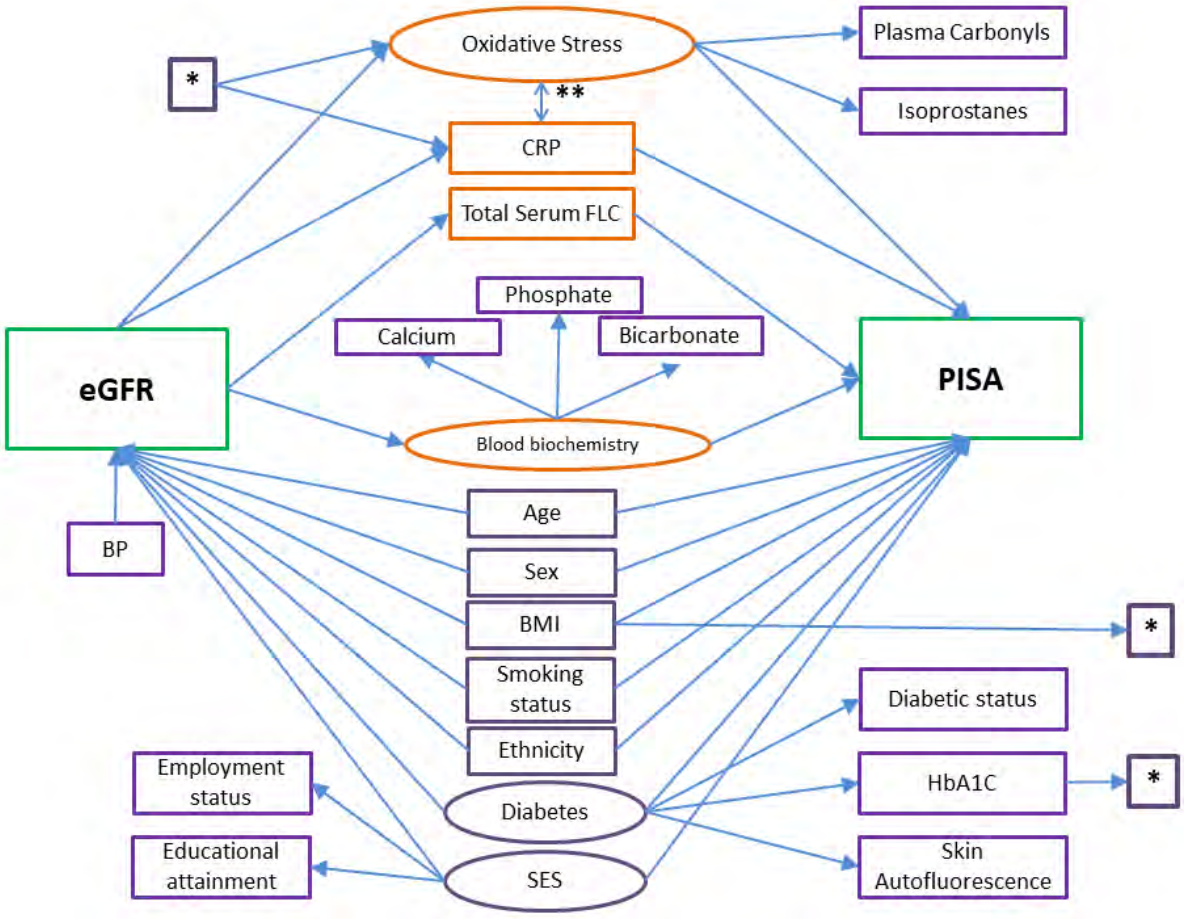

Figure 5: Visual representation of structural equation model with periodontal inflammation, PISA score, as the outcome.

Rectangles: Observed variables; Ovals: Latent variable
Green: Exposure and outcomes of interest; Purple: confounders; Orange: Mediators

*- paths included in Model 2, in addition to the base model. **- paths included in Model 3, in addition to Model 2.

BMI- body mass index; BP- blood pressure; CRP- c-reactive protein; eGFR- estimated glomerular filtration rate; FLC- free light chain; HbA1C- glycated haemoglobin; PISA- periodontal inflamed surface area; SES- socio-economic status

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INfluence of Successful Periodontal Intervention in REnal Disease (INSPIRED): study protocol for a randomised controlled pilot clinical trial [72], [page number 128]

The need for randomised trials to investigate the impact of treatment of periodontitis on the cardio-renal health of patients with CKD has been highlighted by the previous studies in this thesis [44, 55, 63]. Aside from the evidence gathered as part of this thesis, the need for an RCT to investigate the effect of periodontal therapy on outcomes in patients with CKD also stems from meta-analyses of cohort studies assessing the association between periodontitis and mortality in patients with CKD [73], meta-analyses of studies reporting the association between periodontitis and CKD [59, 60, 74] as well as longitudinal studies [47, 75, 76] showing an association between periodontitis and progression of CKD. The cumulation of such observational evidence prompted researchers to conclude that the interventional studies, investigating the effects of treatment of periodontitis in patients with CKD, were now justified [77].

In order the address this, the “INSPIRED” pilot was conceived and developed. The protocol for this trial was published in the following manuscript. This ongoing pilot RCT aims to inform a larger, adequately powered, follow-up study in regard to changes to the trial protocol, particularly relating to improvements in volunteer recruitment and retention, suitable primary/secondary outcomes as well as methods and need for data and sample collection.

For the INSPIRED trial, patients are recruited from the RIISC cohort. In addition, patients who meet the inclusion/exclusion criteria of the RIISC study, but are not included in the RIISC study, are recruited from renal out-patients' clinics. As well as meeting the criteria for inclusion in the RIISC study (Table 5), participants in the INSPIRED trial also need to have sufficient severity/extent of periodontal inflammation, defined as minimum cumulative probing depth of 30mm. Cumulative probing depth is defined as the sum of the deepest PPD per tooth, excluding PPDs<5mm. Patients are excluded if they have received specialist periodontal treatment in the past year or are not amenable to periodontal therapy.

The INSPIRED trial employs a parallel group design with patients randomised to either immediate non-surgical periodontal treatment or non-surgical periodontal treatment at a delay of approximately 12 months. Thirty patients are to be randomised to each arm. The periodontal intervention, in both arms, includes detailed, personalised oral hygiene instructions, as well as non-surgical root surface debridement, under local anaesthesia, carried out over multiple-visits, depending upon clinical need. Patients in both arms are reviewed every 3 months with remedial periodontal intervention offered to patients randomised in the immediate treatment arm, if indicated, until their last visit at 18-months. The general and oral health of patients in the delayed treatment arm is closely monitored on a 3-monthly basis for the first 12 months. After 12 months, patients in this arm receive the same periodontal treatment intervention as the immediate treatment arm, followed by a review, with remedial periodontal

treatment, at the 15- and 18-month time-points. The flow of patients through this trial (Figure 6) and the schedule for patients in this trial (Figure 7) are illustrated.

At baseline, and subsequent 3-monthly visits, patients in either arm have medical and dental assessments performed and sampling undertaken. The medical station involves completion of the OHIP-14 (oral health impact profile) questionnaire to assess the impact of oral health on general wellbeing. This is followed by a non-invasive measurement of skin auto-fluorescence, as a surrogate for the detection of advanced glycation end-products in skin, recording of blood pressure and carotid-femoral pulse wave velocity. Following this, patients' height, weight and hip and waist circumferences are measured and urine and venous blood samples are taken for assessment of renal health, glycated haemoglobin levels and markers of systemic inflammation and oxidative stress. Patients then proceed to the dental station where, following an extra- and intra oral examination, saliva and gingival crevicular fluid (GCF) sampling are performed. This is followed by assessments of gingival inflammation, sampling of sub-gingival plaque and detailed periodontal charting (DPC). The DPC involves recording of PPD and recession on 6 sites per tooth for all teeth present. In addition, BOP at each site is recorded dichotomously as yes/no. Finally, full mouth plaque and marginal bleeding scores are completed.

The INSPIRED study recruited its first patient in June, 2015 and, as of the end of October 2018, has recruited 37 participants.

Figure 6: Flow of patients through the INSPIRED trial

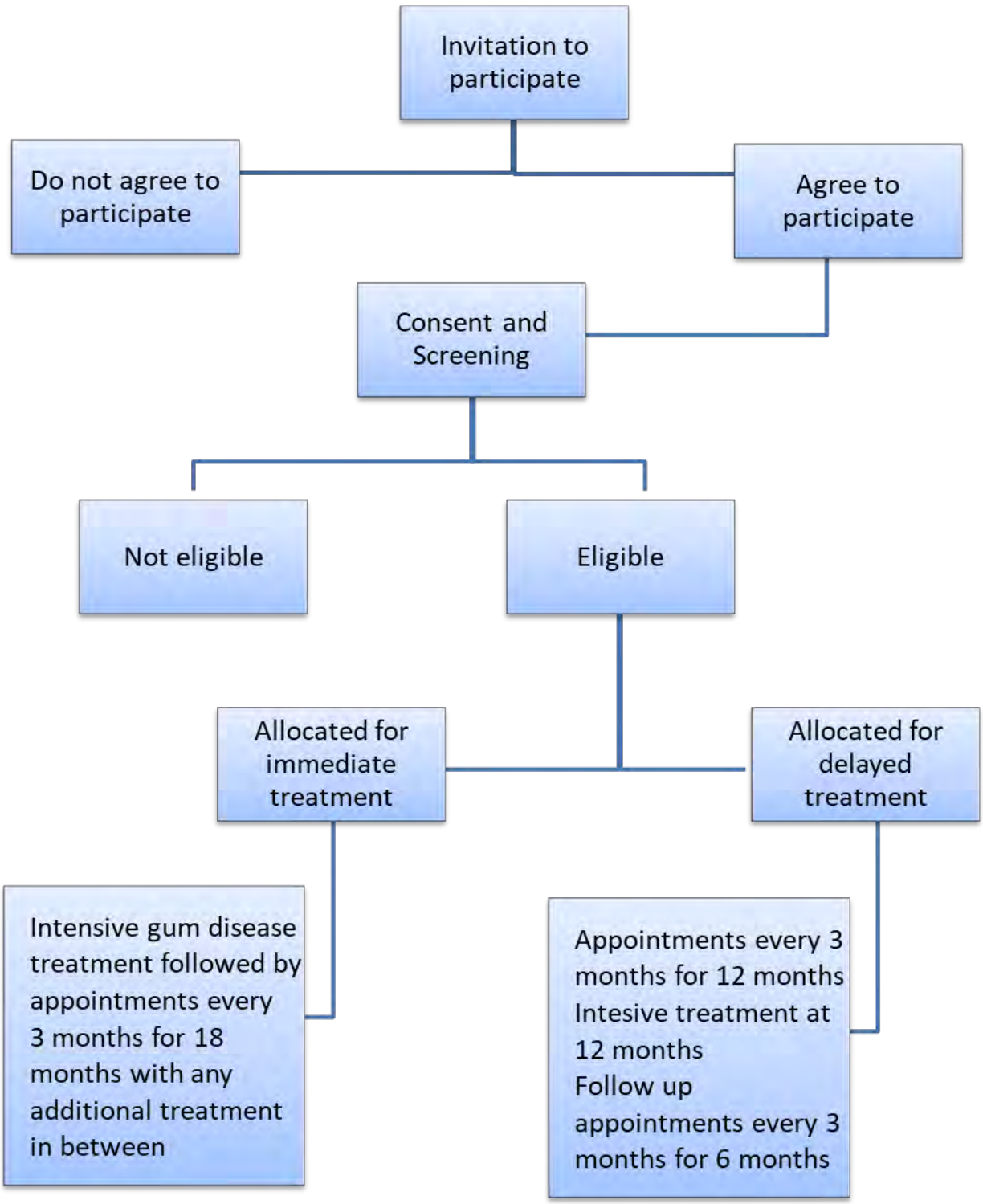

Figure 7: SPIRIT figure for INSPIRED trial

	STUDY PERIOD									
	Enrolment	**Allocation**	**Post-allocation**							**Close-out**
TIMEPOINT**	$-t_1$	0	P_i	t_1	t_2	t_3	t_4	P_d	t_5	t_6
ENROLMENT:										
Eligibility screen	X									
Informed consent	X									
Allocation		X								
INTERVENTIONS:										
Immediate treatment			X							
Delayed treatment								X		
ASSESSMENTS:										
Screening periodontal examination	X									
Demographic, anthropomorphic, cardiovascular data, sample collection, QoL		X		X	X	X	X		X	X

P_i- Immediate periodontal treatment; P_d- Delayed periodontal treatment

0-Baseline assessment; t_1-3-month recall, t_2- 6-month recall, t_3-9-month recall, t_4-12-month recall, t_5-15-month recall, t_6-18-month recall

CONCLUSIONS:

Mapping the manuscripts in this doctoral research project to the Bradford Hill criteria, this research has strengthened the acceptance of a causal nature to the associations observed between periodontitis and CKD. The researcher appreciates that “proving” causality is not a simple exercise of fulfilling a set of criteria. As a future aim, studies investigating the impact of treatment of periodontitis in patients with CKD, along with the health-economic implications of such interventions are an avenue of great interest to the researcher.

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Summary sheet

1. Dietrich T, **Sharma P**, Walter C, Weston P, Beck J. The epidemiological evidence behind the association between periodontitis and incident atherosclerotic cardiovascular disease. Journal of Clinical Periodontology. 2013;40:S70-S84.

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The epidemiological evidence behind the association between periodontitis and incident atherosclerotic cardiovascular disease

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Dietrich T, Sharma P, Walter C, Weston P, Beck J. The epidemiological evidence behind the association between periodontitis and incident atherosclerotic cardiovascular disease.

Abstract

Objectives: The objective of this study was to systematically review the epidemiological evidence for an association between periodontitis (PD) and incident atherosclerotic cardiovascular disease (ACVD), including coronary heart disease (CHD), cerebrovascular disease and peripheral arterial disease.

Methods: Systematic review of cohort and case-control studies on the association of clinically or radiographically diagnosed PD and ACVD.

Results: Overall, 12 studies were included in this study (six studies on CHD, three studies on cerebrovascular disease, two studies on both coronary heart and cerebrovascular disease mortality and one study on peripheral arterial disease). All but one study reported positive associations between various periodontal disease measures and the incidence of ACVD, at least in specific subgroups. The association was stronger in younger adults and there was no evidence for an association between PD and incident CHD in subjects older than 65 years. Only one study evaluated the association between PD and secondary cardiovascular events.

Conclusions: There is evidence for an increased risk of ACVD in patients with PD compared to patients without. However, this may not apply to all groups of the population. There is insufficient evidence for an association between PD and the incidence of secondary cardiovascular events.

Key words: atherosclerosis; cardiovascular disease; epidemiology; periodontal disease; periodontitis; systematic review

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The possible association between periodontitis (PD) and atherosclerotic cardiovascular disease (ACVD) has received much attention over the past two decades, and a significant number of epidemiological studies have been conducted during this time. The evidence has been systematically reviewed several times during that period (Hujoel 2002, Janket et al. 2003, Khader et al. 2004, Bahekar et al. 2007, Humphrey et al. 2008, Blaizot et al. 2009). Most recently, a comprehensive review was performed by an American Heart Association (AHA) working group (Lockhart et al. 2012), which concluded that "periodontal disease is associated with atherosclerotic

vascular disease independent of known confounders". It further concluded that there was no evidence for a causal link and that, therefore, "statements that imply a causative association between periodontal disease and specific atherosclerotic vascular disease events […] are unwarranted". The review further highlighted several research gaps and methodological issues relevant to further research, including the need for uniform criteria for PD measures and case definitions but mainly with regard to the need of well-designed controlled interventional studies with standard treatment protocols and considerations for issues such as the sustainability of treatment response over time.The aim of this review was to systematically review the evidence for the association between PD and incident ACVD, focusing on the most robust studies in terms of definition of the endpoint (incidence of ACVD) and exposure (clinically or radiographically assessed PD). In light of this evidence, we also discuss some additional issues relevant to further research not discussed in the AHA scientific statement.

Methods

The aim of this systematic review was to evaluate the evidence for an association between PD [defined by clinical attachment loss (CAL) /alveolar bone loss] and the incidence of ACVD For this review, we use the term "atherosclerotic cardiovascular disease" to include atherosclerotic diseases of the heart and the vasculature (coronary heart disease (CHD), cerebrovascular disease, peripheral arterial disease).

Objectives and review questions

Primary: What is the association between clinical PD and incident primary or secondary ACVD?

Secondary: What are the modifying effects, if any, of age, gender and smoking?

Eligibility Criteria for Studies

Types of studies

We considered all types of longitudinal studies, either cohort studies or case-control studies. Based on the time and resources available for this review, a pragmatic decision was made to limit this review to studies published in English and German, as these were the languages represented within the team of authors.

Types of exposure measures

To minimize the effect of misclassification of exposure, we only considered studies that employed either periodontal probing to measure periodontal probing depths (PPD) / CAL and/or radiographic assessment of alveolar bone loss. Hence, studies employing self-reported measures of PD or examination findings not based on periodontal probing were excluded. We further excluded studies that used surrogate measures of PD (such as antibody titres to periodontal pathogens) and studies that used composite measures of PD and other oral health conditions (e.g. gingivitis, caries, periapical disease), if the specific effect of PD could not be discerned. Reports had to clearly indicate how dichotomous or categorical definitions of PD were derived.

Types of outcome measures

To compare the risk of ACVD in individuals with PD to the risk of ACDV in patients without PD, we considered studies that evaluated incident CHD (angina, myocardial infarction, CHD death), incident cerebrovascular disease (transient ischaemic attack, stroke) and peripheral arterial disease. For primary disease, patients had to be free of the outcome of interest at baseline (cohort studies) or prior to suffering a cardiovascular event (case-control studies). For example, case-control studies recruiting patients based solely on angiographic findings were not eligible, unless it was specifically stated that angiography was in the context of an incident cardiovascular event. We also considered studies that evaluated the incidence of secondary ACVD events in patients with established ACVD. We did not include studies that used surrogate markers of ACVD (e.g. intima-media thickness, measures of endothelial function) or risk factors for ACVD as outcome measures.

Data presentation/analysis

To qualify for inclusion, studies had to report a measure of relative risk (e.g. risk ratio, rate ratio, hazard ratio, odds ratio) for the association between PD and incident ACVD. As a minimum, studies had to control for the confounding effects of age and gender, either by design (restriction) or statistical analysis (stratification/adjustment). Studies that used matching had to appropriately account for the matching factors in the analysis. We excluded studies where relative risk estimates were not readily interpretable due to inclusion of more than one exposure measure into the same model. For example, we excluded studies that included both a variable for PD status (e.g. none, moderate or severe) and an extent measure (e.g. >4 pockets with PD >4 mm) in the same regression model.

Literature Search

The electronic literature search was designed to be sensitive aiming to identify all relevant cohort and case-control studies (Table 1). Moreover, the references of studies examined for inclusion were thoroughly analysed searching for further studies. We did not actively search the grey literature; this was a pragmatic decision based on the time and resources available for this review.

Review Methods

One single reviewer (P. S.) screened all abstracts to eliminate publications that were clearly irrelevant. The full text of studies that appeared to satisfy the eligibility criteria or where insufficient information was available from the abstract to make a decision was screened by two of four reviewers (C. W., P. S., P. W. and T. D.). Disagreements were resolved by discussion between the reviewers. Data abstraction forms were developed and amended following pilot testing with five studies. Data abstraction was then performed for all full-text papers in duplicate. Disagreements were resolved by discussion. Data were extracted on the general characteristics of the studies in terms of authors, year of publication and country of study as well as population characteristics. Furthermore, specifics regarding exposure assessment and operationalization of PD, if applicable, were abstracted. We

Table 1. Search syntax for articles

Search syntax
1. exp Periodontitis/ or exp Chronic Periodontitis/
2. ("chronic periodont$" or periodont$).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
3. 1 or 2
4. exp heart arrest/ or exp myocardial ischemia/ or exp coronary disease/ or exp myocardial infarction/ or exp cerebrovascular disorders/ or exp peripheral vascular diseases/
5. (stroke or cerebrovasc$ or brain vasc$ or cerebral vasc$ or cva$).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
6. ((brain$ or cerebr$ or cerebell$ or intracran$ or intracerebral$) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
7. ((brain$ or cerebr$ or cerebell$ or intracran$ or intracerebral$ or subarachnoid) adj5 (h?emorrhage or h?ematoma or bleed$)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
8. (cardio$ or cardiac or infarction or "coronary heart disease" or "isch$emic heart disease").mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
9. ("peripheral arter$ diseas$" or "peripheral vascular diseas$").mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
10. 4 or 5 or 6 or 7 or 8 or 9

abstracted the relative risk estimates for the full population, and, if reported, any subgroup analyses for age, gender and smoking. If several models with varying levels of confounder control were presented, we chose the estimates from the model with the most extensive control for confounding. No formal assessment of inter-rater reliability was made for any element of data abstraction. Meta-analysis was not attempted because of the significant heterogeneity of studies in terms of virtually all study characteristics, including but not limited to study populations, assessment and definition of the exposure and outcomes and ascertainment and statistical adjustments made for confounders.

Results

The electronic search outlined in Table 1 yielded 1395 potentially eligible records. After screening of titles and, if available, abstracts, 62 full-text articles were reviewed. This resulted in the exclusion of 50 articles, yielding 12 articles for inclusion in this review according to the inclusion/exclusion criteria. The flow of inclusion/exclusion of articles is summarized in Fig. 1. The principle reason for omission of each excluded full-text article is given in Table S1 (available as online supplement). The most common reasons for exclusion of articles were related to studies not evaluating incident ACVD, the exposure measure used (e.g. self-reported diagnosis of PD or composite measures of oral health), or issues with data analysis and presentation.

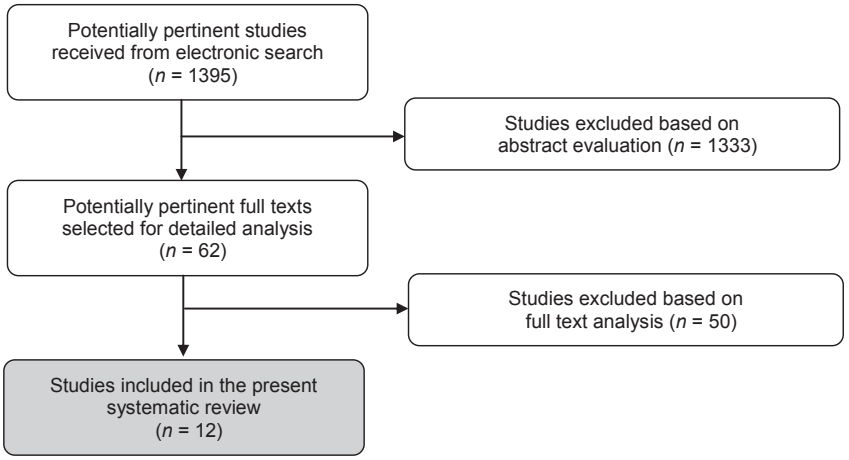

Fig. 1. Selection process of the studies included.

Types of outcomes and studies

We identified three cohort studies and three case-control studies exclusively on CHD (Table S2a), one cohort study and two case-control studies exclusively on cerebrovascular disease (Table S3a) and one cohort study exclusively on peripheral arterial disease (Table S5a). There were two additional cohort studies on ACVD mortality, including both CHD and cerebrovascular disease as causes of death (Table S4a). Tables S2a, S3a, S4a and S5a are available as Online Supplements.

There were several study reports that were based on the same study population but reported on different ACVD outcomes. Data from the Department of Veterans Affairs (VA) Normative Ageing and Dental Longitudinal Studies in Boston, MA, USA were reported in separate publications for CHD (Dietrich et al. 2008), cerebrovascular disease (Jimenez et al. 2009) and peripheral arterial disease (Mendez et al. 1998). Furthermore, the study population sampled for a population based case-control study on myocardial infarction (Andriankaja et al. 2007) was then longitudinally followed up for the incidence of secondary events (Dorn et al. 2010). The latter study was the only study that evaluated secondary cardiovascular events.

Three of the cohort studies (Ajwani et al. 2003b, Tuominen et al. 2003, Xu & Lu 2011) were exclusively on cardiovascular mortality assessed based on linkage of periodontal baseline data with death registry data. All case-control studies were restricted to non-fatal ACVD.

Details regarding the population characteristics of included studies are listed in Tables S2b, S3b, S4b and S5b (available as online only supporting information).

Exposure measurements and operationalization

Exposure measure characteristics of included studies are listed in Table 2. Alveolar bone loss as determined from periapical radiographs was used in all three reports based on the VA Normative Aging Study/Dental Longitudinal Study. All but one study (Mendez et al. 1998) used clinical measures of PD based on periodontal probing (probing depth and/or attachment loss). Partial-mouth recording protocols were utilized in four studies (Ajwani et al. 2003b, Cueto et al. 2005, Sim et al. 2008, Xu & Lu 2011). This included half-mouth recordings of randomly selected quadrants and the use of index teeth, for example, according to Ramfjord and Community Periodontal Index of Treatment Needs (CPITN) protocols.

There was little consistency in terms of the operationalization of PD. Six of the reports used dichotomous PD definitions. These were based on thresholds of either mean CAL (Andriankaja et al. 2007, Dorn et al. 2010), extent scores based on CAL (Cueto et al. 2005), mean bone loss scores according to Schei (Mendez et al. 1998) or based on a minimum number of teeth exhibiting CAL (Sim et al. 2008) or PD (Ajwani et al. 2003b) above a certain threshold (Table 2). Furthermore, studies used either continuous measures of PPD or CAL (Lopez et al. 2002, Dietrich et al. 2008, Jimenez et al. 2009, Dorn et al. 2010), or generated multiple exposure categories based on mean bone loss scores (Dietrich et al. 2008, Jimenez et al. 2009), extent of CAL (Sim et al. 2008), mean of CAL (Grau et al. 2004) or number of teeth exhibiting CAL and/or PD above a certain threshold (Tuominen et al. 2003, Xu & Lu 2011).

Confounder control

In addition to age and gender, all studies included adjusted for a wide range of confounders using statistical modeling (Table 3).

Association between PD and ACVD as represented by incident CHD, cerebrovascular disease and peripheral arterial disease

Relative risk estimates reported in the included studies are listed in Table 3. Overall, with the exception of one study (Tuominen et al. 2003), all studies report significantly higher incidences of ACVD in subjects with PD compared to subjects without PD, or in subjects with more severe PD (worse periodontal status) compared to subjects with no or less severe PD (better periodontal status), albeit not in all subgroups.

There are several studies that report subgroup analyses by age and sex groups. For subgroup analyses by age, cut-offs vary between 60 years (Grau et al. 2004, Dietrich et al. 2008, Sim et al. 2008) and 65 years (Jimenez et al. 2009, Xu & Lu 2011). All studies that stratify by age report stronger associations in younger subjects compared to older subjects. Indeed, for CHD, the majority of studies failed to demonstrate an association between PD and CHD incidence in older subjects. The results with regards to effect-modification by sex are less consistent. Two studies on cerebrovascular disease (Grau et al. 2004, Sim et al. 2008) and one on both CHD and cerebrovascular disease (Xu & Lu 2011) suggest that the association is stronger in men than women, whereas one study on CHD (Andriankaja et al. 2007) found a stronger association in women compared to men and one study found no association between PD and CHD in either sex (Tuominen et al. 2003).

The only study that investigated the incidence of secondary ACVD events found a significant association only in never-smokers, but not in ever smokers (Dorn et al. 2010).

Discussion

This systematic review identified 12 studies that report on the association between clinically or radiographically diagnosed PD and incident ACVD. With the exception of one study (Tuominen et al. 2003), all identified studies report a positive association between PD or PD severity or extent and the incidence of ACVD, at least in selected subgroups, independent of established cardiovascular risk factors. However, the evidence base for an association between PD and peripheral arterial disease, or secondary cardiovascular events in patients who had experienced a cardiovascular event before was very scarce, with only one study addressing each endpoint, respectively.

There is evidence from some studies that the association is stronger in men and younger individuals, although this was not specifically investigated in several of the included studies.

The potential association between PD and ACVD has received much attention in the scientific community since the late 1980s/early 1990s, and several narrative and systematic reviews have summarized the evidence that has emerged over the years, including the pathophysiological pathways that could underpin this association (Kebschull et al. 2010, Lockhart et al. 2012). We therefore chose relatively strict inclusion criteria, focusing on incident ACVD and also focusing on studies that used periodontal probing or radiographic assessment of PD to only include the most robust evidence. The latter criterion is particularly relevant in this field of research, as it excluded studies that used surrogate measures of PD, composite measures or self-reported periodontal measures, excluding several large cohort studies that employed self-reported measures of PD or that were based on the Russell periodontal index. Self-reported PD is associated with significant misclassification, resulting in marked attenuation of relative risk estimates (Dietrich & Garcia 2005).

This review also occasionally highlighted some problems with study design and/or data analysis that reflect the lack of appropriate epidemiological and/or statistical input in design and analysis of the studies. For example, while many of the case-control studies employed matching for some factors in the design, the need to address the

Table 2. Exposure measure characteristics (all studies)

Publication	Method of ascertainment	Partial-mouth recording	Definition(s) of PD(dichotomous)	Other exposure measure
Dietrich et al. 2008	Periapical radiographs (Schei score, ranging from 0 to 5), PPD	N		Categories based on Mean bone loss score, cumulative probing depth [explain in footnote]
Dorn et al. 2010	PPD/CAL	N	Mean CAL $\geq$3 mm	Mean CAL (continuous)
Tuominen et al. 2003	PPD	N		No PPD <4 mm $\geq$1 tooth PPD 4–6 mm $\geq$1 tooth PPD 6+ mm
Andriankaja et al. 2007	PPD/CAL	N	mean CAL $\geq$3 mm	
Cueto et al. 2005	PPD/CAL	Y (Ramfjord teeth only)	% of sites with CAL >3 mm (1) $\leq$ 33% sites No/mild PD (2)>33% moderate and severe PD	
Lopez et al. 2002	PPD/CAL	N		Mean CAL Mean PPD
Jimenez et al. 2009	Periapical radiographs (Schei score, ranging from 0 to 5), PPD	N		Mean bone loss score, Cumulative probing depth [explain in footnote]
Grau et al. 2004	PPD/CAL	N		Stratification into absence of PD or mild PD (defined as mean CAL $\leq$3 mm) and steps of 1.5 mm (mean CAL, 3 to 4.5, 4.5 to 6, and >6 mm). Severe PD: Mean CAL >6 mm.
Sim et al. 2008	PPD/CAL	Y (Two teeth per sextant)	$\geq$1 tooth with $\geq$6 mm CAL	%CAL $\geq$5mm: <48.6%, 48.6%–<73%, $\geq$73%
Xu & Lu 2011	PPD/CAL	Y (Two quadrants, one maxillary and one mandibular)		*Modest PD* One site CAL >4 mm or at least one site with PD >5 mm; *Severe PD* One site with CAL $\geq$6 mm and one or more sites with PD $\geq$5 mm
Ajwani et al. 2003b	CPITN	Y	$\geq$1 pocket $\geq$4 mm (CPITN codes 3 and 4)	
Mendez et al. 1998	Periapical radiographs (Schei score, ranging from 0 to 5)	N	Mean bone loss score >1	

matching in the analysis appropriately was ignored in some studies (Persson et al. 2003). We also had to exclude some studies in which investigators had included more than one measure of PD simultaneously in to the same regression model, rendering the resulting estimates not readily interpretable (Starkhammar Johansson et al. 2008, Pradeep et al. 2010, Holmlund et al. 2011).

The lack of universally accepted recording protocols and criteria for PD classification in clinical research (Tonetti et al. 2005, Page & Eke 2007) is also reflected in the wide variability of criteria evident in this review. Although this variability undoubtedly makes direct comparisons across different studies difficult (and this was one of the main reasons for the authors not to attempt meta-analysis in this review), several points are worthy of consideration. First, it should be noted that the effect of different classification criteria and/or partial-mouth recording protocols on measures of association (where PD is the exposure of interest) is uncertain. This is in contrast to studies on PD prevalence, where for example the underestimation of prevalence associated with partial-mouth recording protocols is well established (Eke et al. 2010). Second, in the context of PD and systemic disease associations, the comparison of results with different PD measures and/or classification criteria may give insight into the underlying mechanisms (Beck et al. 2005). For example, many papers make reference to the fact that the area of the periodontal wound, that is, the ulcerated pocket epithelium is 8–20 cm^2 (Hujoel et al. 2001b). Therefore, measures have been proposed that aim to quantify the size of this wound area (Schwahn et al. 2004, Dietrich et al. 2008, Nesse et al. 2008), such as "cumulative probing depth" utilized in one of the included studies (Dietrich et al. 2008). In contrast, CAL or alveolar bone loss reflect historic disease experience, and may thus be better measures of disease susceptibility rather than current periodontal inflammation. However, comparisons of the results across such dis-

Table 3. Results (all studies)

Publication	Type of Study	Measure of association RR (95% CI)		Factors adjusted for
Dietrich et al. 2008	Cohort	**Mean Bone loss score (MBLS):**		
		Age <60 years		Age, education, income and occupation at baseline and time-varying effects of smoking, body mass index, high density lipoprotein cholesterol, total cholesterol, triglycerides, hypertension, mean systolic blood pressure, mean diastolic blood pressure, diagnosis of diabetes, fasting glucose level, 2 hour glucose level, alcohol consumption and marital status.
		0–≤0.5	HR 1.01	
		(Ref)		
		0.5–≤1	HR 1.7	
		(1.1, 2.5)		
		1–≤1.5	HR 1.6	
		(0.9, 2.6)		
		>1.5	HR 2.1	
		(1.3, 3.6)		
		Edentulous	HR	
		1.9 (0.9, 3.9)		
		Age 60+ years		
		0–≤0.5	HR	
		1.0 (Ref)		
		0.5–≤1	HR	
		0.8 (0.6,1.3)		
		1–≤1.5	HR	
		1.0 (0.7, 1.5)		
		>1.5	HR	
		1.0 (0.6, 1.6)		
		Edentulous	HR	
		1.6 (1.0, 2.7)		
		Cumulative probing depth (CPD):		
		Age <60 years		
		0–<4 mm	HR	
		1.0 (Ref)		
		4–19 mm	HR	
		1.3 (0.8, 2.0)		
		20–40 mm	HR	
		1.4 (0.9, 2.3)		
		41 + mm	HR	
		1.9 (1.2, 3.0)		
		Edentulous	HR	
		1.7 (0.8, 3.6)		
		Age 60+ years		
		0–<4 mm	HR	
		1.0 (Ref)		
		4–19 mm	HR	
		1.1 (0.8,1.6)		
		20–40 mm	HR	
		1.2 (0.8, 1.9)		
		41+ mm	HR	
		0.7 (0.5, 1.2)		
		Edentulous	HR	
		1.7 (0.8, 3.6)		

Table 3. (continued)

Publication	Type of Study	Measure of association RR (95% CI)		Factors adjusted for
Dorn et al. 2010	Cohort	*Never Smokers:*		Age, gender, education, diabetes
		NO PD: (Ref)	HR 1	
		PD: (0.9, 4.5)		Age, gender, education, diabetes, cholesterol, lipid medication, hypertensive medication, BMI, physical activity, fruit and vegetable intake
		Mean CAL (per mm): 1.4 (1.1, 1.9)	HR	
		Ever Smokers:		
		Mean CAL (per mm): 1.0 (0.9, 1.2)	HR	
Tuominen et al. 2003	Cohort	*Men:*		Age, other oral health indicators, education, hypertension, hypercholesterolaemia, smoking, diabetes
		No PPD 4+ mm: 1.0 (Ref)	HR	
		PPD 4–6 mm: 1.0 (0.6, 1.6)	HR	
		PPD 6+ mm: 1.0 (0.6, 1.6)	HR	
		Women:		
		No PPD 4+ mm: 1.0 (Ref)	HR	
		PPD 4–6 mm: 0.9 (0.3, 2.1)	HR	
		PPD 6+ mm: 1.5 (0.6, 3.8)	HR	
Andriankaja et al. 2007	Case-control	*Men:* 1.3 (1.1, 1.6)	OR	Age, BP, cholesterol, diabetes, BMI, physical activity, smoking
		Women: 2.1 (1.5, 2.9)	OR	Age, sex, BP, cholesterol, diabetes, BMI, physical activity
		Never-Smokers: 1.4 (1.1, 1.9)	OR	
		Ever Smokers: 1.5 (1.3, 1.8)	OR	
Cueto et al. 2005	Case-control	No/Mild PD 1.0 (Ref)	OR	Age, sex, smoking, BP, diabetes, cholesterol, regular exercise,
		Moderate/Severe PD 3.3 (1.4, 7.7)	OR	[the following were considered but rejected based of 10% rule: BMI, family history CVD, education, social level, residence (urban/rural), employment, marital status]
Lopez et al. 2002	Case-control	Mean PPD: 8.6 (1.2, 61)	OR	Age, sex, diabetes, BP, smoking, [income, job power/prestige, BMI not included in final model but also considered but not associated with outcome $p > 0.25$]
		Mean CAL: 3.2 (1.3, 7.7)	OR	

Table 3. (continued)

Publication	Type of Study	Measure of association RR (95% CI)		Factors adjusted for
Jimenez et al. 2009	Cohort	*All Age groups*		Age, BMI, HDL, total alcohol, TG, BP, diabetes, alcohol consumption, smoking, marital status, education, occupation, income.
		Mean Bone loss score:		
		≤0.5	HR	
		1.0 (Ref)		
		0.5–≤1	HR	
		1.7 (0.8, 3.7)		
		1–≤1.5	HR	
		2.3 (1.1, 5.0)		
		>1.5	HR	
		3.5 (1.6, 7.8)		
		Cumulative probing depth:		
		0–<4 mm	HR	
		1.0 (Ref)		
		4–30 mm	HR	
		0.9 (0.5, 1.6)		
		31 mm +	HR	
		1.1 (0.6, 1.9)		
		Age <65 years		
		Mean bone loss score:		
		≤0.5	HR	
		1.0 (Ref)		
		0.5–≤1	HR	
		2.7 (0.8, 9.1)		
		1–≤1.5	HR	
		3.6 (1.0, 13)		
		>1.5	HR	
		5.8 (1.6, 21)		
		Cumulative probing depth:		
		0–<4 mm	HR	
		1.0 (Ref)		
		4–30 mm	HR	
		0.8 (0.3, 2.1)		
		31 mm +	HR	
		1.1 (0.4, 2.8)		
		Age ≥65 years		
		Mean bone loss score:		
		≤0.5	HR	
		1.0 (Ref)		
		0.5–≤1	HR	
		1.1 (0.4, 3.0)		
		1–≤1.5	HR	
		1.5 (0.6, 4.0)		
		>1.5,	HR	
		2.4 (0.9, 6.3)		
		Cumulative probing depth:		
		0–<4 mm	HR	
		1.0 (Ref)		
		4–30 mm	HR	
		0.9 (0.5, 1.9)		
		31 mm +	HR	
		1.1 (0.5, 2.3)		
		All study participants		

Table 3. (continued)

Publication	Type of Study	Measure of association RR (95% CI)		Factors adjusted for
Grau et al. 2004	Case-control	Mean CAL:		
		≤3 mm	OR 1.0	Age, sex, dental visits, PI, missing teeth, caries, BP, diabetes, smoking, alcohol consumption, AF, CHD/PAD, previous stroke/TIA, family history of stroke, education, occupation, father's profession
		(Ref)		
		3–4.5:	OR 1.4	
		(0.8, 2.4)		Age, sex, BP, diabetes, smoking, previous stroke, father' profession, dental visits, number of teeth
		4.5–6:	OR 2.7	
		(1.4, 5.3)		
		>6:	OR 4.3	
		(1.8, 10)		Age, sex, BP, diabetes, smoking, previous stroke,father' profession, dental visits, number of teeth
		Age ≤60 years		
		Mean CAL		
		<=3 mm:		
		(Ref)	OR 1.0	Age, sex, BP, diabetes, smoking, previous stroke,father' profession, dental visits, number of teeth
		3-4.5:		
		(0.8, 2.4)	OR 1.8	
		4.5-6:		
		(1.4, 8.5)	OR 3.4	Age, sex, toothbrushing frequency, dental visits, missing teeth, DMFT, income, education, smoking, alcohol, BP, diabetes, cardiac disease, BMI, family history of BP, DM, cardiac disease
		>6:		
		(1.6, 23)	OR 6.1	
		Age >60		
		Mean CAL		
		<=3 mm:	OR 1.0	
		(Ref)		
		3-4.5:	OR 0.9	
		(0.4, 2.2)		
		4.5-6:	OR 1.7	
		(0.7, 4.5)		
		>6:	OR 1.8	
		(0.6, 5.3)		
		Female		
		Mean CAL		
		≤ 3mm:	OR 1.0	
		(Ref)		
		3–4.5	OR 1.3	
		(0.6, 2.9)		
		4.5-6:	OR 1.7	
		(0.6, 5.3)		
		>6:	OR 1.6	
		(0.3, 8.6)		
		Male		
		Mean CAL		
		≤3 mm:	OR 1.0	
		(Ref)		
		3–4.5:	OR 1.6	
		(0.8, 3.2)		
		4.5-6:	OR 3.4	
		(1.5, 7.7)		
		>6:	OR 4.9	
		(1.9, 13.1)		

Table 3. (continued)

Publication	Type of Study	Measure of association RR (95% CI)		Factors adjusted for
Sim et al. 2008	Case control	No PD:	OR 1.0	Age, Sex, toothbrushing frequency, dental visits, missing teeth, DMFT, income, education, smoking, alcohol, BP, diabetes, cardiac disease, BMI, family history of BP, DM, cardiac disease
		(Ref)		
		PD:	OR 4.0	
		(2.3, 7.0)		
		Based on %extent CAL		
		$\geq$5 mm:		
		No/mild, 0% to <48.6%		
		OR 1.0 (Ref)		
		Moderate, 48.6% to 73% :		
		OR 2.6 (1.4, 4.8)		Sex, toothbrushing frequency, dental visits, missing teeth, DMFT, income, education, smoking, alcohol, BP, diabetes, cardiac disease, BMI, family history of BP, DM, cardiac disease
		Severe, >73%:		
		OR 4.3 (2.3, 8.2)		
		Age 40–59 years		
		CAL <6 mmz		
		OR 1.0 (Ref)		
		CAL $\geq$6 mm		
		OR 6.0 (2.1, 16.8)		
		CAL $\geq$5 mm %		
		0–48.6%		
		OR 1.0 (Ref)		
Sim et al. 2008 (continued)	Case control	48.8–73%:		Age, toothbrushing frequency, dental visits, missing teeth, DMFT, income, education, smoking, alcohol, BP, diabetes, cardiac disease, BMI, family history of BP, DM, cardiac disease
		OR 7.9 (2.2, 29)		
		$\geq$73%:		
		OR 5.7 (1.9, 17)		
		Age 60–79 years		
		CAL <6 mm		
		OR 1.0 (Ref)		
		CAL $\geq$6 mm:		
		OR 2.6 (1.2, 5.5)		Age, toothbrushing frequency, dental visits, missing teeth, DMFT, income, education, smoking, alcohol, BP, diabetes, cardiac disease, BMI, family history of BP, DM, cardiac disease
		CAL $\geq$5 mm %		Sex, toothbrushing frequency, dental visits, missing teeth, DMFT, income, education, smoking, alcohol, BP, diabetes, BMI, family history of BP, DM
		0-48.6%		
		OR 1.0 (Ref)		
		48.8–73%:		
		OR 1.4 (0.6, 3.2)		
		$\geq$73%:		
		OR 2.9 (0.9, 5.8)		
		Male		Age, race, household income, education, smoking status, alcohol use, total to HDL cholesterol ratio, obesity, diabetes, hypertension and a history of CHD or stroke.
		CAL <6 mm		
		OR 1.0 (Ref)		
		CAL $\geq$6 mm		
		OR 5.4 (2.4, 12)		
		CAL $\geq$5 mm %		
		0–48.6%		
		OR 1.0 (Ref)		
		48.8–73%:		

Table 3. (continued)

Publication	Type of Study	Measure of association RR (95% CI)	Factors adjusted for
		OR 2.2 (0.9, 5.5) $\geq$73%: OR 6.4 (2.4, 17) *Female* CAL <6 mm OR 1.0 (Ref) CAL $\geq$6 mm OR 3.8 (1.6, 9.0) CAL $\geq$5 mm % 0–48.6% OR 1.0 (Ref) 48.8–73%: OR 3.8 (1.4, 9.9) $\geq$73%: OR 4.1 (1.6, 11) *Smoking ever* CAL <6 mm OR 1.0 (Ref) CAL $\geq$6 mm OR 7.4 (2.4, 23) CAL $\geq$5 mm % 0–48.6% OR 1.0 (Ref) 48.8–73%: OR 1.8 (0.5, 7.1) $\geq$73%: OR 6.8 (2.0, 24) *Smoking never n = 325* CAL <6 mm OR 1.0 (Ref) CAL $\geq$6 mm: OR 3.3 (1.8, 6.7) CAL $\geq$5 mm % 0-48.6% OR 1.0 (Ref) 48.8-73%: OR 3.4 (1.6, 7.1) $\geq$73%: OR 3.8 (1.7, 8.5) Subgroup analyses for different outcome definitions: *Age 40–59 years* Ischaemic Stroke: OR 25.9 (5.8, 117) Haemorrhagic Stroke: OR 2.3 (0.5, 10.0) *Age 60–79 years* Ischaemic Stroke: OR 2.5 (1.1, 5.7) Haemorrhagic Stroke: OR 2.8 (0.8, 9.7) *Men 30–64 years*	

Table 3. (continued)

Publication	Type of Study	Measure of association RR (95% CI)		Factors adjusted for
Xu & Lu 2011	Cohort	No PD		Age, sex, history of CVD, social class, BMI, smoking, BP, serum cholesterol
		1.0 (Ref)	HR	
		Modest PD		
		1.3 (0.9, 1.7)	HR	
		Severe PD		
		2.1 (1.4, 3.3)	HR	
		65+ years		
		No PD		
		1.0 (Ref)	HR	
		Modest PD		
		1.0 (0.8, 1.2)	HR	
		Severe PD		
		1.1 (0.8, 1.8)	HR	
		Women 30–64 years		
		No PD		
		1.0 (Ref)	HR	
		Modest PD		
		0.9 (0.6, 1.4)	HR	
		Severe PD		
		1.6 (0.7, 3.3)	HR	
		65+ years		
		No PD		
		1.0 (Ref)	HR	
		Modest PD		
		1.0 (0.8, 1.2)	HR	
		Severe PD		
		0.9 (0.5, 1.6)	HR	
		Dentate no PD:		
Ajwani et al. 2003b	Cohort	1.0 (Ref)	HR	
		Dentate PD:		
		2.0 (1.0, 3.8)	HR	Age, sex, BMI, family history of heart disease, smoking
		Edentate:		
		1.4 (0.8, 2.6)	HR	
Mendez et al. 1998	Cohort	No PD:	OR	
		1.0 (Ref)		
		PD:	OR	
		2.3 (1.3, 3.9)		

ACVD, Atherosclerotic Cardiovascular Disease; AF, Atrial Fibrillation; BMI, Body Mass Index; BP, Blood Pressure; CAL, Clinical Attachment Loss; CHD, Coronary Heart Disease; CI, Confidence Interval; CPITN, Community Periodontal Index of Treatment Needs; CVD, Cardiovascular Disease; DM, Diabetes Mellitus; DMFT, Decayed, Missing or Filled Teeth; ECG, Electrocardiogram; HDL, High-Density Lipoprotein; HR, Hazard Ratio, ICD, International Classification of Diseases; MI, Myocardial Infarction; NHANES, National Health And Nutrition Examination Survey; OR, Odds Ratio; PAD, Peripheral Arterial Disease; PD, Periodontitis; PI, Plaque Index; PPD, Periodontal Probing Depth; PVD, Peripheral Vascular Disease; Ref, Reference; RR, Relative Risk, TG, Triglyceride; TIA, Transient Ischaemic Attack.

ease measures have only recently been made (Andriankaja et al. 2007, Dietrich et al. 2008, Jimenez et al. 2009). Third, the fact that the results – across different study types and PD measures – of the studies included in this review are relatively consistent can be seen as reassuring. Interestingly, the authors of one of the included studies also demonstrated in an additional paper that the results across various periodontal measurements and case definitions were remarkably consistent (Andriankaja et al. 2006).

A different but related problem is the impact that missing teeth have on the assessment and operationalization of PD, and subsequently the estimation of PD -ACVD associations. This problem has not been systematically investigated. As PD is a major cause of tooth loss, the resulting misclassification of PD is differential, and depending on the PD measure used may result in over- or underestimation of exposure, with uncertain effects on measures of association. However, measures of the periodontal wound area such as "cumulative probing depth" appropriately account for missing teeth, as the wound area associated with a missing tooth is zero (Schwahn et al. 2004, Dietrich et al. 2008, Nesse et al. 2008).

For CHD and cerebrovascular disease, several cohort and case-control studies were included in this review. Although in theory case-control studies are nested in cohort studies and differ only in using a more efficient sampling strategy (Rothman & Greenland 1998), in practice case-control and cohort studies have different specific strength and limitations. For example, for logistic reasons, case-control studies are typically limited to non-fatal disease but allow the detailed ascertainment and specification of the outcome of interest. In contrast, cohort studies can include both fatal and non-fatal outcomes, but there are often limitations in the level of detail available on the outcomes (e.g. data derived from death certificates). The fact that overall both case-control and cohort studies yielded remarkably consistent results also increases confidence in the reported associations.

Many of the included studies perform age-stratification and, across all studies included in this review, there is consistent evidence that the association between PD and incident ACVD is stronger in younger individuals. Indeed, there appears to be little evidence for any association between PD and CHD in older individuals, which may have important implications for intervention studies as discussed elsewhere in the article. The evidence for effect-modification by gender and smoking is less consistent, although some studies suggest that the association may be stronger in men than women. However, it should be noted that investigators very rarely state whether subgroup analyses were specified *a priori* or whether they were informed by previous analyses (i.e. data driven), raising some concerns regarding the validity of the findings of these subgroup analyses.

Perhaps the most surprising finding of this review was that it included only one study evaluating the association of PD with secondary cardiovascular events, showing a moderate association only in a subgroup of never smokers.

Much of the debate over the past decade regarding the implications of the apparent association between PD and ACVD has obviously been regarding the question whether or not the association is causal, and, if so, whether periodontal treatment in patients with PD can reduce the risk of cardiovascular events. It is widely recognized that the latter question could only be answered by a randomized controlled clinical trial. The significant gaps in our knowledge with regards to this question have also been identified in the recent AHA scientific statement (Lockhart et al. 2012). However, due to the relatively low incidence of ACVD in the general population, it is reasonable to assume that any intervention study would have to be limited to a population with a high absolute risk of a cardiovascular event to be feasible (i.e. affordable). For example, the only pilot interventional study conducted to date, the Periodontitis and Vascular Events (PAVE) study (Beck et al. 2008), was a secondary prevention study, that is, limited to patients with existing ACVD in which the risk of a subsequent cardiovascular event was higher than in the general population. Interestingly, it appears from the findings of this review that the evidence for an association between PD and secondary cardiovascular events, and thus the evidence supporting a secondary prevention trial, is extremely scarce. In addition, since age is the most important predictor of ACVD risk, any high-risk population would be more likely to include older individuals. However, given the weak, if any, association between PD and ACVD in older subjects, an intervention study in a population older than 65 years is not supported by the current epidemiological evidence. In addition to the issues raised in the AHA scientific statement (Lockhart et al. 2012), these findings present yet another formidable challenge for the design and conduct of future clinical trials that aim to address the question of benefits of periodontal therapy on adverse cardiovascular events.

Conclusions and clinical relevance

We conclude that the current evidence supports the notion that the incidence of ACVD, as represented by incident CHD, cerebrovascular disease and peripheral arterial disease is higher in subjects with PD and/or worse periodontal status, compared to subjects without PD or with better periodontal status, independent of many established cardiovascular risk factors. However, this may not be the case in all groups of the population. Further epidemiological evidence is needed to establish if PD is associated with the incidence of secondary cardiovascular events in patients with established ACVD.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Full texts excluded including reasons.
Table S2. (a) Outcome Coronary Heart Disease-Study/population characteristics. (b) Outcome Coronary Heart Disease- Demographics.
Table S3. (a) Outcome Cerebrovascular Disease- Study/population characteristics. (b) Outcome Cerebrovascular Disease- Demographics.
Table S4. (a) Outcome cardiovascular and cerebrovascular diseases- Study/population characteristics. (b) Outcome cardiovascular and cerebrovascular diseases- Demographics.
Table S5. (a) Outcome peripheral arterial diseases- Study/population characteristics. (b) Outcome peripheral arterial diseases- Demographics.

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Clinical Relevance

Scientific rationale for the study: Periodontitis has been implicated in the pathogenesis of atherosclerotic vascular diseases.
Principal findings: The incidence of atherosclerotic cardiovascular disease is higher in subjects with periodontitis compared to patients without periodontitis. The association is stronger in younger individuals, and there may be no association in older individuals.
Practical implications: Well-designed epidemiological and interventional studies are required to elucidate the implications of poor periodontal health on atherosclerotic cardiovascular disease risk in different populations.

Manuscript number 2

The relationship between general health and lifestyle factors and oral health outcomes

P. Sharma,*[1] M. Busby,[2] L. Chapple,[3] R. Matthews[4] and I. Chapple[5]

In brief

Presents evidence from a large group of patients (attending general dental practices) demonstrating that worsening oral health correlates with worsening general health

Provides further evidence from this group on the association between high-risk lifestyle factors such as smoking and heavy drinking and poor oral health outcomes

Aim The primary research question addressed in this paper was 'are lower than average oral health scores observed for those patients who report problems with general health and high-risk lifestyle factors?' **Methods** A population analysis was conducted on the first 37,330 patients, assessed by 493 dentists in the UK, to receive a Denplan PreViser Patient Assessment (DEPPA) at their dental practice. The Oral Health Score (OHS) was generated using a mixture of patient-reported factors and clinical findings and is an integrated component of DEPPA. Patients' self-reported risk factors included diabetes status, tobacco use and alcohol consumption. Patients' general health was measured by self-report, that is, a yes/no answer to the question 'have you experienced any major health problems in the last year for example a stroke, heart attack or cancer?' Multivariable linear regression analysis was employed to study the association between the OHS and general health and risk factors for patients in the DEPPA cohort. **Results** The mean age of participants was 54 years (range 17-101; S.D. 16 years) and the mean OHS for the group was 78.4 (range 0-100; S.D. 10). 1,255 (3%) of patients reported experiencing a major health problem in the previous year. In the fully adjusted model, diabetes, tobacco use, excessive alcohol consumption (three or more drinks per day), and poor overall health in the preceding year were all associated with a statistically significant drop in the mean OHS of patients. Having diabetes was associated with a 1.7 point (95% CI 1.3-2.1, P <0.001) drop in OHS, tobacco use was associated with a 2.7 point (95% CI 2.5-2.9, P <0.001) drop in OHS, and excessive alcohol consumption was associated with a 1.8 point (95% CI 1.3-2.4, P <0.001) drop in OHS. The mean OHS in patients who reported a major health problem in the preceding year was 0.7 points (95% CI 0.2-1.2, P = 0.006) lower than that of patients who did not report a major health problem in the preceding year. **Conclusion** The current study has demonstrated that patient reported general health and risk factors were negatively associated with an overall composite oral health score outcome in a large population of over 37,000 patients examined by 493 dentists. While the clinical significance of some of the reported associations is unknown, the data lend support to the growing body of evidence linking the oral and systemic health of individuals. Therefore, GDPs may be in a unique position to influence the lifestyle and general health of patients as part of their specific remit to attain and maintain optimal oral health.

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Background

There is now substantial literature describing the relationship between systemic health and oral, particularly periodontal, health.[1]

As part of Denplan's PreViser Patient Assessment[2] (DEPPA), the oral health status of patients is recorded using the composite 'Oral Health Score'[3,4] (OHS). Such measures offer potentially valuable signposts for patient engagement, education and motivation towards behaviour change.[5] Standard clinical practice commonly employs separate measurements for each aspect of oral health, however, validated composite measurements are valuable in providing patients with a holistic summary of their oral health outcomes and facilitate oral health improvement targets.

The development, validity, reliability and reproducibility of the OHS has been reported previously by an expert panel of dentists in a pilot study[3] and also in another study aimed specifically at the OHS;[4] it has also been studied in comparison with the Adult Dental Health Survey.[6] Recently elements of DEPPA have been shown to impact favourably on the factors influencing behaviour change in dental patients.[7]

To date, the association between general health, risk factors and oral health in patients within the DEPPA cohort remains unclear. The existence within the DEPPA database of patient-reported systemic health measures along with current oral health status based upon the OHS, provides the opportunity to explore the association between the two for a large population of patients in a primary dental care setting.

The aim of this paper is to report on the association between current oral health status of patients, as measured using the OHS, and patient-reported risk factors and general health in the preceding year. The primary research question addressed in this paper was 'are lower than average oral health scores observed for those patients who report problems with general health and high risk lifestyle factors?'

As these measures, the OHS and self-reported general health and risk factor status, are computed independently of each other, the null hypothesis is that there is no association between the OHS and such factors within the DEPPA cohort.

Methods

Data from the first 37,330 patients to receive a DEPPA at their dental practice was analysed. A total of 493 different dentists contributed patient assessments to this population.

The OHS is generated based upon:

- Patient-report of oral pain, function (eating) and appearance
- Clinical dental examination (Table 1 and Fig. 1).

These are recorded online in DEPPA and are then used by the embedded algorithms to produce the composite OHS for each patient based upon their current oral health status. These scores are out of a maximum of 100 which equates to perfect oral health and lower scores indicate worse oral health status.

The remaining general health and lifestyle questions (questions 4 to 17, Fig. 1) inform the Previser future disease risk scores, which are an important part of a full DEPPA report.[4] Inputting of this information is either by:

- The patient completing a paper version of the DEPPA patient questionnaire before their examination and the dental team entering the data online, or
- The patient completing the questionnaire directly online before their examination, or
- A dental team member questioning the patient and entering the data online.

Oral Health Assessment: Confidential DEPPA Patient Questionnaire

Your dental team are here to help if you have difficulty in answering any of these questions

Date..... Patient.....

Denplan
At the heart of dental care

For each question, please (circle) the most appropriate answers

1) Are you experiencing any pain or discomfort in your mouth?	Yes	Some	No	
2) Do your teeth allow you to eat an unrestricted diet?	Yes	Mainly	No	
3) How do you feel about the appearance of your teeth?	Happy	Some concerns	Unhappy	
4) Are you diabetic?	No	Yes: fair control	Yes: good control	Yes: poor control
5) How many different times during a typical day do you eat sugar containing food & drink, **other** than at meal times?	Four times a day or more	Less than four times a day		
6) Do you use fluoride toothpaste and/or fluoride mouthwash?	Yes	No		
7) Is there fluoride in your water supply at home?	Yes	No		
8) Have you experienced a major health problem during the last year for example a stroke, heart attack or cancer?	Yes	No		
9) How often, **other than** at mealtimes, do you consume acidic food and drinks?	A few times a week	Daily	Several times a day	
10) Do you have any conditions that cause you to vomit (be physically sick) at least once a week?	Yes	No		
11) Do you grind your teeth?	Yes	No		
12) Do you suffer from acid reflux (heartburn) into your mouth?	Yes	No		
13) With regards to cigarette smoking, circle all those that apply to you.	Never Smoked	**Smoke(d)** less than 10 cigs/day	**and used for** less than 10 years	**and quit** less than 10 years ago
		10 or more cigs/day	**and used for** 10 or more years	**and quit** 10 or more years ago
14) With regards to cigars or pipe smoking, circle all those that apply to you.	Never Smoked	**Smoke(d)** less than 1 cigar or pipe/day	**and used for** less than 10 years	**and quit** less than 10 years ago
		1 or more cigars or pipes/day	**and used for** 10 or more years	**and quit** 10 or more years ago
15) With regards to smokeless tobacco (chewing products), circle all those that apply to you.	Never used	**Use(d)** occasionally	**and used for** less than 10 years	**and quit** less than 10 years ago
		Daily use	**and used for** 10 or more years	**and quit** 10 or more years ago
16) Which one of the following best describes your average alcohol intake?	None	1 drink per day	**Note: 1 drink equals**	
	Less than 1 drink per day	2 drinks per day	Beer 1 pint 5% alcohol	
		3+ drinks per day	Wine 175 ml 12% alcohol	
			Spirits 25 ml 40% alcohol	
17) Have you ever had oral cancer?	Yes	No		

Fig. 1 DEPPA patient questionnaire

In some instances, the dental team need to assist patients with the questionnaire. The clinical inputs required to complete a DEPPA are usually made directly as the patient is examined.

The data submitted by practices during a DEPPA are held centrally in an encrypted and de-personalised form so that only the submitting practice can identify individual patients. However, as reported by Busby *et al.*,[6] reports can be generated in order to produce a national benchmark, audit tables, and for population level analytics.

The DEPPA database was interrogated to report the OHS for each patient as well as lifestyle factors including diabetes, tobacco use, alcohol consumption and any major health problems in the preceding year (question 8, Fig. 1) as a surrogate for the overall general health of patients in the preceding year.

Statistical analysis

Differences in categorical and continuous data were assessed for statistical significance using Pearson Chi-square, t-test and Fisher's exact test as appropriate.

The association between the self-reported general health and OHS of patients within the DEPPA database is reported unadjusted and adjusted for the following covariates: age; self-reported diabetes status (yes or no); tobacco use (ever smoked cigarettes, cigars or pipe or used smokeless tobacco); alcohol status (none, <1 drink/day, 1 drink/day, 2 drinks/day, 3 or more

drinks/day); presence of acid reflux (yes or no); and conditions causing vomiting at least once a week (yes or no) (Fig. 1). Also included as covariates were dental assessments of inadequate saliva flow (yes or no) and dental attendance (less than recommended or as recommended). These covariates were included as they could possibly confound the association between general health and OHS by influencing both.

Results

All 37,330 patients in the DEPPA database at the census point for data extraction were included in the analysis. The mean age of participants was 54 years (range 17–101; S.D. 16 years) and the mean OHS for the group was 78.4 (range 0–100; S.D. 10). A total of 1,255 (3%) of patients reported experiencing a major health problem in the last year, 1,875 (5%) reported having diabetes, 22,925 (61%) reported no tobacco use ever, 7,723 (21%) reported no alcohol intake, 345 (1%) reported a health condition that predisposes to vomiting at least once a week and 4,463 (12%) reported acid reflux into the mouth. The dentists assessed inadequate saliva flow in 608 (2%) patients and less than recommended dental attendance in 2,213 (6%) of patients (Table 2).

Patients who self-reported to have experienced a major health problem within the previous year (N = 1,255) were significantly older and had a lower OHS than patients who did not report experiencing a major health problem in the last year. Such patients were also more likely to have diabetes, use tobacco, be teetotal, experience reflux or vomiting, have inadequate saliva flow and be infrequent attenders to their dentist.

Multivariable regression analysis, accounting for age, diabetes status, alcohol consumption, tobacco use, reflux, vomiting, salivary flow and dental attendance attenuated the association between OHS and risk factors and major health problems in the preceding year.

The multivariable analysis demonstrated that, accounting for all other covariates mentioned, having diabetes was associated with a 1.7 point drop in OHS compared to no diabetes, tobacco use was associated with a 2.7 point drop in OHS compared with no tobacco use, excessive alcohol consumption (three or more glasses) was associated with a 1.8 point drop in OHS compared with no alcohol consumption and less than recommended dental attendance was associated with a 7.3 point drop in OHS compared with recommended dental attendance. The OHS also decreased in a dose-dependent manner with age with each increase in decade being associated with a 2 point drop in OHS.

Table 1 A guide to the generation of the OHS in DEPPA

	Max score	Possible scores
Comfort	8	0 (pain) 4 (some pain) 8 (no pain)
Function	8	0 (problems) 4 (minor problems) 8 (no problems)
Appearance	8	0 (unhappy) 4(some concern) 8(happy)
Occlusion	8	0 (less than 10 teeth in each jaw opposed) 8 (at least 10 teeth in each jaw opposed)
Soft tissues	8	0 (needs treatment or referral) 4 (needs observation) 8 (healthy)
Tooth health	24	24 (no restorations, caries free) 18 (sound restorations, caries free) 12 (less than 10% teeth need treatment) 6 (10–30% of teeth need treatment) 0 (more than 30% of teeth need treatment)
Tooth wear	12	0 (much more wear than expected for age) 6 (more wear than expected for age) 12 (normal wear for age)
Gum health	24	0 (severe periodontal disease) 6 (moderate periodontal disease) 12 (mild periodontal disease) 18 (gingivitis only) 24 (healthy)
Total	**100**	

Table 2 Description of the whole DEPPA cohort and sub-groups

Variable	Whole cohort N = 37,330	Patients with no major health problem in last year N = 36,075	Patients with major health problem in last year N = 1,255	P-value*
Mean age (SD) in years	54 (16)	54 (16)	61 (15)	<0.001
Mean OHS (SD)	78 (10)	79 (10)	75 (12)	<0.001
Diabetes (%)	5	5	10	<0.001
No tobacco use (%)	61	62	57	0.002
No alcohol use (%)	21	20	29	<0.001
Conditions leading to vomiting at least once a week (%)	0.9	0.7	8.5	<0.001
Reflux (%)	12	12	23	<0.001
Inadequate saliva flow (%)	1.6	1.4	7.3	<0.001
Infrequent dental attendance (%)	6	6	9	<0.001

*P value of comparison between patients who reported a major health problem in the last year and those that did not.

In the fully adjusted model, patients who reported major health problems in the last year had a mean OHS that was 3.5 points, 0.7 points (95% CI 0.2-1.2, P = 0.006) lower than those that did not report such problems.

Discussion

This study reports upon the relationship between an established and validated composite oral health assessment system (DEPPA Oral Health Score) and individual lifestyle and general health factors, within general dental practices, from a large population of 37,330 patients. Unlike the Adult Dental Health Survey,[8] the study cohort cannot be regarded as a representative sample of the UK population. The dentists conducting these examinations are a self-selecting group of enthusiasts who are the relatively early adopters of DEPPA. Nevertheless, it has previously been demonstrated that headline oral health outcomes in DEPPA are largely consistent with the findings of the ADHS for patients who report regular dental attendance.[6]

The study demonstrates an association between the OHS and patient reported risk factors and general health in a large cohort of patients. This association was statistically significant after adjusting for major confounders, which was also the case for the association between the OHS and self-reported major health problems in the preceding year, although the effect size was small for the latter. Assuming accurate data entry and self-reporting on behalf of patients, there are a number of potential explanations for the smaller than expected observed association:

- Self-reported major health problems in the preceding year may be a poor surrogate for overall general health
- Only 3% of patients reported having such health problems limiting the power of the analysis despite the large sample size
- The OHS is a composite score of oral health and the association between systemic health and some aspects of such a composite score, for example patient-reported outcomes, is currently poorly understood.

There is currently an international move towards recognising and embracing patient-centred outcomes in research studies, in service planning and evaluation, because optimal clinical health does not necessarily equate to optimal patient-perceived outcomes or improved quality of life. For example, a disease free mouth with a retentive lower partial denture may be regarded as clinically optimal, but the patient concerned may find the lower prosthesis adversely affects their speech, function and self-confidence. DEPPA records patient perceptions of their oral health in the form of function, comfort and aesthetics and embeds these as significant factors in deriving the composite oral health score.

The literature is now replete with data on the relationship between general health and periodontal health in particular.[1] Significantly, lower overall oral health scores were also observed in this study group for diabetes patients, consistent with the established negative relationship between diabetes and periodontal disease in particular.[9]

Busby *et al.*[10] reported how the average OHS tends to fall with increasing age, which may relate in part to the lifetime accumulation of local oral exposures, or may indeed be influenced by chronic systemic conditions of ageing or lifestyle factors. The present data suggest a significantly negative relationship between oral health outcomes and smoking. Furthermore, significantly better oral health scores were observed in patients who have never smoked. There is widespread evidence in the literature (Bergstrom *et al.*[11]) supporting a negative effect of smoking specifically on periodontal health and Axlesson *et al.*[12] also observed a negative impact of smoking more generally on oral health.

A negative relationship between periodontal health and drinking alcohol was also reported by Tezal *et al.*,[13] and drinking three or more alcoholic drinks daily also seems to be related to significantly poorer oral health outcomes than average for this study group. Patients who reported having a major health problem in the preceding year were more likely to report being teetotal (29% *vs* 20%, Table 2). The correlations between the OHS and alcohol intake are interesting and warranted further investigations. In investigating the categories of alcohol consumption, <1 drink per day, 1 drink per day, 2 drinks per day or 3 or more drinks per day, patients who reported having a major health problem were more likely to report drinking three or more drinks per day (4.5% *vs* 2.8%, data not included in Table 2) or being teetotal (29% *vs* 20%, Table 2). In examining the relative differences in OHS, compared to teetotallers, patients who reported drinking <1 unit per day had an associated 0.5 point higher OHS (P <0.001), patients reporting drinking 1–2 units per day had roughly similar OHS (P = 0.658 and P = 0.126 respectively) whereas patients reporting drinking three or more units per day had exhibited a 1.8 point lower in OHS (P <0.001). The association between no alcohol intake and poorer oral health scores is consistent with the medical literature, which demonstrates that light to moderate intake of alcohol such as wine reduces all-cause mortality and mortality due to cancer and coronary heart disease, whereas high alcohol intake increases mortality risk.[14]

The poorer OHS evident in those with lower than expected dental attendance is unsurprising and consistent with data from the ADHS.[15]

As the DEPPA cohort grows (now over 65,000), future analyses of oral health and risk trends and their prospective association with oral and general health outcomes will be interesting to analyse.

While the data presented provide initial insights into the relationship between a composite oral health score and general health and behaviours, longitudinal data analysis is necessary to enable the directionality of the association to be more appropriately analysed. Other limitations of the study include:

- Missing key covariates (gender, ethnicity, socio-economic status, etc) adjusting for which may attenuate the statistical significance of these findings
- Data entry was performed by a self-selecting group of professionals, who are likely not representative of the wider general dental service. The reliability of the data entered and the comprehensive nature of patient selection are unknown and comparison with ADHS data in the future may be helpful.
- General health status is only gauged by one question relating to 'major health problems in last year' and while a pragmatic question is necessary for logistical reasons, it limits the granularity of the analysis.
- The findings cannot be generalised to a community dwelling population who are not DEPPA patients, although the disease patterns in this cohort are similar to the ADHS group as previously reported.[6]

Conclusions

The current study has demonstrated that patient reported general health and risk factors were negatively associated with an overall composite oral health score outcome, in a large population of over 37,000 patients examined by 493 dentists. While the clinical significance of

some of the reported associations is unknown, the data lend support to the growing body of evidence linking the oral and systemic health of individuals. Therefore, GDPs may be in a unique position to influence the lifestyle and general health of patients as part of their specific remit to attain and maintain optimal oral health.

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EPIDEMIOLOGY (COHORT STUDY OR CASE-CONTROL STUDY)

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Association between periodontal health status and patient-reported outcomes in patients managed in a non-specialist, general dental practice

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Abstract

Aims: To explore the associations between periodontal status and patient-reported outcomes (PROs) in a large cohort of patients based in non-specialist general dental practice.

Materials and methods: Analysis was conducted using data from 14,620 patients, in 233 non-specialist dental practices across the UK. As part of routine clinical care, data on periodontal probing depths (PPD), alveolar bone loss (ABL), bleeding on probing (BoP) as well as PROs (oral pain/discomfort, dietary restrictions and dental appearance) were recorded using an online database. The associations between periodontal status and PROs were investigated using logistic regression analysis, adjusting for confounders.

Results: We found a positive association between worse periodontal health and the prevalence of PROs. After adjustment for confounders, 13.8% of patients in the healthiest category (PPD < 5 mm, ABL < 2 mm, no BoP) reported pain/discomfort, compared to 20.7% of patients in the worst category (PPD > 7 mm, ABL > 4 mm). A similar trend was seen with reporting a restricted diet and unhappiness with appearance.

Conclusion: This study provides novel insights into the associations between periodontal status and PROs in a non-specialist, general dental practice, highlighting the benefits of prevention and management of periodontitis.

KEYWORDS
dental practice, patient-reported outcomes, periodontitis

1 | INTRODUCTION

Patient-reported outcomes (PROs) are defined as "any report of the status of a patient's health condition that comes directly from the patient, without interpretation of the patient's response by a clinician or anyone else" (U. S. Department of Health and Human Services, 2009, U.S. Department of Health and Human Services). PROs are a way for the patient's perception of their disease or health to be incorporated into their clinical assessment and by doing so, ensuring that all dimensions of health are incorporated in the diagnosis and care for the patient (WHO, 2018).

The WHO defines oral health as a state of being free from diseases that "limit an individual's capacity in biting, chewing, smiling, speaking and psychosocial well-being" (WHO, 2012). However, often

the classification of oral health or oral disease is based on assessments carried out by the dental practitioner with little input from the patient. In the field of periodontology, disease status and treatment outcomes are measured using clinical measures such as periodontal probing depths (PPDs), clinical attachment loss, bleeding on probing (BOP) and radiographic bone loss. Clearly, these parameters may not be directly relevant to patients, who are thought not to be affected by their periodontal condition for many years before symptoms such as pain or tooth mobility present. Hence, periodontitis has widely been regarded as a "silent disease". However, this notion has recently been called into question, as several studies have suggested that periodontitis patients report worse oral health-related quality of life (Buset et al., 2016).

The aim of the present study was to explore the associations between clinical and radiographic periodontal parameters and patient-reported experience of oral pain, dietary restrictions and dental appearance in a large, non-specialist dental practice patient cohort.

Clinical Relevance

Scientific rationale for the study: To date, no studies have sought to investigate the relationship between patient-reported outcomes in a non-specialist dental setting.
Principal findings: The probability of reporting pain or restrictions in diet or unhappiness with dental appearance increases with worsening periodontal status in a dose-dependent manner. Patients with alveolar bone loss but no deep periodontal pockets are less likely to report dental pain or restrictions in diet compared to patients with deep periodontal pockets.
Practical implications: This study highlights the impact of periodontitis on the well-being of patients and hints at the potential improvements in well-being that may be obtained by periodontal intervention.

2 | METHODS

2.1 | Patient population

This study used data of 14,620 dentate patients, collected as part of routine clinical care by 355 dentists in 233 non-specialist dental practices across the UK. The dentists were part of a dental payment plan scheme (Denplan), which enables them to charge their patients a fixed monthly fee that covers regular examinations and treatments (Busby, Chapple, Matthews, Burke, & Chapple, 2014). Participating dental practices comply with a range of key performance/quality outcome measures, deemed consistent with "optimal" dental service provision (Busby, Chapple, Matthews, & Chapple, 2013). Beginning in 2013, dentists enrolled in the scheme have used an online tool, the Denplan PreViser Patient Assessment (DEPPA). The use of DEPPA is free to dentists who are Denplan Excel Certified, which is a voluntary quality assurance programme. Hence, the vast majority of DEPPA users are Denplan Excel Certified. The DEPPA system is used by participating dentists to record data on a patient's demographics, risk factors for oral diseases and clinical and radiographic findings. In addition, DEPPA also contains a short patient questionnaire that captures data on the patient's perceptions of their oral health and behaviours. For the present analyses, data of the first data entry for each patient were used, which may represent either an initial consultation for a patient new to the practice or a check-up appointment for a previously seen patient. These two types of appointment cannot be distinguished from the data available. While the DEPPA system uses these data to derive a number of scores as previously reported (Busby et al., 2013; Busby, Chapple, et al., 2014; Busby, Martin, Matthews, Burke, & Chapple, 2014; Newton & Asimakopoulou, 2017; Sharma, Busby, Chapple, Matthews, & Chapple, 2016), only the raw data entered by clinicians were used for the analyses described in this paper.

All personal identifiers are anonymized and collected in an encrypted format, and the system is used as part of routine clinical care. The UK Data Commissioner has confirmed that collected data are non-personalized. Ethical review was therefore not required for this analysis. As data were not recorded for research purposes, no formal calibration and no standardization of clinical or radiographic procedures were performed.

2.2 | Periodontal/exposure variables

As part of the clinical examination, dentists recorded the deepest PPD per sextant (<5 mm, 5–7 mm or >7 mm), any BoP in each sextant (yes/no) and the maximum radiographic alveolar bone loss (ABL) per sextant (<2 mm, 2–4 mm, >4 mm) as measured from the cemento-enamel junction to the alveolar crest, from available radiographs. Third molars were excluded from the periodontal assessment.

2.3 | Patient-reported outcome variables

Patient-reported outcomes were assessed on a 3-point Likert scale using questions on pain ("Are you experiencing any pain or discomfort in your mouth?" [Yes/Some/No]), dietary restriction ("Do your teeth allow you to eat an unrestricted diet?" [Yes/Mainly/No]) and appearance ("How do you feel about the appearance of your teeth?" [Happy/Some concerns/Unhappy]).

2.4 | Other data

Data were collected on the following variables based on patients' self-report: age in years and sex (male/female); smoking status (ever/never smoker); diabetes status (yes/no); tooth grinding habits (yes/no) and frequency of sugar intake (less than four times/four or more times in a typical day). Data on numbers of teeth with

TABLE 1 Demographics of the cohort

	Whole cohort (*n* = 14,568)	Cohort categorized by periodontal parameters							
		I (PPD < 5 mm, ABL < 2 mm, BoP −) (*n* = 2,693; 18.5%)	II (PPD < 5 mm, ABL < 2 mm, BoP +) (*n* = 3,081; 21.2%)	III (PPD < 5 mm, ABL 2–4 mm, BoP −) (*n* = 2,330; 18.0%)	IV (PPD < 5 mm, ABL 2–4 mm, BoP +) (*n* = 2,898; 19.9%)	V (PPD 5–7 mm, ABL 2–4 mm, BoP +/−) (*n* = 1,225; 8.6%)	VI (PPD < 5 mm, ABL > 4 mm, BoP +/−) (*n* = 911; 6.3%)	VII (PPD 5–7 mm, ABL > 4 mm, BoP +/−) (*n* = 868; 6.0%)	VIII (PPD > 7 mm; ABL > 4 mm, BoP +/−) (*n* = 532; 3.7%)
Age (years)	55 (16)	48 (16)	46 (16)	62 (12)	59 (13)	59 (13)	66 (11)	64 (11)	63 (12)
Male (%)	43	40	43	43	43	48	44	46	49
Diabetic (%)	5.7	3.8	3.4	5.5	6.3	6.6	9.1	9.6	11.5
Never smoker (%)	63	69	70	62	62	58	51	48	48
Teeth present[a]	25 (4)	26 (4)	26 (3)	25 (4)	25 (4)	25 (4)	23 (5)	23 (5)	23 (5)
Restored teeth	11 (6)	9 (6)	9 (6)	13 (5)	13 (5)	12 (5)	12 (5)	12 (5)	11 (5)
Teeth needing restoration	0.3 (1.0)	0.2 (0.9)	0.3 (1.1)	0.3 (0.7)	0.4 (1.0)	0.3 (1.0)	0.4 (1.1)	0.4 (1.1)	0.4 (1.1)
Frequency of dental visits less than recommended (%)	5	3	6	4	4	7	3	7	8
Improvement in oral hygiene needed (%)	44	18	54	27	58	52	58	56	64
High frequency of sugar intake (%)	11	12	14	10	10	11	11	10	14
Patient-reported outcomes (%)									
Pain	15	14	15	14	15	17	14	18	21
Diet restrictions	11	11	9	10	11	13	13	16	20
Unhappiness with appearance	26	23	26	26	26	27	30	31	33
Any patient-reported concerns	40	36	38	39	39	42	43	46	52
More than one patient-reported concerns	11	9	10	10	11	12	11	15	18

Notes. Data are unadjusted and are expressed mean (*SD*) unless otherwise stated.

ABL: Alveolar bone loss; Bleeding on Probing: BoP, either present (+) or absent (−); PPD: periodontal probing depth.

[a]Not including wisdom teeth.

TABLE 2 Probabilities (and 95% CI) of reporting various outcomes by periodontal parameters

Adjusted probability (%) of reporting	Periodontal parameters						
	I (PPD < 5 mm, ABL < 2 mm, BoP –)	II (PPD < 5 mm, ABL < 2 mm, BoP +)	III (PPD < 5 mm, ABL 2–4 mm, BoP –)	IV (PPD < 5 mm, ABL 2–4 mm, BoP +)	V (PPD 5–7 mm, ABL 2–4 mm, BoP +/–)	VI (PPD < 5 mm, ABL > 4 mm, BoP +/–)	VII (PPD 5–7 mm, ABL > 4 mm, BoP +/–)	VIII (PPD > 7 mm; ABL > 4 mm, BoP +/–)
Oral pain	13.8 (12.4–15.2)	13.9 (12.7–15.2)	14.9 (13.4–16.4)	15.0 (13.7–16.3)	17.3 (15.2–19.3)	13.7 (11.5–16.0)	17.7 (15.1–20.3)	20.7 (17.2–24.2)
Restricted diet	10.8 (9.5–12.0)	9.7 (8.5–10.8)	9.5 (8.3–10.7)	11.2 (10.1–12.4)	12.5 (10.7– 14.3)	12.1 (10.0–14.2)	15.5 (13.1–17.9)	19.2 (15.9–22.5)
Unhappiness with appearance	22.2 (20.6–23.8)	24.5 (23.0–26.1)	26.6 (24.8–28.4)	26.0 (24.4–27.6)	27.3 (24.8–29.8)	31.7 (28.6–34.8)	32.5 (29.4–35.6)	34.3 (30.3–38.4)
Reporting any concerns	35.6 (33.7–37.4)	37.2 (35.5–39.0)	39.1 (37.1–41.4)	39.1 (37.3–40.9)	42.3 (39.6–45.0)	43.8 (40.5–47.1)	46.7 (43.4–50.0)	52.4 (48.2–56.7)
Reporting more than one concern	9.2 (8.1–10.4)	9.6 (8.5–10.7)	9.9 (8.7–11.2)	10.8 (9.6–11.9)	12.2 (10.4–14.0)	11.4 (9.3–13.5)	15.3 (12.8–17.7)	17.5 (14.3–20.8)

Adjusted for age, sex, smoking status, diabetic status, number of teeth with restorations, number of teeth needing restorations, oral hygiene, dental attendance, abrasion, grinding habits, frequency of sugar intake and salivary flow rate.
ABL: Alveolar bone loss; Bleeding on Probing: BoP, either present (+) or absent (–); PPD: periodontal probing depth.

restorations and number of teeth needing restorations; patient's oral hygiene (adequate/inadequate); patient's dental attendance not as regularly as advised by the dentist (yes/no); presence of cervical tooth wear as a measure of abrasion (yes/no) and salivary flow (adequate/inadequate) were collected, as judged by the clinician.

2.5 | Statistical analysis

The following statistical analysis plan was defined a priori. To avoid sparse strata and facilitate interpretability, binary PRO variables were generated for pain (yes/some versus no), dietary restriction (yes versus mainly/no) and appearance (happy versus some concerns/unhappy). In addition, we created binary outcome variables based on the number of positive PRO responses (at least one versus none and more than one versus one or none).

Based on the worst sextant score of PPD, BoP and ABL, patients were categorized into 8 different periodontal status (exposure) categories (Table 1).

Logistic regression analysis was used to investigate the associations between each PRO as the outcome variable and the various categories of periodontal parameters as independent variables. In multivariable analyses, adjustments were made for variables which might confound these associations. These included age, sex, smoking and diabetes status, number of teeth with restorations, number of teeth needing restorations, oral hygiene, dental attendance, abrasion, grinding habits and frequency of sugar intake. In addition, salivary flow was included as a covariate in the association between periodontal health status and dental pain and diet and but not for the association between periodontal health and appearance.

The adjusted probabilities and two-sided 95% CI of reporting various outcomes were calculated by periodontal status. We carried out a sensitivity analysis by investigating these associations in a subset of patients with no outstanding restorative needs.

To assess whether the reporting of oral pain, dietary restriction and unhappiness with appearance was clustered in a subgroup of patients, we investigated the correlation between reporting pain and restricted diet, reporting restricted diet and unhappiness with appearance and reporting unhappiness with appearance and pain using tetrachoric correlation coefficients.

Additional sensitivity analyses were conducted including comparisons of findings of the above associations between patients requiring no restorations and patients requiring at least one restoration. In addition, a sensitivity analysis was conducted omitting the variables "oral hygiene", "dental attendance" and "salivary flow".

Patients with missing data were not included in the analysis.

3 | RESULTS

3.1 | Sample characteristics

The final analytical sample consisted of 14,568 dentate patients, following the exclusion of 52 patients (0.003%) with missing data. The mean age of patients in this cohort was 55.5 years (*SD* 15.7,

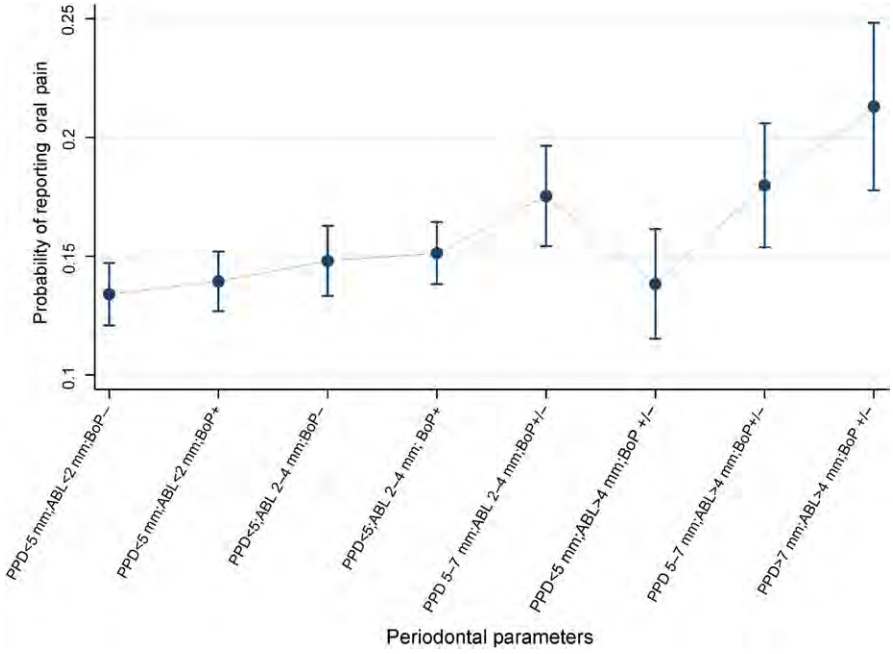

FIGURE 1 Probability of reporting oral pain versus Periodontal parameters. ABL: Alveolar bone loss; Bleeding on Probing: BoP, either present (+) or absent (−); PPD: periodontal probing depth

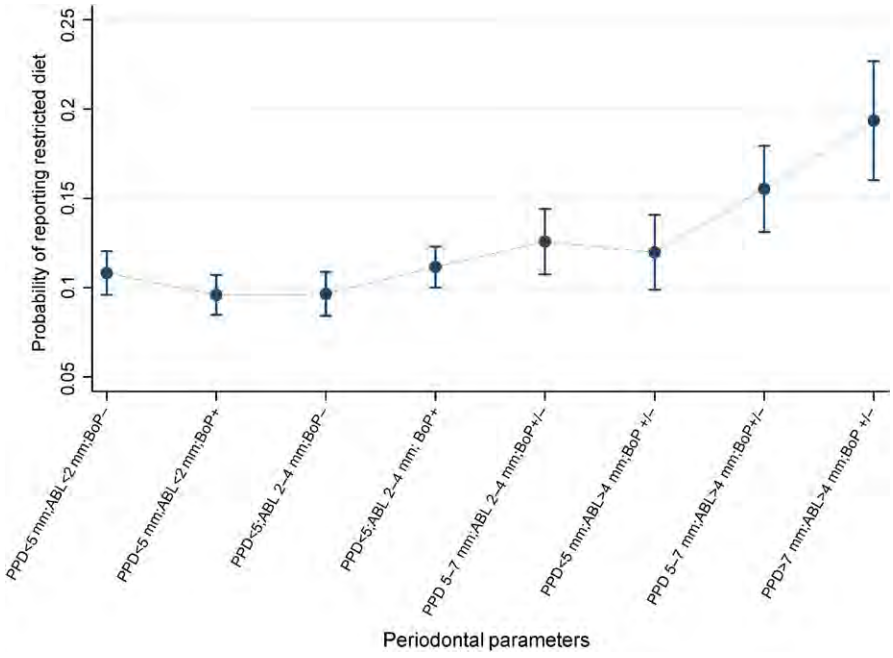

FIGURE 2 Probability of reporting restricted diet versus Periodontal parameters. ABL: Alveolar bone loss; Bleeding on Probing: BoP, either present (+) or absent (−); PPD: periodontal probing depth

range 17–106), 6,280 (43%) were male, 830 (5.7%) reported being diagnosed with diabetes and 9,146 (63%) were never smokers. The mean number of teeth present, not including wisdom teeth, was 25 (*SD* 4, range 1–28). Of these, a mean of 11 (*SD* 5.7, range 0–28) was restored and 0.3 (*SD* 1, range 0–26) needed restorations. A total of 696 (5%) did not attend the dentist as regularly as recommended, 6,469 (44%) had inadequate oral hygiene and 1,656 (11%) reported a high frequency of sugar intake (Table 1).

With regard to their periodontal parameters, 2,693 (18.5%) were in the healthiest group, 3,081 (21.2%) had BoP with no or limited periodontal tissue loss, with the remainder exhibiting various levels of periodontal tissue loss evidenced by PPD 5+mm and/or ABL 2+mm with or without BoP (Table 1).

3.2 | Unadjusted prevalence of PROs

A total of 2.7% of patients reported experiencing oral pain or discomfort and 12.3% reported experiencing some oral pain or discomfort; 3.5% of patients reported a restricted diet and 7.8% reported a somewhat restricted diet; 2.7% of patients reported being unhappy

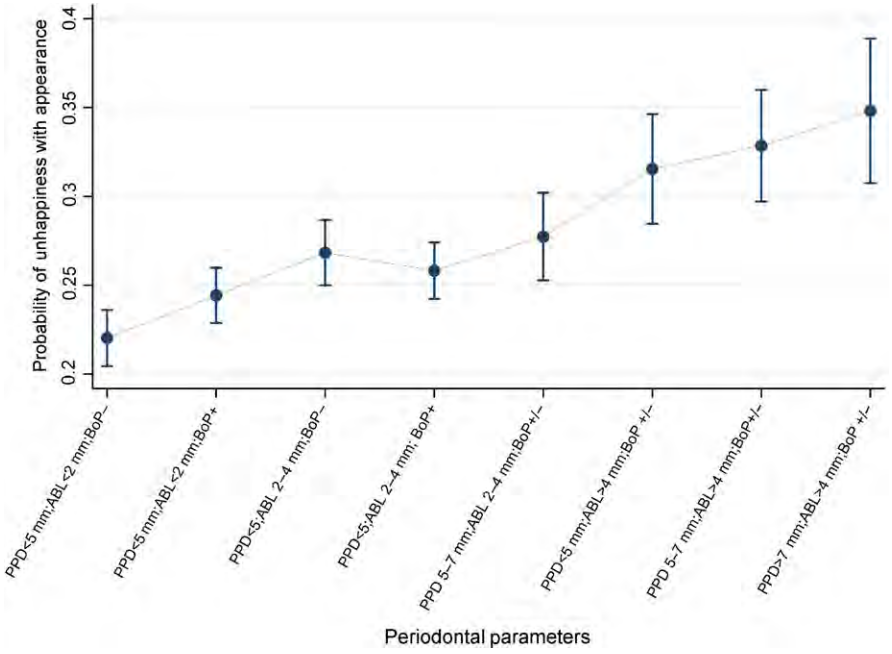

FIGURE 3 Probability of reporting unhappiness with appearance versus Periodontal parameters. ABL: Alveolar bone loss; Bleeding on Probing: BoP, either present (+) or absent (−); PPD: periodontal probing depth

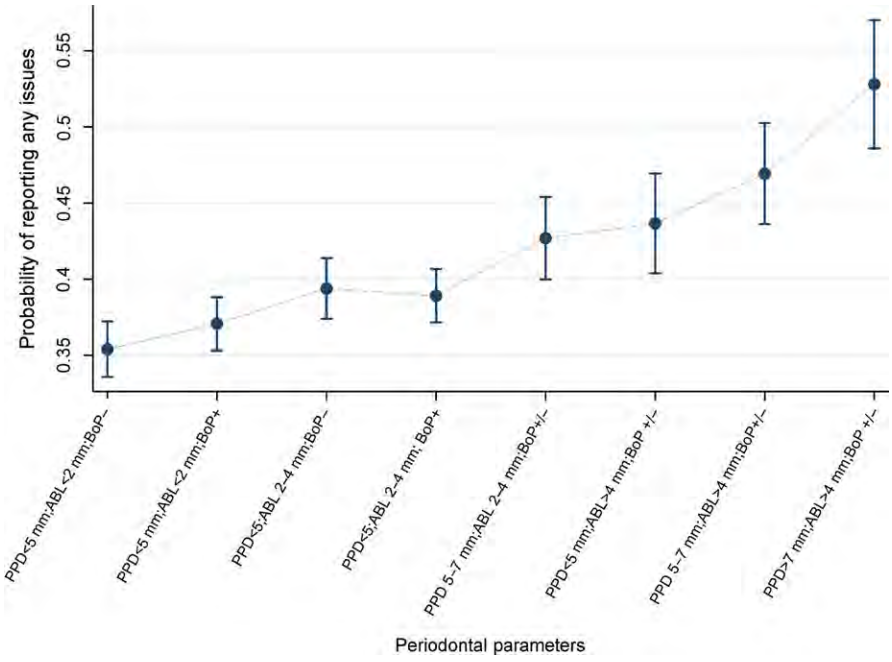

FIGURE 4 Probability of reporting oral pain or restricted diet or unhappiness with appearance versus Periodontal parameters. ABL: Alveolar bone loss; Bleeding on Probing: BoP, either present (+) or absent (−); PPD: periodontal probing depth

with the appearance of their teeth and 23.5% had some concerns with the appearance of their teeth. In addition, 40% of the cohort reported at least one of these concerns and 11% reported more than one of these concerns (Table 1). Patients' demographics, lifestyle factors and number of teeth with restorations, number of teeth needing restorations, oral hygiene, dental attendance, abrasion, grinding habits and frequency of sugar intake, were very similar regardless of whether they reported oral pain, diet restrictions or being unhappy with the appearance of their teeth (data not shown).

There were statistically significant correlations between the PROs with tetrachoric correlation coefficients being 0.3514 ($p < 0.0001$), 0.3110 ($p < 0.0001$) and 0.2940 ($p < 0.0001$) for the correlations between reporting pain and restricted diet, restricted diet and unhappiness with appearance and unhappiness with appearance and pain, respectively.

3.3 | Adjusted prevalence of PROs

Overall, the prevalence of all reported PROs tended to increase with worsening periodontal parameters (Tables 1 and 2, Figures 1–5).

Specifically, the adjusted prevalence estimates, that is, predicted probabilities from multivariable logistic models, for pain ranged from 13.8% (95% CI: 12.4–15.2%) in the periodontally healthiest group to 20.7% (95% CI: 17.2–24.2%) in patients with

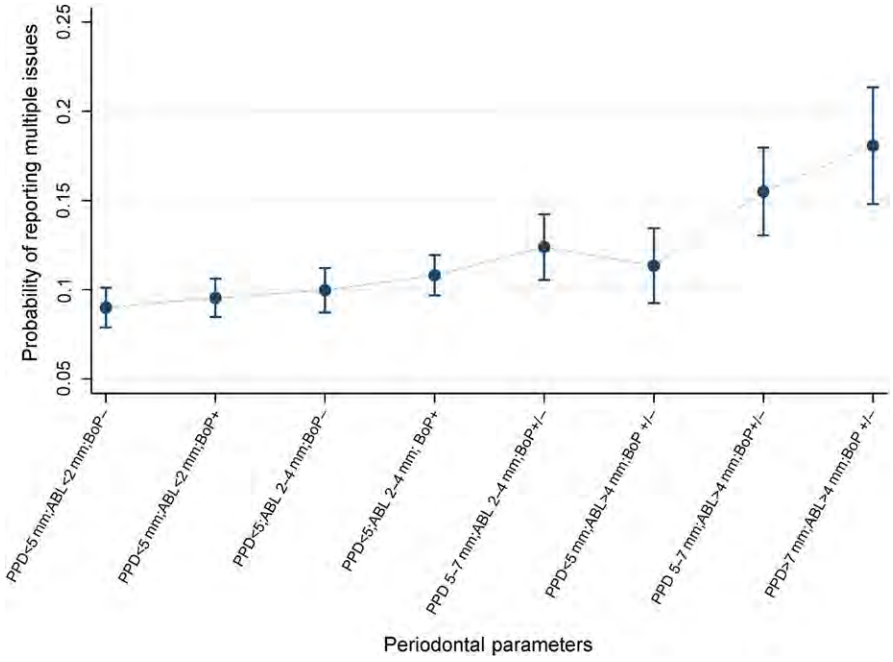

FIGURE 5 Probability of reporting more than one of oral pain or restricted diet or unhappiness with appearance versus Periodontal parameters. ABL: Alveolar bone loss; Bleeding on Probing: BoP, either present (+) or absent (–); PPD: periodontal probing depth

the worst periodontal parameters (PPD > 7 mm and ABL > 4 mm, Table 2 and Figure 1). Similarly, the periodontally healthiest patients had a 10.8% (95% CI: 9.5–12.0%) probability of reporting a restricted diet, compared to 19.2% (95% CI: 15.9–22.5%) for patients with PPD > 7 mm and ABL > 4 mm (Table 2 and Figure 2). For appearance, 22.2% (95% CI: 20.6–23.8%) of the periodontally healthiest patients compared to 34.3% (95% CI: 30.3–38.4%) of patients with the worst periodontal parameters reported an impact (Table 2 and Figure 3).

In addition, a similar trend was found in the adjusted probability of any impact, with 35.6% (95% CI: 33.7–37.4%) and 9.2% (95% CI: 8.1–10.4%) of the periodontally healthiest patients compared to 52.4% (95% CI: 48.2–56.7%) and 17.5% (95% CI: 14.3–20.8%) of patients with the worst periodontal parameters reporting at least one or more than one impact, respectively (Table 2; Figures 4 and 5).

3.4 | Results from sensitivity analysis

A similar trend to the whole-group analyses, in regard to all PROs, was observed in analyses restricted to the 11,744 (81%) patients not requiring any restorations at the time of assessment. Similarly, omission of oral hygiene, dental attendance and salivary flow from the model did not yield appreciably different results.

4 | DISCUSSION

In the present cross-sectional study of a large, general dental practice-based population, we found a dose-dependent association between worsening periodontal status and the probability of reporting pain or restrictions in diet or unhappiness with dental appearance.

The large sample studied here allows for some meaningful and interesting comparisons between categories of different periodontal parameters. For example, a large proportion of patients (44.2%) studied here had evidence of radiographic bone loss of 2+mm, but no periodontal pockets deeper than 4 mm, with approximately 20% exhibiting no BoP. While several causes of ABL other than periodontitis must be considered, the absence of deep periodontal pockets in the presence of ABL is consistent with the periodontal parameters one would expect to see following successful periodontal therapy, or resolution of active disease. Hence, our results are at least consistent with a beneficial effect of successful periodontal therapy and maintenance on the PROs evaluated here, in particular given the specifics of this population, that is, patients enrolled in a quality-assured, prevention-oriented, capitation-based payment plan scheme.

These results are in line with previous research on the association between periodontal disease and its treatment on oral health-related quality of life. A recent systematic review summarizing data from 14,087 patients in 37 studies, with sample sizes ranging from 21 to 3,122 patients, found that patients with periodontal disease had poorer oral health-related quality of life compared to periodontally healthy patients and that the impact on quality of life was greater with increasing extent and severity of periodontal disease (Buset et al., 2016). All but one (Andersson, Hakeberg, Karlberg, & Ostberg, 2010) of the included 37 studies were based in hospital or specialist practices. The effect of treatment of periodontal disease on quality of life was reviewed by Shanbhag, Dahiya, and Croucher (2012), who reviewed 11 studies with sample sizes ranging from 32 to 183 patients, and concluded that periodontal treatment can improve quality of life (Shanbhag et al., 2012). The authors reported an improvement in all domains of oral health-related quality of life following periodontal therapy.

All of the 11 trials (seven prospective case-series or uncontrolled studies, one controlled study and three RCTs with a total of 639 participants) reviewed in this article were conducted in hospital or specialist practices.

There are several important strengths of this study. Firstly, we were able to analyse data from a large, general practice-based sample, resulting in precise estimates generalizable to a setting in which a large number of patients receive care. Secondly, we were able to adjust for several important potential confounders.

Several limitations of this study need to be considered. Firstly, the study used data from practices that were part of a dental payment capitation scheme. As a result, this study likely included patients with above average oral health motivation and compliance. For example, only 5% of patients were classed as irregular attenders (Table 1). Therefore, results may not be generalizable to the entire UK population. Secondly, as this is a pragmatic study using data collected as part of routine clinical care, some measurement error and misclassification may arise due to lack of calibration, use of different periodontal probes and use of routine radiographs. In this respect, assessment of oral hygiene, dental attendance and salivary flow may be particularly subjective. These were included in the statistical model as they are important confounders of the relationship between periodontitis and PROs studied here. However, a sensitivity analysis conducted, omitting these three variables from the model, resulted in findings that were not appreciably different. Thirdly, there is a potential bias due to unmeasured and residual confounding. This is likely to be most relevant for common risk factors for caries and periodontal disease. For example, we had no data on some potential confounders, such as socio-economic status. However, the fact that this is a somewhat more homogeneous population in terms of oral health behaviours and socio-economic status reduces the risk of confounding relative to the general population. Our sensitivity analysis, limited to patients who needed no restorations, showed similar results.

In conclusion, our results demonstrate that in a large, non-specialist, general practice-based population, worse periodontal health as measured by increased probing depth, ABL and BoP is associated with adverse PROs including pain, dietary restriction and unhappiness with appearance in a dose-dependent fashion. Hence, prevention and successful management of periodontitis may have direct benefits on PROs.

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CONFLICT OF INTEREST

Mr Michael Busby acts as a Denplan advisor, and Prof Iain Chapple acts as an advisor to Oral Health Innovations, the licence holder for DEPPA in the UK and Ireland.

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Association between periodontitis and mortality in stages 3–5 chronic kidney disease: NHANES III and linked mortality study

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Abstract

Introduction: Periodontitis may add to the systemic inflammatory burden in individuals with chronic kidney disease (CKD), thereby contributing to an increased mortality rate. This study aimed to determine the association between periodontitis and mortality rate (all-cause and cardiovascular disease-related) in individuals with stage 3–5 CKD, hitherto referred to as "CKD".

Methods: Survival analysis was carried out using the Third National Health and Nutrition Examination Survey (NHANES III) and linked mortality data. Cox proportional hazards regression was employed to assess the association between periodontitis and mortality, in individuals with CKD. This association was compared with the association between mortality and traditional risk factors in CKD mortality (diabetes, hypertension and smoking).

Results: Of the 13,784 participants eligible for analysis in NHANES III, 861 (6%) had CKD. The median follow-up for this cohort was 14.3 years. Adjusting for confounders, the 10-year all-cause mortality rate for individuals with CKD increased from 32% (95% CI: 29–35%) to 41% (36–47%) with the addition of periodontitis. For diabetes, the 10-year all-cause mortality rate increased to 43% (38–49%).

Conclusion: There is a strong, association between periodontitis and increased mortality in individuals with CKD. Sources of chronic systemic inflammation (including periodontitis) may be important contributors to mortality in patients with CKD.

*Contributed equally to this publication.

Key words: chronic kidney disease; NHANES; periodontitis; survival

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Chronic kidney disease (CKD) affects between 8 and 13% of the global population (Jha et al. 2013) and is associated with increased morbidity and mortality. Cardiovascular disease (CVD)-related events are the main cause of mortality in patients with CKD (Go et al. 2004) and systemic inflammation is recog-

Conflict of interest and source of funding statement

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nized as a non-traditional risk factor associated with increased risk of CVD events in such patients (Menon et al. 2005).

Severe periodontitis is the sixth most common human disease (Kassebaum et al. 2014) causing micro-ulceration of the investing sulcular and pocket lining epithelium of affected teeth. The estimated surface area of this ulcerated epithelium approximates 40 cm^2 in severe disease (Nesse et al. 2008). Consequently, individuals with periodontitis have elevated systemic markers of acute-phase (C-reactive protein/CRP, Interleukin-6/IL-6) and oxidative stress (peripheral neutrophil hyperactivity) responses. This has potential systemic consequences and co-morbid effects that have been implicated in other disease processes such as diabetes and CVD (Chapple & Genco 2013, Tonetti & VanDyke 2013).

We have reported that patients with CKD have an increase in prevalence of periodontitis compared with community dwelling adults (Sharma et al. 2014). This finding is supported by a recent systematic review, reporting an association between periodontitis and CKD in several populations with a combined odds-ratio (OR) of 1.65 (95% confidence interval/CI: 1.53–2.01) (Chambrone et al. 2013).

Successful periodontal treatment can reduce levels of systemic inflammation in patients with and without CKD (D'Aiuto et al. 2004, Vilela et al. 2011, Siribamrungwong et al. 2014, Fang et al. 2015). However, the only investigations into associations between periodontitis and mortality rates (all-cause and CVD) in patients with CKD have involved relatively small numbers of patients (ranging from 122–253 patients) on haemodialysis and with a short follow-up period (ranging from 18 months to 6 years) (Kshirsagar et al. 2009, Chen et al. 2011, de Souza et al. 2014). In epidemiological studies reporting mortality outcomes from non-CKD populations some, (Garcia et al. 1998, Xu & Lu 2011, Linden et al. 2012) but not all, (Avlund et al. 2009, Kim et al. 2013) report a significant positive association between periodontitis and an increased mortality rate.

The aim of this study was to evaluate the association between periodontitis and other traditional risk factors (diabetes, hypertension and smoking status) and mortality (all-cause and CVD) in individuals with stage 3–5 CKD, compared to those without using robust, large-scale, population-based data.

Materials and Methods

Data source

Data were derived from the Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994), a representative survey of the civilian, non-institutionalized US population conducted by the National Center for Health Statistics (NCHS) of the Center for Disease Control and Prevention. Details of the survey design and methodology are available elsewhere (NCHS, 2006a). Briefly, individuals were interviewed at home, then invited to a mobile examination centre (MEC) for further interviews, tests and examinations.

Assessment of periodontal health

Details of the oral health component of NHANES III are published elsewhere (Drury et al. 1996). Briefly, detailed periodontal measurements were taken from volunteers aged 13 and over. The teeth were divided into two maxillary and two mandibular halves and measurements were taken from two sites per tooth (mid-buccal and mesio-buccal) for all teeth (excluding third molars) in one randomly chosen upper and lower quadrant. These measurements included periodontal probing depth (PPD), gingival recession and bleeding on probing (BOP). Clinical attachment loss (CAL) was calculated as the sum of the recession and PPD. Individuals receiving renal replacement therapy (through dialysis or kidney transplant) were excluded from periodontal examination.

Periodontitis was defined using the 2007 CDC/AAP (Centre for Disease Control and Prevention/American Academy of Periodontology) classification (Page & Eke 2007). In addition, continuous periodontal parameters were also employed such as mean PPD, mean CAL, cumulative periodontal probing depth (C-PPD), number of teeth present and proportion of sites that bled upon probing. Cumulative PPD was calculated as the sum of the maximum probing pocket depths ≥4 mm of each tooth and as such is a surrogate measure of the potential extent of biofilm exposed connective tissues (Dietrich et al. 2008). Edentulous individuals were included in the analyses but formed a group distinct from individuals with periodontitis.

Assessment of CKD

The serum creatinine levels recorded in the NHANES III survey were recalibrated to be traceable to an isotope-derived mass spectroscopy method using the equation below (NCHS, 2006b):

$$\text{Standardized creatinine} = (0.960 \times \text{serum creatinine}) - 0.18$$

Age, sex, ethnicity and standardized serum creatinine were incorporated in the CKD Epidemiology Collaboration (CKD-EPI) equation to calculate estimated glomerular filtration rate (eGFR) (Levey et al. 2009). This equation improves mortality risk stratification in individuals with CKD compared with the Modification of Diet in renal Disease (MDRD) equation (Shafi et al. 2012). Based on an eGFR<60 ml/min/1.73 m^2, individuals were classified as having stage 3–5 CKD, hitherto referred to as "CKD".

Urinary albumin and creatinine levels were employed to calculate the albumin-creatinine ratio (ACR). Details of the laboratory assays can be found elsewhere (NCHS, 2006b). Albuminuria was classified as ACR <30 mg/g; ACR ≥ 30 mg/g and <300 mg/g; and ACR ≥ 300 mg/g.

Assessment of traditional risk factors

Individuals were classed as hypertensive if their mean (of three consecutive measurements) systolic blood pressure (BP) was ≥140 mmHg or mean diastolic BP was ≥90 mmHg.

Individuals were classed as diabetic by self-reporting (excluding gestational diabetes) or if their glycated haemoglobin (HbA1C) was ≥6.5%.

Individuals' smoking status was determined from self-reporting and

classified into current, former or never smokers (cigarettes only).

Covariate data

Data on covariates employed in the statistical analyses included information on age, sex, ethnicity (Non-Hispanic White, Non-Hispanic Black, Mexican American or Other), alcohol consumption (never, not in last year, between 0–14 drinks/week, more than 14 drinks/week) and self-reported history of previous cardiovascular events (stroke, heart attack or heart failure). Pulse pressure was calculated as the difference between the mean systolic and diastolic BP. Self- reported measures of socio-economic status (household income, marital status and educational attainment) were coded as follows. Household income (less than $20,000 or $20,000 or more); marital status (married or living as married, never married, divorced or separated or widowed); educational attainment (less than high school, high school diploma or more than high school). Body mass index (BMI) was coded as a categorical variable with BMI <18.5 kg/m^2 as underweight; ≥ 18.5 kg/m^2 and <25 kg/m^2 as normal; ≥ 25 kg/m^2 and <30 kg/m^2 as overweight and ≥ 30 kg/m^2 as obese. Laboratory tests including serum cholesterol (total and high-density lipoprotein/HDL) were performed. Serum cholesterol levels were classified into binary variables (total serum cholesterol ≥ 24 mg/L or <24 mg/L and serum HDL cholesterol ≤ 3.5 mg/L or >3.5 mg/L). Physical activity was self-reported by individuals and reclassified as "recommended or more" if they reported moderate activity five or more times a week or vigorous activity three or more times a week. Physical activity was also classified as "recommended or more" if individuals reported moderate physical activity four or more times a week and vigorous activity one or more times a week or reported moderate activity three or more times a week and vigorous activity two or more times a week. Individuals' physical activity was classified as "none" if they reported no leisure time physical activities. Individuals who reported some level of physical activity but less than recommended were classed as "less than recommended" (Beddhu et al. 2009).

Mortality data

The NCHS provide mortality data for NHANES III participants up to 31st December 2006, linked by probabilistic record matching with the National Death Index (NDI). The publicly available data set contains information on the mortality status of individuals aged 17 years or older. For individuals who are classified as "assumed deceased", information is available on 113 underlying cause of death categories, based on the ninth and tenth revisions of the International Classification of Diseases (ICD-9 and ICD-10). CVD mortality was limited to cases where the underlying cause of death was coded between 53 and 75 (inclusive) (Anderson et al. 2001). Details of the linked mortality data have been published elsewhere (NCHS, 2010).

Statistical analyses

Analyses performed followed guidelines for NHANES III (NCHS, 1996), accounting for the complex survey design and sampling weights to yield estimates generalizable to the US population. Differences in categorical and continuous data were assessed for statistical significance using Pearson Chi-square, *t*-test, Fisher's exact test and analysis of variance (ANOVA) as appropriate. Cox proportional hazards (PH) regression models were fitted to evaluate the association between periodontal status, traditional risk factors (diabetes, hypertension and smoking status) and all-cause and CVD mortality, independent of potential confounders. The fully adjusted model adjusted for age, sex, ethnicity, CKD status, periodontal status, diabetic status, hypertensive status, smoking status, pulse pressure, history of CVD (heart attack or stroke or heart failure), alcohol consumption, ACR, hypercholesterolaemia and low-HDL, BMI, physical activity and measures of socio-economic status (household income, marital status and educational attainment). The PH assumption was tested using Schoenfeld residuals, scaled Schoenfeld residuals and graphical methods. Variables were chosen to minimize missing data. Any individuals with missing covariate data were not included in the analyses (listwise deletion). Thus out of a possible 13,784 individuals eligible for analyses, 1379 (10%) individuals were excluded due to incomplete covariate data (Table S1).

We considered the effect measure modification of mortality (all-cause and cardiovascular) in individuals with CKD according to their periodontal health status. We conducted formal tests of interaction between periodontal variables and CKD case definition by entering interaction terms in the model. Further formal tests of interactions between CKD, periodontitis or edentulism and age, gender and ethnicity were also carried out.

Analyses were carried out using Stata/IC version 12.1 (StataCorp LP, College Station, TX, USA).

Results

Description of whole population and subpopulations

We analysed data from individuals in NHANES III aged 20 years and older with complete data on serum creatinine, periodontal status and mortality follow-up ($n = 13{,}784$) and with a median follow-up time of 14.3 years (mean 13.5 years, range 1 month–18.2 years). Of the 13,794 individuals included in the analyses, 861 (6%) were classified as CKD and 12,923 as non-CKD. Individuals with CKD were more likely to be older, have different ethnic and socio-economic mix, non-smokers (never or ex- smokers), diabetic, hypertensive, with higher total serum cholesterol and lower levels of serum HDL, report lower levels of physical activity and consume less alcohol and report a history of CVD (stroke, heart attack and congestive heart failure) compared to those without CKD. Individuals with CKD were more likely to suffer from periodontitis (or be edentulous) and have fewer teeth compared to individuals without CKD. When examining continuous variables of periodontal health, patients with CKD were more likely to have a greater mean CAL and greater BOP (Table 1).

Among individuals with CKD, those with periodontitis were more likely to be older, of non-white eth-

Table 1. Demographics of study population divided by CKD and periodontal status. Values are percentages (standard error) unless stated

Characteristics	No CKD (eGFR ≥ 60 ml/min/1.73 m^2) N = 12,923			CKD (eGFR<60 ml/min/1.73 m^2) N = 861			*p*-values*	*p*-values†
	Periodontal status			Periodontal status				
	Healthy n = 10,089 (78%)	Periodontitis n = 1637 (13%)	Edentulous n = 1197 (9%)	Healthy n = 357 (41%)	Periodontitis n = 172 (20%)	Edentulous n = 332 (39%)		
Assumed deceased							<0.001	<0.001
All-cause mortality	11	35	56	70	88	87		
Cardiovascular mortality	4	14	23	39	48	44		
Mean (SE) age (years)	41 (0.2)	55 (0.4)	67 (0.4)	73 (0.6)	75 (0.7)	77 (0.5)	<0.001	0.03
Female	55 (0.4)	37 (1.2)	54 (1.4)	54 (2.6)	45 (3.8)	55 (2.7)	0.95	0.07
Ethnicity							<0.001	<0.001
Non-Hispanic White	37 (0.5)	32 (1.2)	60 (1.4)	72 (2.4)	54 (3.8)	70 (2.5)		
Non-Hispanic Black	27 (0.4)	34 (1.2)	23 (1.2)	16 (2.0)	27 (3.4)	20 (2.2)		
Mexican American	31 (0.4)	30 (1.1)	13 (1.0)	8 (1.4)	17 (2.9)	6 (1.3)		
Other	4 (0.2)	3 (0.4)	4 (0.5)	3 (1.0)	2 (1.0)	3 (1.0)		
Current Smoker	24 (0.4)	39 (1.2)	29 (1.3)	8 (1.4)	13 (2.6)	12 (1.8)	<0.001	0.03
Diabetic	6.7 (0.2)	17.7 (0.9)	20.3 (1.2)	21.4 (2.2)	29.7 (3.5)	27.1 (2.4)	<0.001	0.04
Hypertensive	16 (0.3)	33 (1.2)	44 (1.4)	54 (2.6)	65 (3.6)	59 (2.7)	<0.001	0.01
Alcohol consumption							<0.001	0.07
Never	17 (0.4)	16 (0.9)	25 (1.3)	22 (2.2)	27 (3.5)	35 (2.6)		
Not in last year	33 (0.5)	40 (1.2)	49 (1.5)	47 (2.7)	51 (3.9)	53 (2.8)		
0–14 drinks/week	44 (0.5)	36 (1.2)	22 (1.2)	31 (2.5)	20 (3.1)	11 (1.8)		
>14 drinks/week	6 (0.2)	8 (0.7)	4 (0.5)	0.6 (0.4)	1 (0.8)	1 (0.5)		
History of stroke	1.1 (0.1)	3.5 (0.5)	4.8 (0.6)	9 (1.5)	10 (2.3)	14 (1.9)	<0.001	0.75
History of heart attack	1.9 (0.1)	5.1 (0.5)	7.8 (0.8)	12 (1.7)	15 (2.8)	17 (2.0)	<0.001	0.31
History of congestive heart failure	1.5 (0.1)	3.1 (0.4)	5.1 (0.6)	9 (1.5)	15 (2.7)	11 (1.7)	<0.001	0.07
Mean (SE) eGFR (ml/min/1.73 m^2)	107 (0.2)	96 (0.5)	87 (0.4)	49 (0.5)	47 (0.9)	48 (0.5)	<0.001	0.005
Mean (SE) ACR (mg/g)	19.8 (1.3)	53.5 (10.0)	63.2 (12.5)	211 (63.6)	276 (74.2)	320 (82.7)	<0.001	0.54
Mean (SE) BMI (kg/m^2)	27.1 (0.06)	27.6 (0.15)	27.0 (0.16)	27.5 (0.27)	26.8 (0.40)	26.5 (0.27)	0.27	0.16
Total serum cholesterol (≥24 mg/L)	25 (0.4)	35 (1.2)	44 (1.4)	50 (2.6)	47 (3.8)	48 (2.8)	<0.001	0.512
HDL cholesterol (≤3.5 mg/L)	11 (0.3)	17 (0.9)	13 (1.0)	18 (2.0)	16 (2.9)	20 (2.2)	<0.001	0.767
Pulse pressure (mm Hg)	47 (0.1)	56 (0.4)	63 (0.6)	68 (1.1)	74 (1.5)	74 (1.1)	<0.001	0.002
Marital status							<0.001	0.53
Married (or living as married)	63 (0.5)	65 (1.2)	57 (1.4)	56 (2.6)	51 (3.8)	45 (2.7)		
Never married	21 (0.4)	9 (0.7)	5 (0.6)	5 (1.2)	5 (1.6)	2 (0.8)		
Divorced or separated	11 (0.3)	13 (0.8)	11 (0.9)	8 (1.4)	11 (2.4)	5 (1.2)		
Widowed	5 (0.2)	12 (0.8)	26 (1.3)	31 (2.5)	33 (3.6)	48 (2.7)		
Household income (<$20,000)	43 (0.5)	57 (1.2)	66 (1.4)	55 (2.7)	68 (3.6)	72 (2.5)	<0.001	0.004
Educational status							<0.001	<0.001
Less Than High School	33 (0.5)	54 (1.2)	63 (1.4)	40 (2.6)	62 (3.7)	75 (2.4)		
High School Diploma (including GED)	33 (0.5)	28 (1.1)	26 (1.3)	30 (2.4)	22 (3.2)	16 (2.0)		
More Than High School	34 (0.5)	18 (1.0)	11 (0.9)	30 (2.5)	16 (2.8)	9 (1.6)		
Physical activity							<0.001	0.15
None	18 (0.4)	25 (1.1)	30 (1.3)	25 (2.3)	33 (3.6)	39 (2.7)		
Less than recommended	44 (0.5)	43 (1.2)	36 (1.4)	36 (2.5)	34 (3.6)	29 (2.5)		
Recommended or more	38 (0.5)	32 (1.2)	34 (1.4)	39 (2.6)	33 (3.6)	32 (2.7)		
Mean (SE) Teeth Present	26 (0.1)	21 (0.2)	0	18 (0.4)	17 (0.5)	0	<0.001	0.10
Mean (SE) CAL (mm)	0.9 (0.008)	3.1 (0.04)	N/A	1.6 (0.06)	3.6 (0.11)	N/A	<0.001	<0.001
Mean (SE) PPD (mm)	1.5 (0.004)	2.2 (0.02)	N/A	1.4 (0.2)	1.9 (0.6)	N/A	0.77	<0.001
Mean (SE) C-PPD (mm)	1.8 (0.04)	11.4 (0.3)	N/A	1.0 (0.2)	7.1 (0.8)	N/A	0.53	<0.001
BOP	11 (0.2)	18 (0.5)	N/A	14 (1.1)	19 (1.8)	N/A	<0.001	0.015

ACR, albumin-creatinine ratio; BMI, body mass index; BOP, percentage of sites that bleed on probing; CAL, clinical attachment loss; C-PPD, cumulative probing depth; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; PPD, periodontal probing depth.

*Comparing no CKD and CKD.

†Within individuals with CKD, comparing healthy and periodontitis.

nicity, current smokers, diabetic and hypertensive and have a lower eGFR compared to periodontally healthy individuals. These individuals also had lower household incomes and educational attainments compared to periodontally healthy individuals. Periodontally healthy individuals were similar to those with periodontitis in terms of their sex, alcohol consumption, marital status, physical activity, history of CVD events and BMI (Table 1).

All-cause mortality

After adjusting for covariates, individuals with CKD had a 44% (95% CI: 28–63%) increased rate of all-cause mortality compared to those without CKD (Table 2). Individuals with periodontitis had a 36% (22–51%) increased rate of all-cause mortality compared to individuals who were periodontally healthy.

The association between periodontitis and all-cause mortality was similar between individuals with or without CKD (p-value for interaction = 0.57). Similarly, the associations between CKD and all-cause mortality did not vary by age, sex or diabetes status (p-values for interaction 0.14, 0.99 and 0.09 respectively). Furthermore, the association between periodontitis and all-cause mortality did not vary by age, gender or diabetes status (p-values for interaction 0.73, 0.51 and 0.51 respectively). In edentulous individuals, there was a significant difference in all-cause mortality by age. Edentulous individuals under the age of 65 had a significantly increased rate of all-cause mortality compared to edentulous individuals 65 years and older, hazard ratio (HR) 1.85 (1.41–2.44) and 1.18 (1.04–1.33) respectively (Tables S2, S3 and Fig. S1).

For continuous measures of periodontitis in fully adjusted models, an increased mortality rate was seen with worsening periodontal health in a dose-dependent manner. For example a 1 mm increase in mean PPD was associated with a 17% (6–28%) increase in incident rate of all-cause mortality (Table 2). Edentulousness was associated with a 32% (17–50%) increased rate of all-cause mortality compared with periodontally healthy dentate individuals.

Diabetes (HR 1.41; 1.27–1.57), hypertension (HR 1.06; 0.93–1.20) and current smoking (HR 2.12; 1.82–2.48) were associated with an increased rate of all-cause mortality although this increase was not significant for hypertension (Table 2).

The 10-year all-cause mortality for individuals with CKD (but without periodontitis or other traditional risk factors) was 32% (29–35%). Addition of periodontitis to the risk profile increased 10-year mortality to 41% (36–47%). This increase in mortality was comparable with that seen in individuals with CKD who had diabetes instead of periodontitis (43%; 38–49%). A similar cumulative effect on mortality is seen with periodontitis and other traditional risk factors (Table 3). These estimates are based on the demographic features of individuals with CKD within NHANES III (e.g. a mean age of 73 years). Estimated survival curves for individuals with CKD and different risk factor profiles is given in Fig. 1.

Cardiovascular mortality

After adjusting for covariates, individuals with CKD had a 60% (32–95%) increased rate of CVD mortality compared to those without CKD (Table 2), independent of confounders specified. Individuals with periodontitis had a 38% (16–65%) increased rate of CVD mortality compared to individuals who were periodontally healthy. The association between periodontitis and CVD

Table 2. Results from Cox proportional hazards regression analyses for all-cause and cardiovascular mortality using an age and sex-adjusted and a fully adjusted model

	Hazard Ratio (95% CI) of All-cause mortality		Hazard Ratio (95% CI) of Cardiovascular mortality	
	Age adjusted	Fully adjusted	Age adjusted	Fully adjusted
CKD	1.58 (1.39–1.80)	1.44 (1.28–1.63)	1.81 (1.55–2.14)	1.60 (1.32–1.95)
Periodontal status				
Healthy	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
Periodontitis	1.78 (1.59–2.00)	1.36 (1.22–1.51)	1.79 (1.52–2.11)	1.38 (1.16–1.65)
Edentulous	1.83 (1.64–2.05)	1.32 (1.17–1.50)	1.47 (1.24–1.73)	1.05 (0.85–1.29)
Continuous periodontal variables				
Mean PPD (per mm)	1.48 (1.35–1.62)	1.17 (1.06–1.28)	1.51 (1.34–1.72)	1.21 (1.05–1.40)
Mean CAL (per mm)	1.20 (1.16–1.25)	1.09 (1.05–1.14)	1.16 (1.11–1.22)	1.05 (0.99–1.12)
C-PPD (per 10 mm)	1.29 (1.20–1.38)	1.08 (1.01–1.17)	1.35 (1.19–1.54)	1.16 (0.99–1.35)
BOP (per 10%)	1.10 (1.07–1.13)	1.05 (1.02–1.08)	1.10 (1.06–1.13)	1.05 (1.01–1.09)
Diabetes	1.85 (1.63–2.10)	1.41 (1.27–1.57)	2.00 (1.71–2.35)	1.45 (1.24–1.70)
Hypertension	1.28 (1.15–1.43)	1.06 (0.93–1.20)	1.52 (1.31–1.77)	1.32 (1.06–1.63)
Smoking status				
Never	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
Former	1.41 (1.23–1.60)	1.25 (1.09–1.43)	1.32 (1.11–1.56)	1.18 (0.98–1.42)
Current	2.70 (2.35–3.09)	2.12 (1.82–2.48)	2.44 (2.05–2.91)	2.10 (1.69–2.62)

BOP, proportion of sites that bleed on probing; CAL, clinical attachment loss; CKD, chronic kidney disease; C-PPD, cumulative periodontal probing depth; PPD, periodontal probing depth.
Fully adjusted model adjusted for age, sex, ethnicity, CKD status, periodontal status, diabetic status, hypertensive status, smoking status, pulse pressure, history of CVD (heart attack or stroke or heart failure), alcohol consumption, ACR, hypercholesterolaemia and low-HDL, BMI, physical activity and measures of socio-economic status (household income, marital status and educational attainment).

Table 3. Ten-year all-cause mortality (percentages) of individuals with CKD by risk factors (along with the addition of periodontitis to the risk factor)

Risk factor	10-year all-cause mortality (95% CI) without periodontitis	10-year all-cause mortality (95% CI) with periodontitis
CKD	32% (29–35%)	41% (36–47%)
CKD + Diabetes	43% (38–49%)	55% (47–63%)
CKD + Hypertension	34% (29–39%)	44% (37–52%)
CKD + Smoking	58% (51–65%)	71% (62–79%)

Fully adjusted model adjusted for age, sex, ethnicity, CKD status, periodontal status, diabetic status, hypertensive status, smoking status, pulse pressure, history of CVD (heart attack or stroke or heart failure), alcohol consumption, ACR, hypercholesterolaemia and low-HDL, BMI, physical activity and measures of socio-economic status (household income, marital status and educational attainment).

mortality was similar between individuals with or without CKD (p-value for interaction = 0.27). The associations between CKD and CVD mortality did not vary by age, sex or diabetes status (p-values for interaction 0.39, 0.82 and 0.34 respectively). The association between periodontitis and CVD mortality did not vary by gender or diabetes status (p-values for interaction 0.77 and 0.17 respectively). There was a trend in patients with CKD and periodontitis to have an increased HR of CVD mortality if they were under the age of 65 compared with 65 and over but this was not significant. In edentulous individuals, there was a significant difference in CVD mortality by age. Edentulous individuals under the age of 65 having a significantly increased rate of CVD mortality, HR 2.03 (1.31–3.13), compared to edentulous individuals 65 years and older who had comparable rates of CVD mortality compared to periodontally healthy individuals, HR 0.89 (0.71–1.10) (Tables S4, S5 and Fig. S2).

For continuous measures of periodontal health, mean PPD and percentage of sites that bleed on probing were associated with a statistically significant increase in the rate of CVD mortality (Table 2). Edentulous and periodontally healthy dentate individuals had comparable rates of CVD mortality (Table 2).

Diabetes (HR 1.45; 1.24–1.70), hypertension (HR 1.32; 1.06–1.63) and current smoking (HR 2.10; 1.69–2.62) were associated with an increased rate of CVD mortality (Table 2).

The 10-year CVD mortality for individuals with CKD (and combinations of risk factors) highlights the similarity in the magnitude of increase in CVD mortality associated with diabetes (24%; 19–30%) compared with periodontitis (22%; 19–27%) (Table 4). Estimated CVD survival for individuals with CKD and

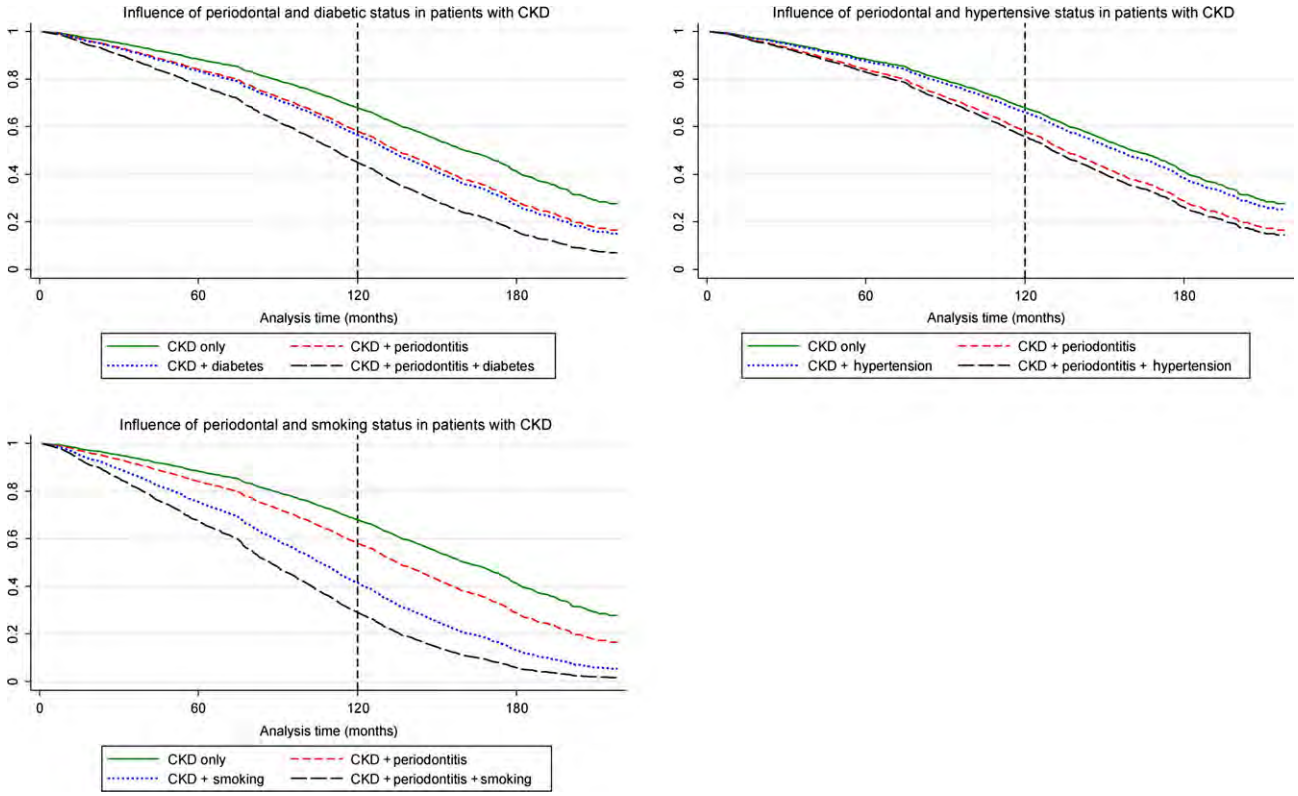

Fig. 1. For all-cause mortality. Cox proportional hazard regression graphs (adjusting for age, sex, ethnicity, pulse pressure, history of CVD, alcohol consumption, ACR, hypercholesterolaemia and low-HDL, BMI, physical activity, household income, marital status and educational attainment) of survival in patients with CKD stratified using periodontitis and other traditional risk factors (diabetes, hypertension and smoking). The reference lines indicate 10 year survival.

Table 4. Ten-year CVD mortality (percentages) of individuals with CKD by risk factors (along with the addition of periodontitis to the risk factor)

Risk factor	10-year CVD mortality (95% CI) without periodontitis	10-year CVD mortality (95% CI) with periodontitis
CKD	16% (14–19%)	22% (19–27%)
CKD + Diabetes	24% (19–30%)	32% (27–39%)
CKD + Hypertension	21% (16–28%)	29% (22–37%)
CKD + Smoking	33% (24–44%)	43% (32–56%)

Fully adjusted model adjusted for age, sex, ethnicity, CKD status, periodontal status, diabetic status, hypertensive status, smoking status, pulse pressure, history of CVD (heart attack or stroke or heart failure), alcohol consumption, ACR, hypercholesterolaemia and low-HDL, BMI, physical activity and measures of socio-economic status (household income, marital status and educational attainment).

different risk factor profiles is given in Fig. 2.

Discussion

In this large cohort, representative of the US population from which it was derived, CKD was associated with increased rates of all-cause mortality and CVD mortality, independent of periodontitis, traditional risk factors and other confounders. Periodontitis was associated with increased rates of all-cause and CVD mortality comparable with, but independent of, that associated with diabetes (Tables 2–4; Figs 1 and 2). There was an increased rate of all-cause mortality but not CVD mortality in edentulous individuals with CKD compared with periodontally healthy dentate individuals. The association between edentulousness and CVD mortality was significant in a subgroup of edentulous individuals under the age of 65. Given the high prevalence of chronic periodontitis in patients with CKD (Chambrone et al. 2013), our results suggest that periodontitis may be an important non-traditional risk factor for CVD and all-cause mortality in these patients, and interestingly contributing to the increased risk to a similar extent as diabetes.

The strengths of this study are its large population-based sampling with robust sampling methodology which allow the results from this analysis to be generalized to the US population. The detailed clinical, demographic and anthropomorphic data collected allows for many of the known covariates to be accounted for in the Cox proportional hazards regression model, generating more accurate point estimates. The length of follow-up for this study is its final strength and allows for the pragmatic assessment of long term, hard outcomes (all-cause and CVD mortality). The

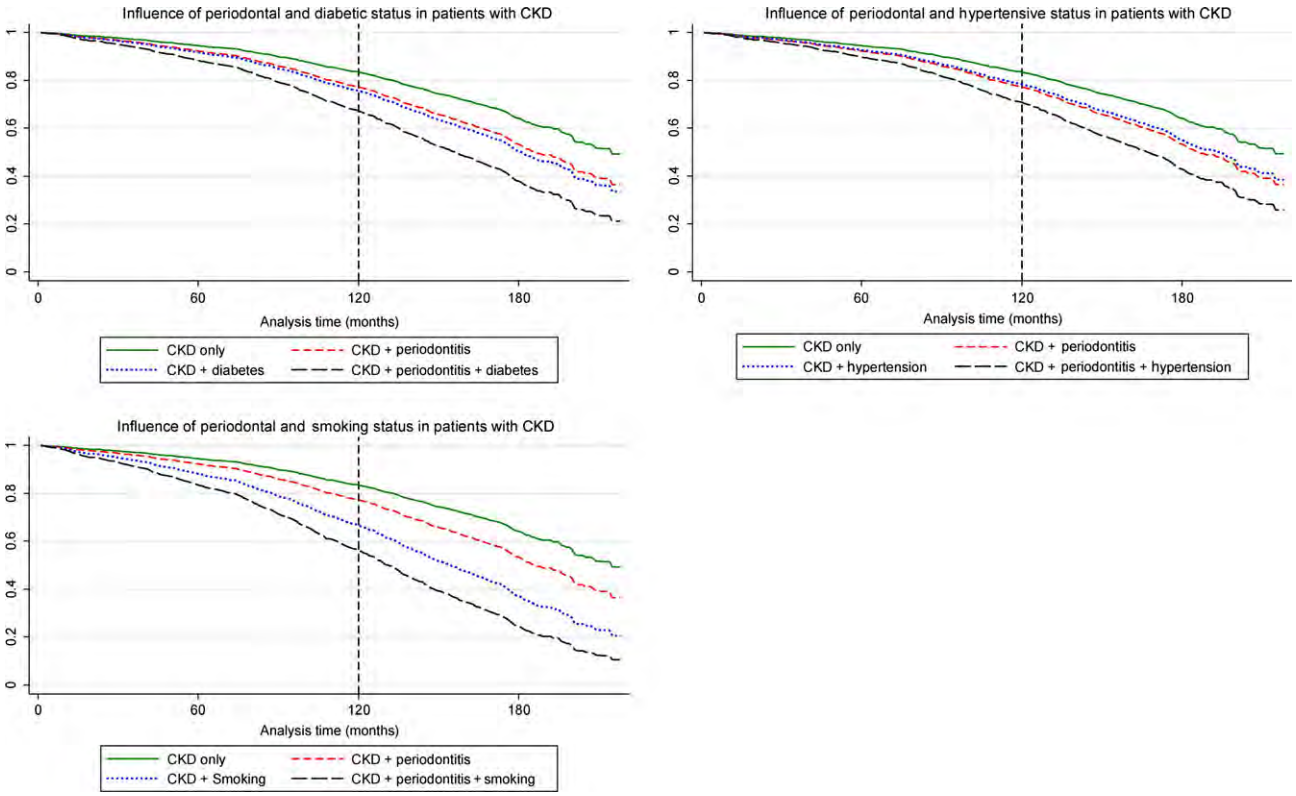

Fig. 2. For cardiovascular mortality. Cox proportional hazard regression graphs (adjusting for age, sex, ethnicity, pulse pressure, history of CVD, alcohol consumption, ACR, hypercholesterolaemia and low-HDL, BMI, physical activity, household income, marital status and educational attainment) of survival in patients with CKD stratified using periodontitis and other traditional risk factors (diabetes, hypertension and smoking). The reference lines indicate 10-year survival.

limitations of this study include the lack of longitudinal examination of individuals. Unfortunately, in NHANES, the longitudinal data is limited to the mortality status of patients derived from the National Death Index. Data on variables were only gathered at inception and therefore changes in variables (periodontal, diabetes, smoking status, etc) are not ascertainable. Analyses were carried out on the assumption that characteristics did not change between inception and time to death or censoring. Some individuals with periodontitis are likely to have received treatment and/or lost teeth during follow-up, resulting in disease misclassification over time. Furthermore, periodontal measurements from NHANES III are known to underestimate the prevalence of periodontitis by 13.4% (absolute) or 60% (relative) (Eke et al. 2010). The results of this study may therefore under-estimate the association between periodontitis and mortality in CKD. Also, as with any multivariable regression analysis, the issue of residual confounding from inaccurate measurement or categorization of variables or confounding from variables not included in the analysis cannot be ruled out.

Previous studies investigating the link between mortality and periodontitis in patients with CKD have done so in patients on haemodialysis (Kshirsagar et al. 2009, Chen et al. 2011, de Souza et al. 2014). Apart from the small sample sizes (122–253 patients) and shorter follow-up period (18 months to 6 years), these studies differed significantly from the present analysis as individuals receiving RRT (through chronic dialysis or a functioning kidney transplant) were not included in the present analysis (RRT was an exclusion criteria for periodontal examination in NHANES III). Hence, even though these studies demonstrate an association between periodontitis and mortality, thereby lending support to the current findings, the results cannot be directly compared.

A putative mechanism for a possible link between periodontitis and increased all-cause and CVD mortality is via the increased systemic acute-phase and oxidative stress burden. This increased burden is seen in individuals with periodontitis and CKD (Ioannidou et al. 2011) and individuals with periodontitis who do not have CKD (D'Aiuto et al. 2004, Chapple & Genco 2013). Increased systemic inflammatory and oxidative stress burdens increase the incidence of CVD events in patients with CKD (Arici & Walls 2001, Mathew et al. 2008, Li et al. 2015). This mechanism is supported by the association demonstrated here between increased risk of CVD mortality and measures of active periodontitis (periodontitis case definition, mean PPD and BOP), as opposed to measures of historical periodontitis (edentulousness and mean CAL), where there was a lack of association (Table 2). However, at least part of the association between periodontitis and CVD may also be due to common risk factors such as smoking and diabetes (Dietrich et al. 2008, Mucci et al. 2009). The increase in all-cause mortality in edentulous individuals compared to periodontally healthy dentate individuals, as reported here and also by other investigators in non-CKD cohorts (Brown 2009), may be due to several factors. Patients are rendered edentulous for a variety of reasons including periodontitis, with approximately 50% of teeth being extracted due to periodontal disease (Phipps & Stevens 1995). As approximately half of all tooth extractions are for reasons other than periodontal disease, edentulousness may act as a surrogate marker of general health attitudes and/or behaviours, limited healthcare access or other socio-economic measures (Joshipura & Ritchie 2005). This might also explain the association between edentulousness and CVD mortality in patients under the age of 65 who might have such characteristics and attitudes towards healthcare that render them edentulous before the age of 65.

The biological mechanisms underpinning the relationship between periodontitis and increased mortality in individuals with CKD form a promising area of research and may produce mechanistic targets leading to risk stratification and novel interventions. Ongoing longitudinal studies (Stringer et al. 2013) investigating large cohorts of patients with pre-dialysis CKD may provide confirmation of this association and shed light upon explanatory mechanisms. Successful treatment of periodontitis has been shown to improve surrogate markers of CVD risk, including serum markers of systemic inflammation (CRP, IL-6) (D'Aiuto et al. 2004), endothelial function as measured by flow-mediated dilatation (FMD) and endothelial-activation markers such as soluble E-selectin and von Willebrand factor (Tonetti et al. 2007). Two randomized controlled trials of periodontal interventions in patients with CKD have been carried out but limited to cohorts of haemodialysis patients. These have produced conflicting results either not demonstrating changes in inflammatory markers following periodontal intervention (Wehmeyer et al. 2013) or demonstrating that significant reductions in inflammatory markers can be achieved following periodontal therapy (Fang et al. 2015). Currently, patients with CKD are managed to strict targets concerning glycaemic control (diabetes) and control of hypertension and smoking cessation to improve outcomes. If a causal link is established between periodontitis and increased rates of adverse outcomes in CKD patients, then establishing and maintaining periodontal health may become an important part of the care pathway of patients with CKD.

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Disclosures

None.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Cox PH regression graphs for all-cause mortality in individuals with periodontitis/edentulism and CKD by age category

Figure S2 Cox PH regression graphs for CVD mortality in individuals with periodontitis/edentulism and CKD by age category.

Table S1 Numbers of participants (and percentage) with missing data in variables included in statistical model.

Table S2 Exploring the interactions between CKD (stage 3–5), periodontal variables and age, gender and diabetes status for all-cause mortality.

Table S3 Hazard ratios (95% CI) and 10-year survival (95% CI) of all-cause mortality by subgroups of age (<65 years of age and ≥65 years of age).

Table S4 Exploring the interactions between CKD (stage 3–5), periodontal variables and age, gender and diabetes status for CVD mortality.

Table S5 Hazard ratios (95% CI) and 10-year survival (95% CI) of CVD mortality by subgroups of age (<65 years of age and ≥65 years of age).

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Clinical Relevance

Scientific rationale for the study: CKD prevalence and complications cannot be entirely explained by traditional risk factors such as diabetes or cardiovascular disease (CVD). Periodontitis is independently associated with CKD and contributes to the systemic inflammatory burden, therefore this study aimed to establish the association between periodontitis and mortality in patients with chronic kidney disease (CKD).

Principal findings: Periodontitis was associated with a 9% (absolute) or 28% (relative) increase in all-cause mortality at 10 years for individuals with CKD, within the limitation of this analysis. This association is of a similar magnitude, but independent of, that seen between diabetes and mortality in individuals with CKD.

Practical implications: Periodontitis may be an important predictor of mortality in patients with CKD and sources of chronic inflammation (including periodontitis) may be important contributors beyond traditional risk factors in patients with CKD.

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The periodontal health component of the Renal Impairment In Secondary Care (RIISC) cohort study: a description of the rationale, methodology and initial baseline results

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Abstract

Introduction: Chronic kidney disease (CKD) is associated with significant morbidity and mortality. There is a need to identify novel and modifiable risk factors in such patients. The periodontal component of the Renal Impairment In Secondary Care (RIISC) study aims to evaluate the association between chronic periodontitis and CKD progression.

Methods: The RIISC study is a prospective, observational cohort study of patients with CKD from a renal clinic at a hospital in the West Midlands region of the UK. Patients undergo a periodontal examination and plaque and saliva sampling. To benchmark the oral health status of the RIISC cohort, we compared it to the Adult Dental Health Survey 2009 (ADHS), a representative survey of the oral health of community dwelling adults in the UK.

Results: Of the first 500 patients recruited into the RIISC study, 469 patients underwent a dental examination and 80 (17%) were edentulous. Among dentate subjects, patients within RIISC were significantly more likely to have any (OR 4.0 95% CI 2.7–5.9) or severe (OR 3.8 95% CI 2.5–5.6) periodontitis compared to the ADHS sample.

Conclusion: The prevalence and severity of chronic periodontitis in this cohort of CKD patients is markedly higher than a geographically matched control population.

Key words: chronic kidney disease; observational cohort study; periodontitis

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Conflict of interest and source of funding statement

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Chronic kidney disease (CKD) affects over 13% of the adult population in the UK (Coresh et al. 2007, O'Callaghan et al. 2011) and is

associated with increasing age (Zhang & Rothenbacher 2008), hypertension and diabetes (Stenvinkel 2010).

CKD is typically classified into stages based upon the estimated Glomerular Filtration Rate (eGFR; Table 1; Levey et al. 1999). Patients with stage 5 CKD (also known as End Stage Renal Disease – ESRD) may require renal replacement therapy (RRT), that is, dialysis or kidney transplantation. In the US, almost 30% of the Medicare budget for over 65s is spent on patients with CKD (Stenvinkel 2010). In 2009–2010, the annual cost for treatment of patients with stages 3–5 CKD in England alone was approximately 1.45 billion pounds [approximately 1.3% of the overall National Health Service (NHS) budget in that period] and more than half was spent on patients requiring RRT (Kerr et al. 2012). The main cause of death in patients with CKD is due to cardiovascular events which are directly related to the severity of kidney disease (Go et al. 2004) and to an increased systemic inflammatory burden. Therefore, biomarkers such as C-reactive protein (CRP) are a reliable marker of cardiovascular and all-cause mortality in patients with CKD (Lacson & Levin 2004). As at least 50% of the increased mortality seen in patients with CKD is not associated with traditional risk factors (diabetes, hypertension and smoking; Matsushita et al. 2010). Therefore, identifying and targeting novel, modifiable risk factors contributing to systemic inflammation in CKD may be an important strategy in reducing morbidity and mortality in these patients.

Periodontitis and CKD

Periodontal diseases are the most common inflammatory conditions in humans (Pussinen et al. 2007). Acute-phase markers (CRP, IL-6) and those of oxidative stress are elevated in patients with periodontitis and reduce following successful periodontal therapy (D'Aiuto et al. 2004). Periodontal inflammation may therefore contribute to the systemic inflammatory burden (Tonetti & Van Dyke 2013).

The association between periodontitis and other systemic diseases (particularly cardiovascular disease – CVD) is established and was recently reviewed in a joint European and American consensus workshop in periodontology (Dietrich et al. 2013). Putative mechanisms include:

1 Metastatic infection from periodontal bacteria,
2 Metastatic inflammation from the local inflammatory-immune response spilling into the circulation,
3 Metastatic injury by periodontal pathogens or their products entering the circulation and subsequently stimulating both acute-phase and oxidative stress responses.

Periodontitis may therefore act as a comorbid inflammatory disease in patients with CKD in promoting the development of CVD (Kshirsagar et al. 2009). This pathway may be amenable to treatment as significant reductions in systemic inflammatory markers (IL-6, CRP) are reported following periodontal therapy in patients with CKD (Vilela et al. 2011).

To date, the majority of studies have been cross-sectional in nature, investigating the prevalence of periodontitis in patients with ESRD, rather than its impact upon CKD progression. For example, a large epidemiological study using data from the US Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994), demonstrated an increased prevalence of moderate periodontitis (14.6%) in patients with CKD compared with control populations (8.7%; Ioannidou & Swede 2011) and a study using more recent NHANES data (2001–2004) reported an association between periodontal disease and CKD after adjusting for key confounders (age, gender, ethnicity, tobacco use, hypertension, diabetes, poverty status, educational attainment and dental care use) with an odds ratio of 1.51 (95% CI: 1.13,2.02; $p = 0.006$; Grubbs et al. 2011). Currently, there are no studies investigating periodontitis as a risk factor for CKD progression, the need for RRT, or mortality in patients with CKD. Given the increased systemic inflammatory burden in patients with periodontitis and the deleterious effect this might have on CKD progression and increased incidence of adverse cardiovascular events in these patients, prospective cohort studies in this field are needed. These will allow the impact of periodontitis on CKD progression in pre-dialysis CKD patients and the resulting morbidity and mortality to be assessed.

With this in mind, a dental component including a periodontal examination was included in the protocol of the Renal Impairment In Secondary Care (RIISC) study (ClinicalTrials.gov reference number NCT01722383).

The aims of this paper are:

1 To provide the methodological details for the oral component of the RIISC study.
2 To report initial descriptive results regarding the periodontal status of the first 500 patients at baseline with benchmarking against a representative regional population sample derived from the latest Adult Dental Health Survey (ADHS), carried out in 2009 (O'Sullivan et al. 2011).

Table 1. Stages of chronic kidney disease

Stage of Chronic Kidney Disease	Estimated glomerular filtration rate (eGFR), ml/min/1.73 m^2
Stage 1: eGFR shows normal kidney function but patients have other signs or symptoms of kidney damage or disease	90 or more
Stage 2: Mildly reduced kidney function	60–89
Stage 3: Moderately reduced kidney function	45–59 (3A) 30–44 (3B)
Stage 4: Severely reduced kidney function	15–29
Stage 5: Very severely reduced kidney function	<15

Methods

RIISC-Overview

The RIISC study is an ongoing, prospective, observational cohort study

of patients with CKD with evidence of, or at high risk of, renal disease progression. The inclusion and exclusion criteria have previously been reported in detail (Stringer et al. 2013). Briefly, patients are recruited if they have stage 3 CKD and a decline in eGFR of ≥5 ml/min/year or ≥10 ml/min/5 years or a urine albumin creatinine ratio (uACR) ≥70 mg/mmol on three consecutive occasions, or stage 4/5 CKD. Glomerular filtration is estimated (eGFR) using the four-variable Modification of Diet in Renal Disease (MDRD) equation with serum creatinine recalibrated to be traceable to an isotope derived mass spectroscopy method (Levey et al. 2005). Patients are excluded if they were receiving dialysis treatment or immunosuppressive drugs for kidney disease. Patients' socioeconomic status (SES) is assessed using (amongst other measures), the Index of Multiple Deprivation (IMD). This score of deprivation (lower being more deprived) is derived using the patient's post code. Seven domains contribute to the overall IMD score, these are: income deprivation, employment deprivation, health deprivation and disability, education skills and training deprivation, barriers to housing and services, living environment deprivation, and crime. From October 2010–November 2013 over 600 patients have been recruited. The study is approved by the South Birmingham Local Research Ethics committee (reference: 10/H1207/6) and is conducted in accordance with the Declaration of Helsinki.

Patients undergo detailed clinical assessment as previously outlined (Stringer et al. 2013). The periodontal component of this assessment is reported here. Patients are excluded from a detailed periodontal examination if there is risk of severe bleeding (e.g. due to warfarin use with INR > 4.0) or a history of infective endocarditis.

As part of the oral assessments, patients undergo a detailed periodontal examination and plaque and saliva samples are collected.

Saliva sampling

The aim of saliva sampling is to enable subsequent investigation of the constituents of saliva as potential biomarkers for periodontal and/or renal and/or general health status of patients in the RIISC cohort.

A stimulated, whole-saliva sample is collected using a sterile marble to stimulate saliva flow (Chapple 1997). Samples are collected in a 15 ml, graduated Falcon tube (Corning®; Corning Incorporated, Corning, NY, USA) over a period of at least 5 min.

The aim is to collect a minimum of 1 ml of saliva. If this minimum volume is not reached by 5 min., saliva sampling continues for a further 5 min. (maximum) to attempt to collect the target volume.

Samples are immediately centrifuged at 4°C and 1000 *g* for 10 min. to remove debris and the supernatant is transferred into a cryogenic vial (Greiner Bio-One, Stonehouse, UK), without disturbing the pellet. A maximum of 1.8 ml is snap frozen in liquid nitrogen and is biobanked at −80°C. Future analysis will include non-presumptive proteomic analysis of saliva by FT-ICR mass spectrometry and data visualization and clustering (e.g. Polysnap) using methods developed by our group (Grant et al. 2010).

Periodontal assessment

Detailed periodontal examination is carried out by a dental hygienist or dentist using a constant force periodontal probe (UB-WHO-CF15 periodontal probe – Implantium.co.uk) to standardize probing forces at 0.2 N. For the first 500 patients presented here, the majority were examined by a single clinician (AS) who was trained in the use of the constant force probe.

For each tooth (including wisdom teeth where present), interproximal probing pocket depth (PPD) and recession are recorded for the buccal and palatal/lingual surfaces (a total of four sites per tooth). The clinical attachment loss (CAL) at each site is recorded as the sum of the probing depths and recession at that site.

On completion of these measurements for a dental quadrant, a dichotomous record of bleeding on probing (BoP) from the base of the gingival sulcus is made for each site probed.

Plaque sampling

The aim of the plaque sampling is to facilitate subsequent molecular microbial analysis by sequencing of the V1–3 region of the 16s rRNA gene followed by PCR to determine whether there are associations between certain oral bacteria and oral status or with progressive CKD. The identity of these bacteria will be determined using non-presumptive next generation molecular sequencing of sub-gingival samples from deep sites, performed collaboratively with the Institute of Microbiology and Infection at the University of Birmingham. Probing sites are divided into 'deep' (probing depth ≥ 6 mm) or 'shallow' (probing depth ≤ 3 mm) sites. Up to three deep and three shallow pockets are selected per patient.

Once representative sites have been identified, four size 40 sterile paper points are introduced sequentially to the selected sites and left in situ for 10 s (Kumar et al. 2005).

The paper points are then removed and placed in a cryotube containing Tris buffer (Tris-EDTA – Cat no. 93302; Sigma Aldrich, Dorset, UK) in DNA/DNase free microcentrifuge tubes (Jencons, protein Lobind/DNA Lobind). Paper points from all deep sites from each patient are pooled and stored as 'deep pocket samples' and paper points from all shallow sites are pooled in a similar manner. The cryotubes are stored at −80°C.

Comparison with regional, representative population

To benchmark the oral health status of the RIISC cohort, we compared the periodontal status of the RIISC population to the results from the ADHS in 2009. The details of this survey have been described elsewhere (O'Sullivan et al. 2011). Briefly, the ADHS provides an assessment of the oral health and attitudes of community dwelling adults in the UK. This survey is commissioned by the NHS Information Centre for health and social care in the UK and has been carried out every 10 years since 1968 with the latest survey preformed in 2009. Of this study, the ADHS 2009 dataset was limited to 876 patients recruited in the West Midlands region, which is where the RIISC population is drawn from.

From here on, this cohort (derived from the ADHS data from West Midlands region in the 2009 cycle of the ADHS) will be referred to as ADHS-2009.

The ADHS-2009 differed in its periodontal assessment from the RIISC assessment in that:

1 PPD was recorded at two interproximal sites (lingual for mandibular, and buccal for maxillary teeth) on each tooth,
2 PPDs were recorded in the following categories and the worst score per sextant was recorded:
   (a) Score 1–0 to 3.5 mm,
   (b) Score 2–4 to 5.5 mm,
   (c) Score 3–6 to 8.5 mm, and
   (d) Score 4 to ≥9 mm.
3 If there was a single tooth in a sextant, the sextant was not recorded and the tooth was considered to belong to the adjacent sextant.

In the present analyses, we derived the same periodontal scores for the RIISC population using the more comprehensive periodontal data collected in RIISC to compare with the ADHS-2009 population. As established case definitions for periodontitis could not be employed in this comparison due to the ordered nature of the ADHS-2009 clinical measurements, we used pragmatic case definitions based upon the worst score recorded in each patient. Of this comparison, a case of periodontitis was defined as having at least one score of 2 or more (probing depth ≥4 mm), and severe periodontitis was defined as at least one score of 3 or more (probing depth ≥6 mm). The ADHS-2009 also recorded the participants' SES using the 2010 IMD score (divided into deciles) and the IMD scores from RIISC were divided into deciles using the same cut-off points as the ADHS-2009.

The periodontal status of the CKD patients enrolled in RIISC was analysed as follows:

1 The prevalence of chronic periodontitis was calculated using the
   (a) Centre for Disease Control and Prevention/American Academy of Periodontology (CDC/AAP; Page & Eke 2007) and European Federation of Periodontology (EFP; Tonetti et al. 2005) case definitions.
2 The periodontal status was described using the following periodontal measures:
   (a) Mean PPD.
   (b) Mean CAL.
   (c) Cumulative PPD: Calculated as the sum of the maximum PPDs ≥4 mm of each tooth (Dietrich et al. 2008).
   (d) Number and proportion of teeth with PPD and CAL above several thresholds (4+, 5+, 6+, 7+ mm).
   (e) Proportion of sites BoP.
   (f) Number of missing teeth.

Statistical analyses

Summary statistics of patient characteristics were calculated as appropriate and comparisons between groups were made using *t*-test for continuous and Pearson's chi-squared test or Fisher's exact test (as appropriate) for categorical variables.

When comparing the RIISC and ADHS-2009 cohorts, age-standardized prevalences of periodontitis and severe periodontitis as well as edentulism were calculated for the RIISC cohort using the ADHS-2009 cohort as the reference population. Multiple logistic regression analyses were performed to provide prevalence odds-ratios adjusted for age, gender, ethnicity (White, Asian, Black or other), smoking status (current vs. never or former smoker) and SES status (measured by IMD score). These covariates were chosen for their biological significance in impacting both periodontitis and CKD. Other covariates were not included in the analyses due to lack of comparable information from the ADHS-2009 cohort. For example, information on a diagnosis of diabetes was not available in ADHS-2009. However, diabetes is an established risk factor for periodontitis and highly prevalent in the RIISC population. We therefore conducted separate analyses restricted to non-diabetic RIISC patients to minimize the confounding effect of diabetes.

Results

RIISC-Prevalence, severity and extent of periodontitis

Thirty-one of the first 500 patients recruited did not have a dental examination for a variety of reasons (Table 2). The remaining 469 patients underwent a dental examination. The demographics of patients who participated in the dental exam did not differ significantly from 31 patients who did not participate in the dental examination (Table 3).

The mean age of examined patients was 63 years (SD 16 years; range 19–92) and 285 (60.8%) patients were male. The majority of patients were White (71%) with 14% of patients reporting to be current smokers (Table 3).

Measures of SES reveal patients in the RIISC cohort to be not currently in employment (72%), reporting no formal educational qualification (49%) and residing in areas of deprivation (Table 3).

The large majority of patients (84%) had stage 3 or stage 4 CKD. The prevalence of diabetes within the RIISC cohort was 39% and 33% reported a history of CVD (Table 3).

Dental examination revealed 80 patients (17%) to be edentulous. Of

Table 2. Reason for not undergoing baseline dental examination

Reason for not attending dental station at baseline	Number of patients	Cumulative number of patients
Could not wait for dental exam	7	7
Missed dental station (Error)	6	13
Dental Team not setup/present	5	18
Did not consent for dental exam	5	23
Previous endocarditis	5	28
Withdrew consent from entire study	2	30
Anxious	1	31

Table 3. Renal Impairment In Secondary Care cohort description

	All patients[a] (n = 469)	Dentate (n = 389)	Edentulous (n = 80)	No dental exam (n = 31)	p Values[b]
Age					
Mean	63	61	77	65	0.59
SD	16	16	9	14	
Range	19–92	19–92	44–91	35–84	
Male (%)	61	62	55	55	0.51
Ethnicity (%)					
White	71	70	78	81	0.40
Asian	16	18	8	13	
Afro-Caribbean	11	11	13	3	
Other	2	2	3	3	
Smoker (%)					
Never	45	47	35	55	0.44
Former	41	38	58	39	
Current	14	15	8	6	
Diabetic	39	39	40	42	0.77
HbA1C (mmol/mol)					
Mean	49	49	48	47	0.58
SD	17	17	12	12	
Range	27–139	27–139	31–81	32–78	
Chronic kidney disease (%)					
Stage 1	2	3	0	3	0.70
Stage 2	5	5	5	10	
Stage 3	24	25	21	26	
Stage 4	60	58	67	58	
Stage 5	8	9	6	3	
Estimated glomerular filtration rate (ml/min/1.73 m^2)					
Mean	27	28	25	31	0.13
SD	12	13	9	12	
Range	5–90	5–90	12–51	14–74	
History of cardiovascular disease (%)	33	31	46	42	0.34
Body mass index (kg/m^2)					
Mean	30	30	29	30	0.86
SD	7	7	6	5	
Range	15–60	15–60	19–49	19–42	
Employed (%)					
Yes	28	33	4	26	0.82
No	20	21	14	16	
Retired	52	46	83	58	
Highest educational qualification (%)					
None	49	43	77	52	0.38
GCSE	21	24	6	19	
NVQ	8	8	11	7	
GCE A-Level	7	8	1	17	
UG	11	12	3	6	
PG	5	6	2	0	
Index of Multiple Deprivation 2010 score (Decile) (%)					
1	29	28	33	39	0.946
2	19	17	26	13	
3	9	9	9	10	
4	9	9	10	13	
5	12	13	8	13	
6	8	8	8	3	
7	5	6	1	3	
8	3	3	3	3	
9	3	3	3	3	
10	3	3	1	0	

[a]Who underwent a dental exam.
[b]For the differences between patients that did and did not undergo a dental exam.
GCE A-Level, General Certificate of Education Advanced Level (aged approximately 18); GCSE, General Certificate of Secondary Education (aged approximately 16); NVQ, National Vocal Qualification (aged approximately 16–18); PG, Postgraduate; UG, Undergraduate.

the remaining 389 dentate patients, 4.4% had no periodontitis, 44.0% had moderate periodontitis and 51.7% had severe periodontitis, resulting in 372 (95.6%) patients with moderate or severe periodontitis according to CDC/AAP criteria. Using the EFP case definitions, 99.2% met the "sensitive" criteria for periodontitis and 59.4% met the "specific" criteria for periodontitis. Other measures of periodontitis severity and extent further describe the disease burden (Tables 4 and 5). For example, patients had a median of seven teeth with CAL ≥5 mm and 92% and 86% of patients had at least one and two teeth with CAL ≥5 mm respectively (Table 5).

Comparison with regional representative population-based sample

The ADHS-2009 examined 876 patients (limited to the West Midlands region), 10% of who were edentulous. Of the remaining 791 dentate participants, 27% did not consent for a dental exam and 11% did not have an examination performed after giving consent (for reasons such as unavailability of a dental member to conduct a detailed examination). The remaining 487 (62%) underwent a detailed periodontal examination (O'Sullivan et al. 2011). Compared to the RIISC cohort, ADHS participants were significantly younger, less likely to be non-white, more likely to be female, more likely to be current smokers and more likely to come from an area of social deprivation (Table 6).

Within the RIISC cohort, patients without diabetes (61% of the total population) did not differ significantly from patients with diabetes in terms of gender, ethnicity, smoking status, SES or the prevalence of edentulism. Patients with diabetes within RIISC were older than patients without diabetes (p = 0.046; Table 6).

The age-standardized prevalence of periodontitis (1+ site with PPD ≥4 mm) and severe periodontitis (1+ site with PPD ≥6 mm) was significantly higher in the RIISC cohort as compared to the ADHS population with odds ratios (OR) of 4.9 [95% confidence intervals (CI) 3.4–7.1] and 4.8 (95% CI 3.3–6.8) respectively.

Table 4. Renal Impairment In Secondary Care cohort periodontal status description for dentate patients

Periodontitis (Centre for Disease Control and Prevention/American Academy of Periodontology classification)	Healthy: 17 (4.4%) Moderate periodontitis: 171 (44.0%) Severe periodontitis: 201 (51.7%)
Periodontitis (European Federation of Periodontology classification)	Sensitive criteria: Healthy 3 (0.8%); Periodontitis 386 (99.2%) Specific criteria: Healthy 158 (40.6%); Periodontitis 231 (59.4%)

	Mean	Proportion of population (n = 389)
Mean probing depths (mm)	Population Mean: 2.9 SD: 0.9 Range: 1.0–6.8	≤2 mm: 13.1% 2 to ≤3 mm: 47.6% 3 to ≤4 mm: 29.3% 4 to ≤5 mm: 8.5% >5 mm: 1.5%
Mean clinical attachment loss (mm)	Population Mean: 3.6 SD: 1.4 Range: 1.0–12.3	≤2 mm: 6.9% 2 to ≤4 mm: 67.1% 4 to ≤6 mm: 19.3% 6 to ≤8 mm: 5.1% >8 mm: 1.5%
Cumulative probing depth of sites ≥4 mm (mm)	Population Mean: 51 SD: 43 Range: 0–238	0 mm: 7.5% 4 to ≤20 mm: 24.4% 20 to ≤40 mm: 16.5% 40 to ≤60 mm: 14.4% 60 to ≤80 mm: 14.9% 80 to ≤100 mm: 8.5% >100 mm: 13.9%
Proportion of sites bleeding on probing	Population Mean: 33.5% SD: 25% Range: 0–100%	≤33%: 11.5% 33 to ≤66%: 32.7% >66%: 55.8%
Number of teeth present (including wisdom teeth)	Population Mean: 21 SD: 8 Range: 1–32	25 to 32: 46.8% 16 to 24: 30.6% 1 to 15: 22.6%

Table 5. Renal Impairment In Secondary Care cohort periodontal status description for dentate patients

Threshold	Proportion (%) of dentate patients above threshold		Number (%) of teeth per patient above threshold				
	≥1 tooth	≥2 teeth	Minimum	25th percentile	Median	75th percentile	Maximum
CAL ≥ 4 mm	99	96	0 (0)	7 (41)	12 (67)	18 (90)	32 (100)
CAL ≥ 5 mm	92	86	0 (0)	3 (15)	7 (36)	11 (70)	32 (100)
CAL ≥ 6 mm	72	60	0 (0)	0 (0)	2 (13)	6 (33)	30 (100)
CAL ≥ 7 mm	50	36	0 (0)	0 (0)	1 (3)	1 (17)	25 (100)
PPD ≥ 4 mm	93	87	0 (0)	3 (20)	9 (47)	16 (75)	32 (100)
PPD ≥ 5 mm	79	67	0 (0)	1 (5)	4 (20)	10 (49)	32 (100)
PPD ≥ 6 mm	51	36	0 (0)	0 (0)	1 (3)	3 (14)	30 (100)
PPD ≥ 7 mm	23	14	0 (0)	0 (0)	0 (0)	0 (0)	25 (100)

Following adjustment for age, gender, smoking status, SES and ethnicity, the odds of having periodontitis were four times higher (95% CI 2.7–5.9) and the odds of having severe periodontitis were 3.8 times higher (95% CI 2.5–5.7) within the RIISC cohort than the ADHS-2009 cohort. The prevalence of edentulism was similar in both populations, after adjustment for confounders (Table 7).

RIISC patients without diabetes had significantly greater odds of having periodontitis (OR 4.4; 95% CI 2.7–7.1) and severe periodontitis (OR 3.0; 95% CI 1.9–4.8) compared to the ADHS-2009 population after adjusting for confounders (Table 7).

Discussion

The RIISC cohort is the first longitudinal cohort of patients with CKD, who are at risk of CKD progression and in whom a comprehensive periodontal examination was undertaken. Monitoring of the periodontal status of this cohort is planned over a 10 year follow-up period. This cohort will therefore provide a unique resource to study the longitudinal association between chronic periodontitis and CKD, which has previously only been reported in cross-sectional studies. Specifically, the cohort will allow us to evaluate whether periodontal status is a risk factor for CKD progression and cardiovascular morbidity and mortality in patients with CKD. The various biological samples available will also enable us to explore biologically plausible mechanisms underpinning an association between the two conditions.

The baseline results described here indicate that periodontal inflammation and previous disease experience (CAL) in this population of patients with CKD is high and substantially higher than in the local population (Table 7). Only a small minority of enrolled patients with CKD were classified as periodontally healthy (4.4% according to CDC/AAP criteria, 0.8% according to the EFP's "sensitive criteria" criteria and 40.6% according to the EFP's specific criteria). The burden of periodontitis in terms of disease severity and extent in this population is remarkably high, with 50% of enrolled patients having at least 36% and 20% of their teeth with a CAL of 5+ mm and PPD of 5+ mm respectively (Table 5). The lack of periodontally healthy patients in this cohort may present future challenges as it may mask the association between periodontitis and CKD progression (if any). It may be possible to visualize a dose–response relationship between continuous measures of periodontal health (such as mean PPD, mean CAL and cumulative PPD) and measures of renal health (such as eGFR).

Table 6. Comparison of the Renal Impairment In Secondary Care (RIISC, whole cohort and non-diabetics) with the Adult Dental Health Survey (ADHS)-2009 cohort

	RIISC (whole cohort) $n = 469$	RIISC (non-diabetic) $n = 285$	ADHS-2009 $n = 876$	p Value[a]	p Value[b]
Age (years)					
Mean	63	62	52	0.046	<0.001
SD	16	17	18		
Range	19–92	19–91	16–85		
Male (%)	60.8	60.4	44	0.818	<0.001
Ethnicity (%)					
White	71	72	93	0.699	<0.001
Asian	16	14	4		
Afro-Caribbean	11	11	2		
Other	2	2	2		
Current smokers	14	16	19	0.132	0.018
Index of Multiple Deprivation 2010 score (Decile) (%)					
1	29	27	12	0.130	<0.001
2	19	20	8		
3	9	8	10		
4	9	7	9		
5	12	12	10		
6	8	10	13		
7	5	6	11		
8	3	3	11		
9	3	2	9		
10	3	2	8		
Edentulous (%)					
Crude	17	17	10	0.883	<0.001
Age-adjusted[c]	9	9	10	0.859	0.524
Periodontitis (%)					
Crude	87	88	55	0.524	<0.001
Age-adjusted[c]	88	90	55	0.590	<0.001
Severe periodontitis (%)					
Crude	39	33	11	0.002	<0.001
Age-adjusted[c]	39	33	11	0.003	<0.001

[a]Comparing RIISC (with diabetics) to RIISC (without diabetics).
[b]Comparing RIISC (whole cohort) to ADHS-2009.
[c]Using the ADHS-2009 population as the referent population.

Table 7. Odds ratios of Renal Impairment In Secondary Care (RIISC, whole cohort and non-diabetics) compared with Adult Dental Health Survey-2009 cohort)

	RIISC (whole cohort)		RIISC (non-diabetics)	
	Age-adjusted OR (95% CI)	Fully adjusted[a] OR (95% CI)	Age-adjusted OR (95% CI)	Fully adjusted[a] OR (95% CI)
Edentulism	0.89 (0.62–1.30)	0.79 (0.51–1.22)	0.94 (0.60–1.46)	0.89 (0.53–1.48)
Periodontitis	4.93 (3.42–7.10)	3.96 (2.65–5.90)	5.43 (3.48–8.48)	4.37 (2.69–7.08)
Severe periodontitis	4.75 (3.31–6.83)	3.77 (2.52–5.65)	3.65 (2.43–5.50)	3.01 (1.89–4.79)

[a]Adjusted for age, gender, ethnicity, smoking status and socioeconomic status.

As very little data on covariates was available for the ADHS-2009 (except for age, gender, ethnicity, SES and smoking status), there may still be confounders that account for the differences in the prevalence of periodontitis between the two populations. The most likely confounder is diabetes which is strongly associated with both CKD and periodontitis (Stenvinkel 2010, Chapple et al. 2013). Within the RIISC cohort, diabetes prevalence is considerably higher than would be expected in the reference population of the ADHS-2009, but the diabetic status of participants in the ADHS-2009 remains unknown. To account for confounding by diabetes, we performed a sensitivity analysis restricted to non-diabetic RIISC participants, showing only moderate attenuation of the odds-ratio comparing periodontitis prevalence between patients with CKD and the ADHS sample (Table 7). It should be noted that the association between periodontitis and CKD will be negatively confounded by diabetes in the comparison between non-diabetic RIISC patients and ADHS participants, as a small proportion of the ADHS participants is expected to be diabetic.

The association between periodontitis and CKD has been reported by previous investigators and was the subject of a recent systematic review and meta-analysis (Chambrone et al. 2013), which showed an increased prevalence of CKD in patients with periodontitis compared with patients without periodontitis (OR 1.65, 95% CI 1.35–2.01). This meta-analysis was based on results from four studies, two of which are based on data obtained from the NHANES database (having a combined weighting of 82.3% in the meta-analysis). The higher prevalence odds-ratios reported in this study may be due to differences in the populations studied or due to bias. For example, it should be noted that the prevalence of periodontitis is underestimated in NHANES studies by 50% or more due to partial mouth sampling (Eke et al. 2010), possibly explaining the higher prevalence odds-ratios reported here. Moreover, the associations reported here have to be interpreted with caution, as we compared data from two different studies with different examination protocols. Furthermore, only a limited number of covariates were available in ADHS population and residual confounding may be present.

Notwithstanding the possibility for confounding, several explanations have been proposed to explain the increased prevalence of periodontitis in patients with CKD. For example, in patients with ESRD, uraemia is associated with altered cell-mediated immunity (Tonelli & Pfeffer 2007) which may make these patients more susceptible to periodontitis (Akar et al. 2011). Also in uraemic patients, changes in salivary constituents (increased urea and

calcium and phosphate ions) may lead to increased calculus formation (Epstein et al. 1980, Nunn et al. 2000) which is likely to promote periodontitis. It is also postulated that patients on dialysis may not have oral care as a high priority, which may result in an increased prevalence of periodontitis (Bayraktar et al. 2007, Cengiz et al. 2009). However, these effects of CKD on periodontitis (immunological or salivary changes caused by uraemia or having oral care as less of a priority) are likely to be more significant in patients with ESRD than in pre-dialysis CKD patients such as in RIISC.

Conversely, there may be an impact of periodontitis on the CKD disease process in a similar manner to other systemic diseases for which periodontitis is an independent risk factor. The longitudinal nature of this cohort study will facilitate analysis of CKD progression with time and with patients' periodontal status as a predictor variable. Currently, if patients are diagnosed with periodontitis, they are informed of their condition and are advised to see their local dentist. At this stage, it is difficult to estimate what the uptake of treatment by patients in the community might be but changes in periodontal health over time can be accounted for in the analyses. In addition, this cohort study also provides a platform for possible intervention studies to evaluate the effect of periodontal therapy on various outcomes in patients with CKD.

In future analyses of plaque and saliva samples, collaborations with national and international experts in the fields of bioinformatics, microbial and "omic" analyses will be sought to unravel the mechanistic links that may correlate the microbial and omic environments with CKD progression.

Finally, in recent years, focus on common features of inflammatory disease such as diabetes, chronic obstructive pulmonary disease and rheumatoid arthritis suggests that some patients may possess a constitutive hyper-inflammatory phenotype, both in terms of reactive oxygen species release by peripheral blood polymorphonuclear leucocytes (Chapple & Matthews 2007, Dias et al. 2013) and also activation of cytokine networks (Preshaw & Taylor 2011). This would render such individuals co-incidentally more susceptible to inflammatory diseases such as CKD and periodontitis.

Contrary to other studies (Fisher et al. 2008), we did not find a difference in the prevalence of edentulism between the cohorts after adjustments for age, gender, ethnicity and smoking status were made. This may be due to the fact that the prevalence of edentulism as recorded by the ADHS was highest in the West Midlands region than for any other region in the UK (Chenery 2011), and this data formed the comparator group for the RIISC study.

Conclusion

The RIISC cohort is unique in that it comprises patient volunteers with pre-dialysis CKD who are at a higher risk for progression of CKD and as a result, higher risk of adverse cardiovascular events. The prevalence, severity and extent of chronic periodontitis among RIISC patients is high, and markedly higher than in a geographically matched control population. The longitudinal nature of the study with repeated oral examinations will facilitate the prospective determination of the impact of periodontal disease and tooth loss upon CKD progression and complications. Furthermore, the cohort may provide the basis for intervention studies to evaluate the effects of periodontal therapy on periodontal, renal and cardiovascular outcomes in patients with CKD.

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Clinical Relevance

Scientific rationale for the study: Periodontitis may be more prevalent in patients with chronic kidney disease (CKD) and have a comorbid effect on patients with CKD.
Principal findings: Patients with CKD have higher odds of having periodontitis and severe periodontitis when compared to a control population of local, community dwelling adults.
Practical implications: The increased prevalence of periodontitis in patients with CKD may have a comorbid effect on these patients. Interventional studies are needed to investigate the effect of treatment of periodontitis on patients with CKD.

Manuscript number 6

TITLE: Periodontal Inflammation Influences Renal Function in Patients With Chronic Kidney Disease

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RUNNING TITLE: Periodontitis and renal function

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Significance Statement: Periodontitis is a highly prevalent, chronic inflammatory disease, affecting tissues surrounding teeth. It is associated with an increase in markers of systemic inflammation and oxidative stress. Inflammation and oxidative stress negatively impact on the course of chronic kidney disease (CKD). Using a cohort of patients with CKD, this study shows an association between periodontal inflamed surface area (PISA) and renal function and measures of oxidative stress. Using structural equation modelling (SEM), the authors confirmed a causal hypothesis that PISA indirectly influences eGFR, via oxidative stress. This reveals that a 10% increase in PISA results in a 2.5% (95%CI: 0.3-4.6%) decrease in eGFR. In future, if confirmed by randomized controlled trials, managing periodontal inflammation may form part of the management of patients with CKD.

Introduction

Chronic kidney disease (CKD) affects 8-16% of the global population[1] and approximately 13% of UK adults[2]. Approximately 7% of adults in the US have stage 3-4 CKD[3]. It is associated with significant morbidity in the form of end-stage renal disease and cardiovascular disease, as well as early mortality. Prognostic factors associated with adverse outcomes in CKD include severity of kidney disease[4] and systemic inflammation and oxidative stress[5, 6].

Periodontitis is a chronic inflammatory disease affecting the connective tissues supporting the teeth. It is initiated by bacteria accumulating between the gingiva and teeth, causing gingival inflammation. In susceptible individuals, this can progress to destruction of periodontal ligament and alveolar bone. Periodontitis is a highly prevalent condition and, in its severe form, affects over 7% of the world's population[7]. Recent systematic reviews of epidemiologic studies have suggested that periodontitis is more common in patients with CKD than those without, with an adjusted odds ratio of 2.3 (95%CI: 1.7-3.0)[8]. Recent systematic reviews also confirm an association between periodontitis and increased all-cause mortality in patients with CKD, with a relative risk of 1.25 (95%CI: 1.05-1.50)[9]. Data from the third National Health and Nutrition Examination Survey (NHANES III) and linked mortality data suggest that, amongst patients with CKD, those who also have periodontitis have a markedly higher 10-year all-cause and cardio-vascular mortality rate than those without periodontitis[10].

There is also growing evidence of the associations between periodontitis and other chronic conditions, most notably cardiovascular disease [11] and diabetes. Treatment of periodontitis in patients with diabetes is associated with reductions in HbA1C of 0.27–0.48%, with implications in patients with diabetic nephropathy [12].

There is growing evidence that local inflammation in periodontitis is associated with an increased systemic inflammatory and oxidative stress burden [13]. Treatment of periodontitis has been shown to reduce plasma concentrations of inflammatory mediators [14-17].Plasma protein carbonyls, generic products from a wide range of protein oxidation reactions, are also found at higher levels in patients with periodontitis [18]. Lipid peroxidation markers, F2-α-isoprostanes, that are formed from oxidation of the abundant fatty acid, arachidonic acid are also reported in high levels in patients with CKD [19]. Protein carbonyls and isoprostanes activate vascular endothelial and inflammatory cells via receptors for advanced glycation endproducts (RAGE) and toll-like receptors (TLR) respectively, increasing interleukin-6 (IL-6) production and are associated with increased cardiovascular disease [20].

The underlying mechanisms for the association between periodontal inflammation and CKD are not fully understood. There are biologically plausible mechanisms by which periodontal inflammation could influence renal function. These mechanisms might involve the dissemination of intact bacteria, bacterial products or inflammatory cytokines from inflamed, ulcerated, periodontal tissues, via the bloodstream, to sites distant to the oral cavity. It is also possible that the associations between periodontal inflammation and renal function are due to the effect of renal function on periodontal inflammation. These may also be mediated via increases in systemic inflammatory or oxidative stress burden. Alternatively, renal function may influence periodontal inflammation via an altered immune response or altered blood chemistry, the latter leading to increased mineral content of saliva and increased susceptibility to calculus formation. It is also possible that the relationship between periodontal inflammation and renal function is bi-directional, as has been suggested [21]. Finally, it is possible that the associations between periodontal inflammation and renal function are artefactual, arising from shared risk factors such as smoking and diabetes or other known or unknown confounders, and not due to a causal relationship between the two.

With these possibilities in mind, this study firstly aims to confirm the associations, seen in other cohorts, between periodontal inflammation and renal function. Secondly, this study aims to assess the associations between periodontal inflammation and measures of systemic inflammatory or oxidative stress burden, in patients with CKD, independent of renal function. Finally, the third aim of this study is to use path analysis structural equation modelling (SEM) to unravel the potential mechanisms by which periodontal inflammation could influence renal function and vice versa, if at all.

Methods

Data presented here are from baseline assessments of an ongoing, longitudinal, observational cohort study, the Renal Impairment In Secondary Care (RIISC) study. The protocol of the RIISC study is published [22] and provide more detail. Briefly, the RIISC study recruited a cohort of patients with CKD, at high risk of adverse outcomes, as defined by the inclusion and exclusion criteria (Table 1). Patients, with a minimum of one-year follow-up, were recruited from secondary care renal clinics. The RIISC study recruited between October 2010 and December 2015 with the aim of identifying novel prognostic factors in CKD. The study was approved by the South Birmingham Local Research Ethics committee (reference 17010/H1207/6) and University Hospitals Birmingham Research and Development department (reference RRK3917) and was conducted following the principles of the Declaration of Helsinki.

Periodontal assessments

Participants underwent a full-mouth detailed pocket chart, recording probing depths (PPD), recession and clinical attachment loss at interproximal sites of all teeth present. Bleeding on probing (BOP) was recorded at each site (present/absent). The PPD and BOP data were used to calculate the periodontal inflamed surface area (PISA) [23], which is an estimate of the size of the periodontal wound area in mm^2.

Renal assessments

Estimated glomerular filtration rate (eGFR) was derived from the CKD-EPI equation, incorporating both creatinine and cystatin-C [24]. Albuminuria was assessed as an albumin-creatinine ratio (ACR).

Assessment of inflammation and oxidative stress

The systemic inflammatory burden was quantified using C-reactive protein (CRP) as a measure of acute-phase response and serum free light chain concentration as a measure of systemic humoral response. Systemic oxidative stress was quantified using products of oxidative stress induced modulation of proteins, protein carbonyls, and lipids, F2-α-isoprostanes.

Protein carbonyls were assessed by ELISA following the method of Augustyniak et al.[25] Carbonyl content was calculated from a standard curve and expressed as nmol carbonyl per mg of protein. The degree of lipid oxidation was determined as 8-isoprostane F2α levels by EIA method according to manufacturer's instructions (Cayman Chemicals, USA).

Other variables

Patient age in years, sex (male/female), ethnicity (White or non-white), smoking status (current/former/never), diabetic status (yes/no), employment status (yes/no) and educational attainment were ascertained from patients' self-report.

In addition, body measurements were carried out to determine body-mass index (BMI) and venous blood samples were taken to assess glycated haemoglobin (HbA1C) and serum concentrations of calcium, phosphate and bicarbonate. Blood pressure (BP) was measured using the BpTRU automated device (BpTRU Medical Devices, Coquitlam, BC, Canada) which obtains six BP readings after a five-minute rest period. The systolic and diastolic BP are derived from the mean of the second to sixth readings. This method has been reported to be comparable to the mean daytime BP from 24-hour ambulatory BP monitoring [26].

Statistical analysis plan

Regression analyses

In order to fulfil the first and second aims of the study, associations between measures of periodontal inflammation, PISA score, measures of renal function, eGFR, and measures of inflammation and oxidative stress, CRP, total serum FLC concentration, protein carbonyls and isoprostanes, were investigated. These were carried out using multiple linear regression analyses adjusting for a priori selected covariates. These included age, sex, ethnicity, diabetic and smoking status, BMI, BP, and socio-economic status, represented by current employment status and highest educational qualification. In addition, eGFR was added as a covariate in the model investigating the association between periodontal inflammation and measures of systemic inflammation or oxidative stress as the aim was to quantify this association, independent of renal function.

Regression diagnostics were carried out in the form of graphical evaluation for the assumption of homoskedasticity, using residual vs fitted plots, and the assumption of linearity, using fractional polynomial regression. In addition, the assumption of linearity was tested more formally using partial F-tests. Missing data were handled using casewise deletion.

Path analyses using SEM

In order to fulfil the third and final aim of this study, theoretical directed acyclic graphs (DAGs) were developed by discussion among authors (Figures 1 & 2). A SEM was specified based on the DAGs with model parameters estimated using maximum likelihood with missing values to account for missing data. Robust estimations of the standard errors were generated using STATA's "vce (robust)" command. Examination of endogenous variables revealed most to have a log-normal distribution. These were log-transformed to aid the assumption of multi-variate normality. The overall goodness of fit of the models was determined using the coefficient of determination (CD) with values >0.9 indicating good overall fit of the model. If needed, the models were modified and re-fit and the overall goodness of fit was re-examined. Finally, path-dependent estimations of the in/direct standardized effects of the exposure on outcome were obtained for each model and results presented along with the 95% confidence interval.

Models of increasing complexity were considered (* and ** in Figures 1 & 2) by, firstly, including HbA1C and BMI as confounders of the relationship between exposure-mediator and mediator-outcome effects due to the potential impact of glycaemic control and BMI on CRP levels (* in Figures 1 & 2). In addition, the interplay between CRP and oxidative stress was specified by adding a feedback loop between these (** in Figures 1 & 2). As these models were non-recursive, the stability of the models was tested using an Eigenvalue stability index of <1 as an indicator of a stable model.

All analyses were carried out using Stata/IC version 15.1 (StataCorp LLC)

Data sharing

Pseudonymized individual participant data, used in preparation of this manuscript, will be available immediately following publication for a period of 36 months. This will be available to researchers providing a methodologically sound proposal and for the purposes of achieving the aims of that proposal only. Proposals should be directed to the corresponding author. To gain access, researchers will need to sign a data access agreement.

Results

Between October 2010 and December 2015, 770 participants were recruited into the RIISC study, of which 93.9% (n=721) underwent a detailed periodontal examination. Of these, 4% (n=29) were periodontally healthy, one patient had mild periodontitis, 40% (n=287) had moderate periodontitis, 41% (n=296) had severe periodontitis, according to the CDC/AAP (Centre for Disease Control and Prevention/American Academy of Periodontology) classification of periodontitis [27] and 15% (n=108) were edentate (i.e., had lost all natural teeth). Edentulous individuals were excluded from further analysis, yielding a final sample size of 613. The mean age was 60.6 $\pm$ 16 years; 62% were male, 67.6% were White, 50% never-smokers and 36% had diabetes. The mean PISA score for this cohort was 483 mm^2 (S.D. 532 mm^2) (Table 2).

Results from multivariable linear regression analyses

In a model with PISA as the main exposure and eGFR as the main outcome of interest, a 1 S.D. increase in PISA score was associated with a 5.4% (95%CI: 1.4 to 9.4%; p=0.009) decrease in eGFR (Table 3). In a model with eGFR as the main exposure and PISA as the main outcome of interest, an increase of 1 S.D. in eGFR was associated with a 9.8% (95%CI: -0.8 to 19.3%; p=0.068) decrease in PISA.

Similarly, a 1 S.D. increase in PISA score was associated with a 4.8% (95%CI: -7.0 to 18.0; p=0.443) increase in serum CRP and a 0.5% (95%CI: -4.0 to 5.3; p=0.814) increase in total serum FLC concentration, the sum of κ and λ FLC concentration. A 1 S.D. increase in PISA score was associated with a 12.0% (95%CI: 3.2 to 21.6%; p=0.007) increase in F2-α-isoprostanes and an 8.8% (95%CI: 1.4 to 16.6%; p=0.018) increase in protein carbonyls (Table 3).

Results from path analyses using SEM

In the base model investigating the effects of periodontal inflammation on renal function, it was found that periodontal inflammation had a significant direct effect on oxidative stress and, in turn, oxidative stress had a significant, direct effect on eGFR. This pathway produced an overall effect of PISA score on eGFR, mediated via oxidative stress, such that a 10% increase in PISA score led to a 2.9% decrease in eGFR (95% CI: 0.8-5.1%; p=0.011). As there was no significant, direct effect of PISA on CRP or total serum FLC concentration, there was no significant indirect effect of PISA score on eGFR mediated via these (Figure 1, Table 4).

There was no significant direct or indirect effect of eGFR on PISA score (Figure 2, Table 5).

These findings were similar between the models lending some credibility to the integrity of the model (Tables 4 and 5) with the most complex model showing that a 10% increase in PISA score leads to a 3.0% (95%CI: 0.6 to 5.3%; p=0.014) decrease in eGFR.

Discussion

This study demonstrates that, in this cohort of patients, periodontal inflammation is associated with decreased renal function, and increased systemic oxidative stress, F2-α-isoprostanes and protein carbonyls. The causal assumption that periodontal inflammation affects renal function, via increased

systemic oxidative stress, also holds with a 10% increase in PISA score leading to a 2.5% decrease in eGFR.

This study represents the largest of its kind with detailed periodontal phenotyping of patients with CKD at an elevated risk of progressing. Collection of detailed demographic and bio-clinical data allowed for adjustment for factors that might confound the relationship between periodontal inflammation, renal function and systemic inflammatory/oxidative stress markers. This also allowed for comprehensive testing of the causal assumptions made in the SEM. The use of SEM allowed for these associations to be examined simultaneously and the assumptions of the effect of periodontal inflammation on renal function, and vice versa to be investigated. This demonstrated an effect of periodontal inflammation on renal function with a 10% increase in PISA resulting in a 2.5% decrease in renal function. This is the second study to ever report links between serum free light chains, a measure of B-cell activity, and periodontitis, and the first in patients with impaired renal clearance. Using SEM, there was no appreciable direct, causal effect of periodontal inflammation on serum FLC concentration (Table 3). A further strength of this study lies in the use of PISA to quantify periodontal inflammation. Previous researchers have highlighted the limitations of using case definitions of periodontitis, designed for epidemiological purposes, in investigating the association between periodontitis and systemic diseases [28, 29].

There are limitations with this study which should be addressed. Primarily, these arise from the causal assumptions made in the DAGs describing the relationships between periodontal inflammation and renal function (Figures 1&2). The presence, and absence, of variables and arrows, along with the direction of arrows are assumptions based on the available data and current thinking. The authors feel that a comprehensive attempt has been made in the inclusion of variables in the SEM which might confound the exposure-outcome, exposure-mediator or mediator-outcome effects. However, even these comprehensive DAGs do not capture the complexity of the biological interactions at play here. In particular, there may be an effect of renal function on periodontal inflammation, via the effect of renal function on immune function. In the absence of data quantifying participants' immune function, this analysis was not possible.

This study adds considerable weight to other studies and systematic reviews finding an association between renal function and periodontal health [30-33]. A previous, pioneering study exploring the links between periodontitis and CKD, using SEM, based on NHANES III data, demonstrated a bi-directional relationship between the two with a significant direct effect of periodontitis on CKD and vice-versa [21]. Such a bidirectional relationship was not found in this study and this study was unable to replicate previous SEM analyses due to the lack of data on diabetes duration. However, the more bespoke, detailed periodontal and bio-clinical phenotyping of patients in this cohort, compared with the NHANES III survey, allow for more robust testing of the mechanisms by which periodontal inflammation and renal function may influence each other. Therefore, this study provides unique evidence in exploring the pathways by which periodontal inflammation may influence renal function via contribution to the systemic oxidative stress burden. This theory is borne in animal studies showing renal tissue damage linked to oxidative stress following periodontitis [34].

Unlike a previous study [35], this study showed no major effect of periodontal inflammation on serum FLC concentration. This may be because the previous study did not use PISA score as a measure of periodontal inflammation, or more likely, because the previous study was conducted in patients with unimpaired renal clearance of FLCs. This may indicate that the increase is systemic FLC concentration seen with decreased renal clearance of FLCs far out-weighs the contribution of periodontal inflammation to a rise in systemic FLC concentration. This is because sufficient numbers of intact bacteria would have to evade the reticulo-endothelial system to trigger a B-cell response, detectable

in patients with impaired renal clearance of FLCs, which may not be the case. Again, contrary to a more robust body of evidence from previous reports and reviews[15, 16, 36], no appreciable association was noted between periodontal health and markers of non-specific immune response, CRP, in this population. Similarly, no appreciable, direct, causal effect of periodontal inflammation on systemic CRP levels was noted, in this population. This may also be due to the higher levels of CRP in patients with CKD, mean CRP being 7.7 ml/L, and hence this study was underpowered to show a small additional increase in CRP that is found in other cohort of patients with periodontitis [16]. Patients with CKD have a greater relative increase in CRP as compared with protein carbonyls or isoprostanes [37]. The less dramatic increase in markers of oxidative stress, compared with CRP may make it possible for the effects of periodontal inflammation to be more readily detected in changes in oxidative stress levels and less so in CRP levels.

Using data from the largest clinical study of its kind, this study highlights the role of periodontal inflammation, as an occult source of increased oxidative stress, in patients with CKD, which adversely affects renal function. In future, studies collecting data on oxidative stress may be able to confirm and elaborate on these results. Furthermore, in the presence of longitudinal data, the effect of periodontal health on decline in renal function can be elucidated. Even so, it is no known whether treatment of periodontitis will improve the oxidative stress burden in patients with CKD and, ultimately, impact on the morbidity and mortality associated with CKD. If, further, high-quality randomized control trials confirm this mechanistic link, management of periodontal inflammation may form a key part of the medical management of patients with CKD.

Tables

Table 1: Inclusion/Exclusion criteria for RIISC

Inclusion criteria	• Secondary care renal clinic follow-up for at least 1 year prior to recruitment; • CKD stage 3, with either: o eGFR decline ≥5mls/min/year or ≥10mls/min/5years; or o Urinary ACR >70mg/mmol on three occasions; or • CKD stage 4 or 5 (pre-dialysis);
Exclusion criteria	• Renal replacement therapy (dialysis or kidney transplant) • Immunosuppression

Table 2: Baseline demographics of this cohort expressed as mean (SD), unless otherwise stated

		% missing data
Age	60.6 (15.8)	0
Male	62%	0
White Ethnicity	67.6%	
Smoker Never Former Current	 50.2% 34.5% 13.5%	1.8
Diabetic	36%	0
HbA1C (mmols/mol)	49 (17.4)	4.7
BMI (kg/m^2)	30 (7)	3.3
eGFR (ml/min/1.73m^2)	37 (20)	1.95
PISA (mm^2)	483 (532)	0.33
CRP (mg/L)	7.4 (12.8)	0.8
Total serum FLC concentration	108 (187)	0.8
Isoprostane (pg/ml)	26 (20)	26.8
Protein carbonyls (nmol/mg of protein)	1.2 (0.7)	25.9
Currently employed	33%	0.5
Highest Educational Qualification None GCSE NVQ GCE A-Level UG PG	 40% 24% 8% 9% 13% 7%	1.6

GCSE- General Certificate of Secondary Education (aged approximately 16); NVQ- National Vocal Qualification (aged approximately 16–18); GCE A-Level- General Certificate of Education Advanced Level (aged approximately 18); UG- Undergraduate ;PG- Postgraduate

Table 3: Magnitude of change (%) in eGFR and markers of systemic inflammation and oxidative stress associated with a 1 S.D. change in PISA score.

	% change	95%CI	p-value
% increase in			
eGFR	-5.4	(-9.3 to -1.4)	0.009
CRP	4.8	(-7.0 to 18.0)	0.443
Total serum FLC (κ+λ)	0.5	(-4.0 to 5.3)	0.814
F2-α-isoprostanes	12.0	(3.2 to 21.6)	0.007
Protein carbonyls	8.8	(1.4 to 16.6)	0.018

Table 4.
Unstandardized path coefficients from structural equation model depicted in Figure 1

	Model 1				Model 2 (model 1 + HbA1C and BMI as confounders of exposure->mediator and mediator->outcome paths)				Model 3 (model 3 + feedback loop between CRP and Oxidative stress)			
Paths	**Path coeff**	**p-value**	**95%CI lower**	**95%CI upper**	**Path coeff**	**p-value**	**95%CI lower**	**95%CI upper**	**Path coeff**	**p-value**	**95%CI lower**	**95%CI upper**
PISA-->FLC-->eGFR	0.000	0.958	-0.015	0.015	0.000	0.960	-0.015	0.015	0.000	0.959	-0.015	0.015
PISA-->OxStress--> eGFR	-0.026	0.011	-0.047	-0.006	-0.026	0.013	-0.047	-0.006	-0.027	0.014	-0.048	-0.005
PISA-->CRP-->eGFR	0.001	0.431	-0.001	0.003	0.001	0.419	-0.001	0.003	0.001	0.347	-0.001	0.004
PISA-->OxStress--> CRP --> eGFR									0.001	0.324	-0.001	0.002
PISA-->OxStress--> CRP --> OxStress--> eGFR									0.000	0.885	0.000	0.000
PISA-->CRP--> OxStress -->eGFR									0.000	0.873	-0.001	0.001
PISA-->CRP--> OxStress -->CRP--> eGFR									0.000	0.875	0.000	0.000

PISA- periodontal inflamed surface area; FLC- free light chain; ox stress- oxidative stress; eGFR- estimated glomerular filtration rate; CRP- c-reactive protein

Table 5.
Unstandardized path coefficients from structural equation model depicted in Figure 2

	Model 1				Model 2 (model 1 + HbA1C and BMI as confounders of exposure->mediator and mediator->outcome paths)				Model 3 (model 3 + feedback loop between CRP and Oxidative stress)			
Paths	**Path coeff**	**p-value**	**95%CI lower**	**95%CI upper**	**Path coeff**	**p-value**	**95%CI lower**	**95%CI upper**	**Path coeff**	**p-value**	**95%CI lower**	**95%CI upper**
eGFR-->FLC-->PISA	0.016	0.805	-0.112	0.144	0.017	0.798	-0.111	0.145	0.000	0.995	-0.119	0.120
eGFR-->OxStress-->PISA	-0.152	0.208	-0.390	0.085	-0.139	0.279	-0.392	0.113	-0.224	0.070	-0.467	0.018
eGFR-->CRP-->PISA	0.010	0.580	-0.025	0.044	0.009	0.587	-0.025	0.044	-0.002	0.869	-0.028	0.024
eGFR-->Blood_biochem-->PISA	-0.099	0.578	-0.445	0.248	-0.100	0.573	-0.447	0.247	0.000	0.000	0.000	0.000
eGFR-->OxStress-->CRP-->PISA									-0.001	0.890	-0.015	0.013
eGFR-->OxStress-->CRP-->OxStress-->PISA									0.000	0.914	-0.001	0.001
eGFR-->CRP-->OxStress-->PISA									0.018	0.067	-0.001	0.037
eGFR-->CRP-->OxStress-->CRP-->PISA									0.000	0.888	-0.001	0.001

PISA- periodontal inflamed surface area; FLC- free light chain; ox stress- oxidative stress; eGFR- estimated glomerular filtration rate; CRP- c-reactive protein; Blood_biochem- blood biochemistry

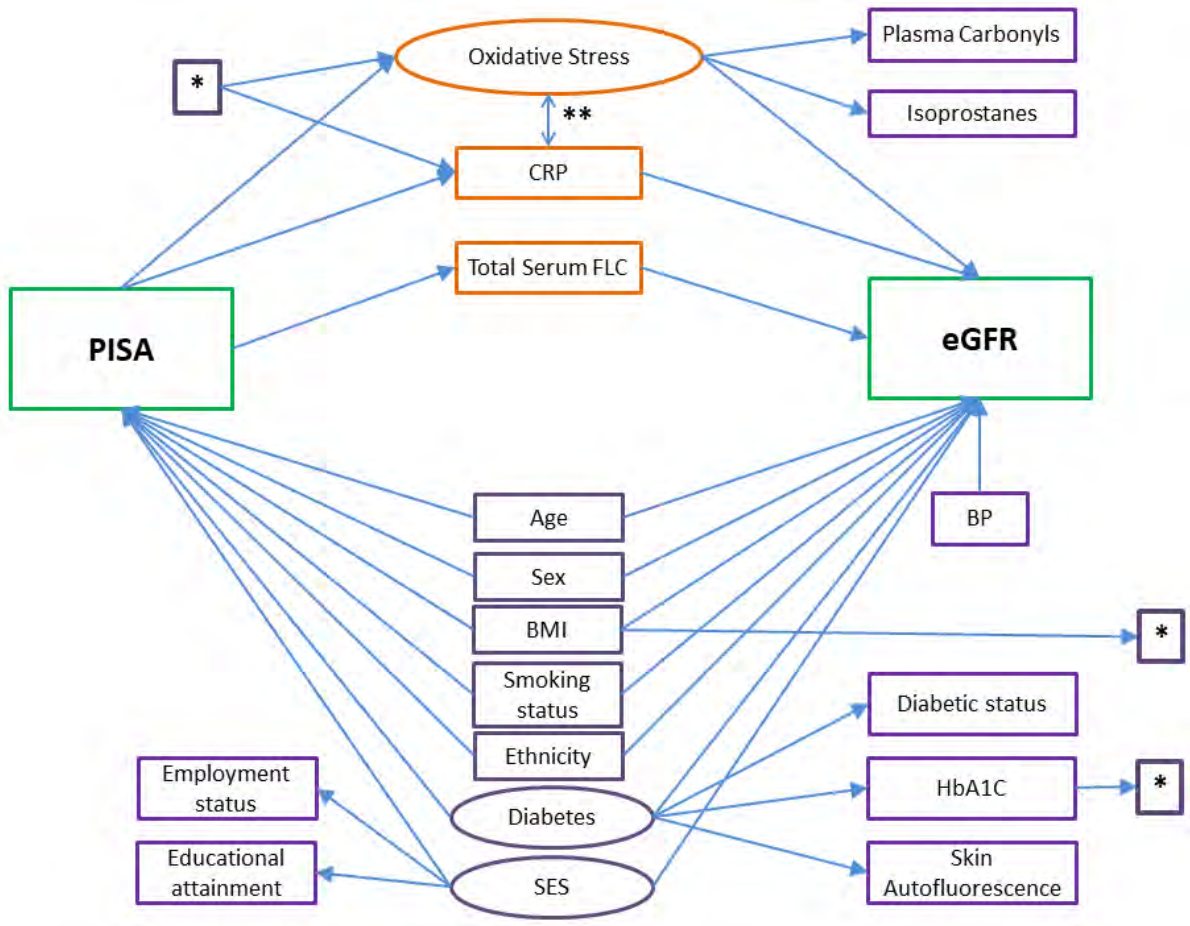

Figure 1: Visual representation of structural equation model with renal function, eGFR, as the outcome.

Rectangles: Observed variables; Ovals: Latent variable; Green: Exposure and outcomes of interest; Purple: confounders; Orange: Mediators

*- paths included in Model 2, in addition to the base model. **- paths included in Model 3, in addition to Model 2.

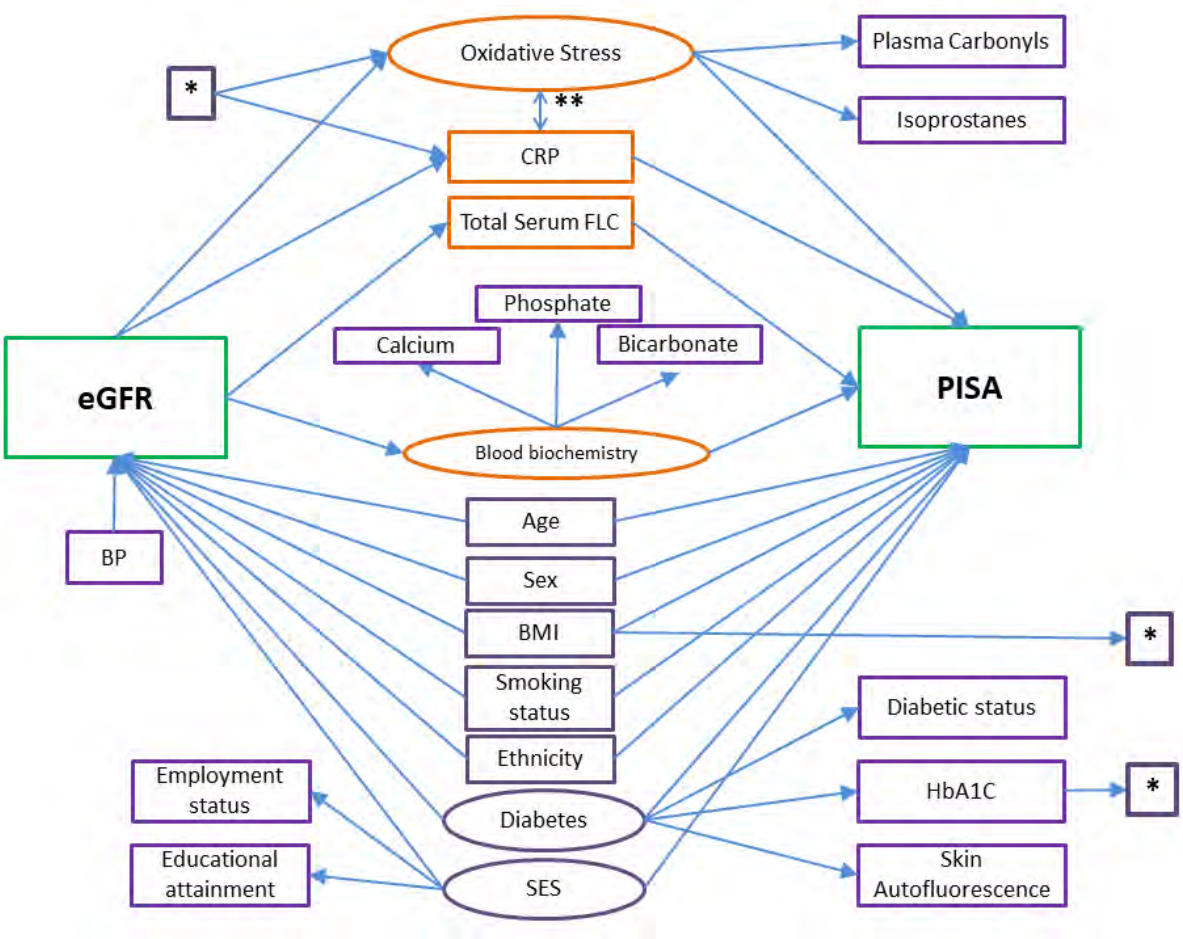

Figure 2: Visual representation of structural equation model with periodontal inflammation, PISA score, as the outcome.

Rectangles: Observed variables; Ovals: Latent variable; Green: Exposure and outcomes of interest; Purple: confounders; Orange: Mediators

*- paths included in Model 2, in addition to the base model. **- paths included in Model 3, in addition to Model 2.

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Trials

STUDY PROTOCOL Open Access

INfluence of Successful Periodontal Intervention in REnal Disease (INSPIRED): study protocol for a randomised controlled pilot clinical trial

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Abstract

Background: Patients with chronic kidney disease (CKD) exhibit increased morbidity and mortality which is associated with an increased systemic inflammatory burden. Identifying and managing comorbid diseases that contribute to this load may inform novel care pathways that could have a beneficial impact on the morbidity/mortality associated with CKD.
Periodontitis, a highly prevalent, chronic inflammatory disease affecting the supporting structures of teeth, is associated with an increased systemic inflammatory and oxidative stress burden and the successful treatment of periodontitis has been shown to reduce both.
This pilot study aims to gather data to inform a definitive study into the impact of successful periodontal treatment on the cardio-renal health of patients with CKD.

Methods/design: This pilot study will employ a randomised, controlled, parallel-group design. Sixty adult patients, with CKD with a high risk of progression and with periodontitis, from the Queen Elizabeth Hospital, Birmingham, will be randomised to receive either immediate, intensive periodontal treatment ($n = 30$) or treatment at a delay of 12 months ($n = 30$). Patients will be excluded if they have reached end-stage renal disease or have received specialist periodontal treatment in the previous year. Periodontal treatment will be delivered under local anaesthetic, on an outpatient basis, over several visits by a qualified dental hygienist at the Birmingham Dental Hospital, UK. Patients in the delayed-treatment arm will continue to receive the standard community level of periodontal care for a period of 12 months followed by the intensive periodontal treatment. Randomization will occur using a centralised telephone randomisation service, following baseline assessments. The assessor of periodontal health will be blinded to the patients' treatment allocation. Patients in either arm will be followed up at 3-monthly intervals for 18 months. Aside from the pilot outcomes to inform the practicalities of a larger trial later, data on cardio-renal function, periodontal health and patient-reported outcomes will be collected at each time point.

Discussion: This pilot randomised controlled trial will investigate the viability of undertaking a larger-scale study investigating the effect of treating periodontitis and maintaining periodontal health on cardio-renal outcomes in patients with CKD.
(Continued on next page)

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Trial registration: National Institute of Health Research (NIHR) Clinical Research Network (UKCRN ID: 18458), ID: ISRCTN10227738. Registered retrospectively to both registers on 23 April 2015.

Keywords: Periodontitis, Chronic kidney disease, Randomised controlled trial, Periodontal treatment, Intervention, Pilot study

Background

Chronic kidney disease (CKD) affects over 13% of the adult population in the United Kingdom [1] and is associated with increasing age [2], hypertension and diabetes [3]. CKD is categorised into five stages, with stage 5 CKD, also known as established renal failure or end-stage renal disease (ESRD), comprising patients who may require renal replacement therapy (RRT) by dialysis or kidney transplantation. In 2009–2010, the annual cost for treatment of patients with stages 3–5 CKD in England was estimated at £1.45 billion, approximately 1.3% of the overall National Health Service (NHS) budget in that period; more than half of this was spent on patients requiring RRT [4].

The primary cause of mortality in patients with CKD is cardiovascular disease (CVD) [5]. Cardiovascular disease mortality in patients with CKD is not only related to the severity of kidney disease but also to an increased systemic inflammatory burden; biomarkers such as C-reactive protein (CRP) and interleukin-6 (IL-6) are reliable predictors of cardiovascular and all-cause mortality in patients with CKD [6, 7].

Consequently, identifying and targeting comorbid disease processes that contribute to systemic inflammation or oxidative stress burden, in patients with CKD, may lead to novel therapeutic approaches to reduce these burdens; an important strategy towards reducing mortality in such patients.

Periodontitis is the most common chronic inflammatory condition in humans [8] and in its severe form is the sixth most common human disease, affecting 11.2% of the global population [9]. Periodontitis is initiated by bacterial accumulation between the gingivae (gums) and teeth, which triggers an inflammatory-immune response within the host. In susceptible individuals, the initial acute inflammatory response fails to resolve and a dysregulated chronic inflammation ensues, which destroys the supporting connective tissues surrounding the teeth. This results in periodontal 'pockets' forming, with chronically ulcerated pocket epithelium exposed to the microbial biofilm (Fig. 1 [10]). In severe disease the surface area of this ulcerated epithelium can be as large as 40 cm^2 [11].

Periodontal inflammation contributes to the systemic inflammatory burden, through acute-phase and oxidative stress pathways [12], as evidenced by increases in CRP, IL-6 and biomarkers of oxidative stress in the serum of patients with periodontitis. Successful periodontal therapy is associated with reductions in these inflammatory mediators [13].

The association between periodontitis and other systemic diseases (particularly CVD) is well established

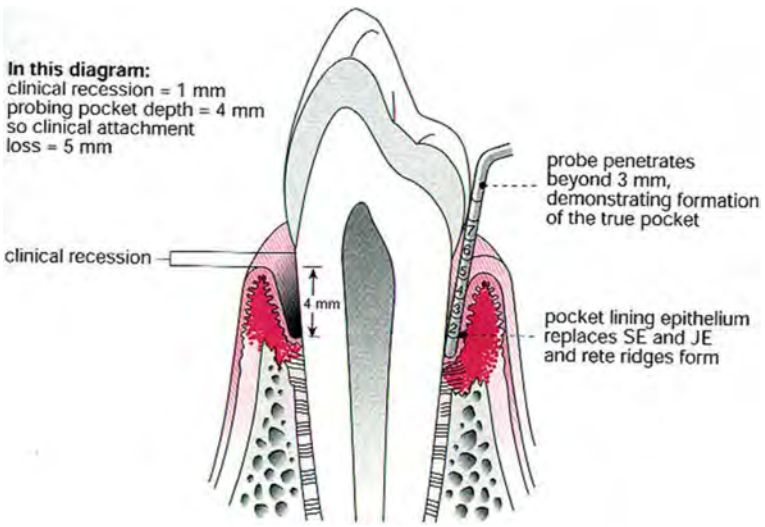


Fig. 1 Anatomy of a tooth and supporting structures depicting destruction of the periodontal architecture due to periodontitis

[14, 15] and was recently reviewed in a joint European and American consensus workshop in periodontology [16].

Periodontitis may act as a comorbid chronic inflammatory disease in patients with CKD, contributing to increased systemic inflammation and the development of CVD. This risk pathway may be amenable to treatment as significant reductions in systemic inflammatory markers (IL-6, CRP) are reported following periodontal therapy in patients with CKD [17].

Periodontitis and CKD

An ongoing longitudinal study investigating novel risk factors in the progression of CKD [18] has reported that patients with CKD at a high risk of progression, had a higher prevalence of periodontitis (odds ratio (OR) 4.0 95% CI 2.7–5.9) or severe periodontitis (OR 3.8 95% CI 2.5–5.6) compared to a local, control population [19].

We have recently analysed the US Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994) database, for associations between periodontitis and mortality in patients with CKD and demonstrated a 10-year all-cause mortality of 41% (95% CI 36–47%) in patients with periodontitis compared with 32% (95% CI 29–35%) in patients without periodontitis [20].

To date, only a limited number of underpowered, non-randomised interventional studies have investigated the effect of periodontal therapy on renal function [21–23]. These studies have not answered the question of whether effective periodontal prevention and treatment may reduce both the morbidity associated with ESRD (dialysis or transplantation) and also the mortality associated with CKD.

Research question

Our long-term, overarching goal is to evaluate whether treatment of periodontitis and periodontal maintenance can reduce renal and cardiovascular morbidity and mortality in patients with CKD.

As the research in this field is lacking, estimates of effect size are not available to adequately inform a sample size calculation. Furthermore, there are methodological considerations that need testing in a small-scale study, prior to embarking on a larger-scale, appropriately powered study. Therefore, the current pilot study was designed.

The specific research questions that this pilot study will address are:

1. Can 60 patients with CKD and periodontitis be recruited, screened and randomised into two treatment arms? What are the challenges in recruitment, screening or the randomisation process that need addressing?
2. Will patients find the intervention and follow-up appointments acceptable?
3. Are the proposed data collection methods acceptable?
4. Are patients willing to attend outpatient clinics for follow-up assessments and complete the trial assessments?
5. Does the data collected allow for the identification of a relevant and practical primary outcome measure for a larger study?
6. What are the barriers to clinical measurements and to collecting, storing and analysing samples?
7. What is an appropriate outcome measure/s to use in a subsequent, larger trial?
8. Are there changes needed in the study design/protocol and/or are there any barriers to a larger-scale study?

Objectives and outcomes

Primary objective

As this is a pilot study, the primary objective is to inform a subsequent definitive trial. The pilot objectives will be achieved in answering the research questions detailed above. Selection of suitable primary and secondary outcomes, from the outcomes of interest listed below will inform subsequent sample size calculations for a pivotal trial.

The outcomes of interest include:

1. Measures of renal function (including estimated glomerular filtration rate (eGFR) and urinary albumin:creatinine ratio (ACR))
2. Measures of cardiovascular function (including blood pressure (BP), pulse wave velocity (PWV))
3. Measures of periodontal health
4. Patient-centred outcomes using the Oral Health Impact Profile-14 (OHIP-14) questionnaire

Methods/design

The INSPIRED (INfluence of Successful Periodontal Intervention In REnal Disease) trial is a randomised, controlled, parallel-group pilot study and designed to address the research questions above. This trial was reviewed and favourably by West Midlands – The Black Country Research Ethics Committee (REC) (REC reference: 15/WM/0006) and is funded by a doctoral research fellowship grant by the National Institute of Health Research (NIHR), UK (grant reference: DRF-2014-07-109). The study is sponsored by the University of Birmingham (ref: RG_14-195). This manuscript is based on the latest version of the INSPIRED protocol (version 2.4, dated 28 Feb 2017) and is subject to

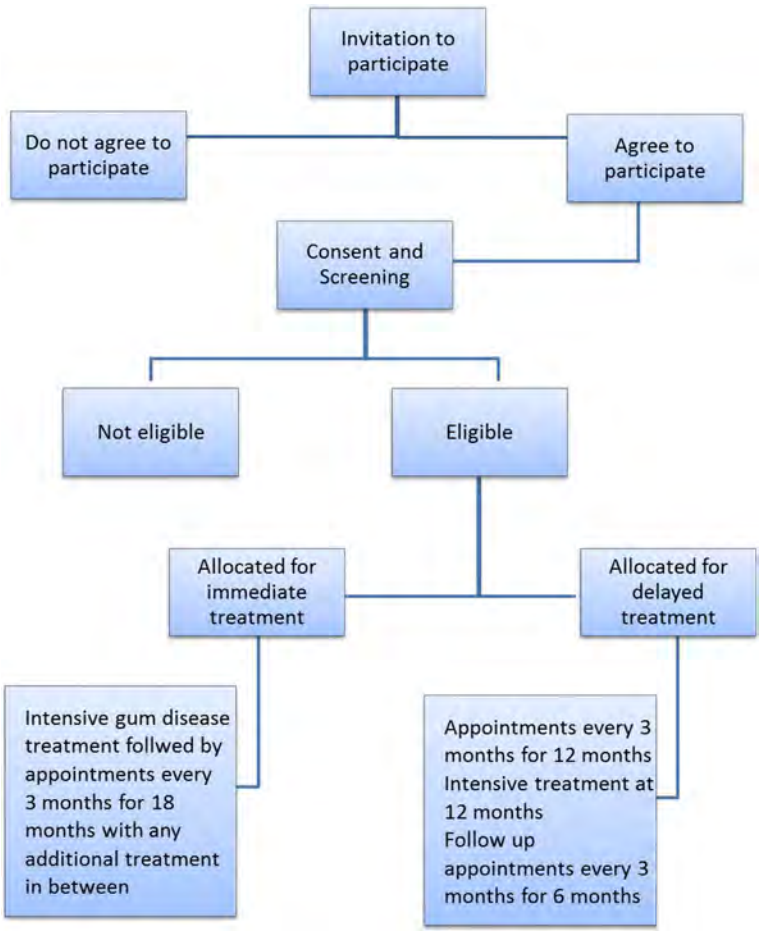


Fig. 2 Flow of patients with chronic kidney disease (CKD) through the INSPIRED trial

change as the trial progresses. Any changes will be communicated to and authorised by the REC. The trial is registered online with the NIHR Clinical Research Network (UKCRN ID: 18458) and has the following ISRCTN identifier: ISRCTN10227738.

Participants

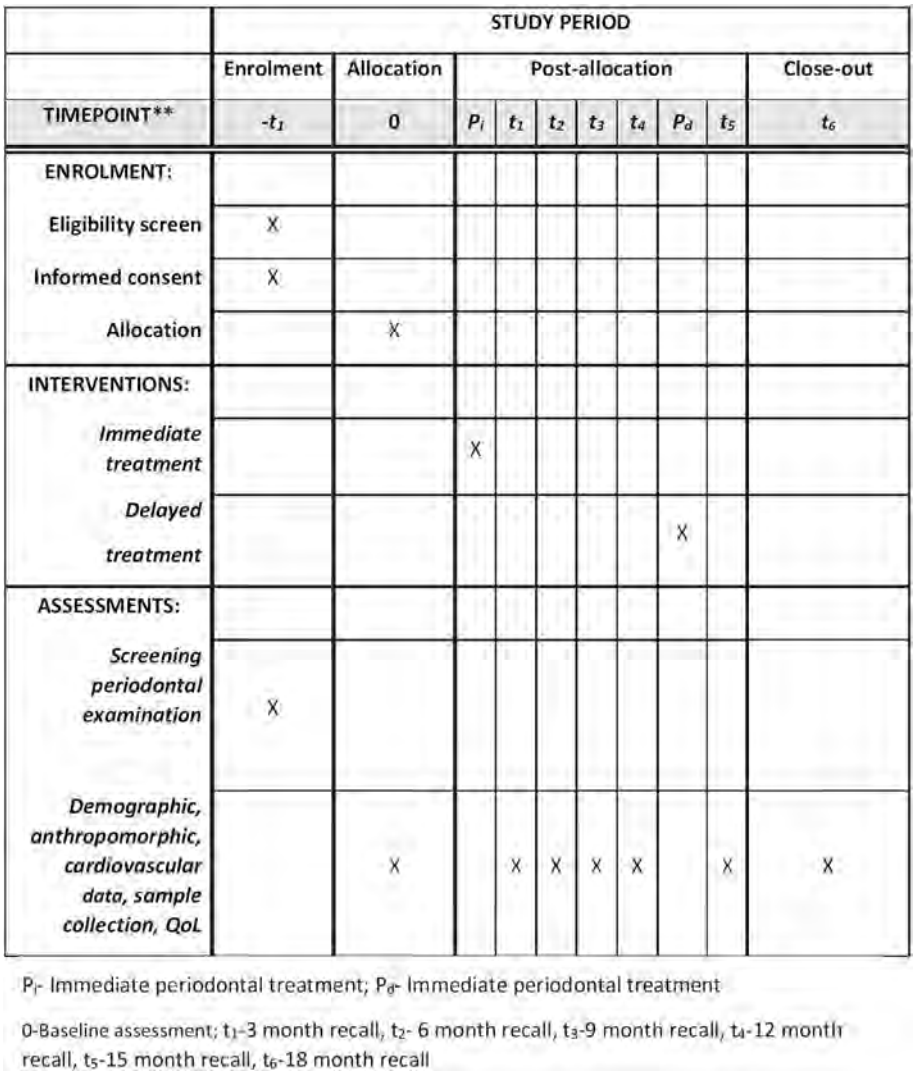
Patients with CKD, with a greater likelihood of progression, as defined in the inclusion/exclusion criteria below, and periodontitis will be invited to participate in the INSPIRED trial. Participants for the INSPIRED trial will be recruited either from an existing, observational study in patients with CKD [24] or from patients with CKD attending clinics affiliated with the Queen Elizabeth Hospital, Birmingham, UK. The patient journey through the trial is illustrated in the flowchart (Fig. 2) as well as the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) Figure (Fig. 3).

Inclusion criteria

1. Patient aged 18 years or older
2. Able to provide informed consent to participate in the trial
3. Secondary care renal clinic follow-up for at least 1 year prior to recruitment
4. High-risk CKD defined as:
   (i) A decline of eGFR of 5 ml/min/year or 10 ml/min/5 years; and/or
   (ii) urinary ACR > 70 mg/mmol on three occasions; and/or
   (iii) CKD stage 4 or 5 (not on dialysis)
5. Generalised moderate-severe periodontitis defined as a minimum cumulative probing depth of 30 mm. This is the sum of the deepest probing pocket per tooth, excluding probing depths < 5 mm

Exclusion criteria

1. ESRD requiring treatment with RRT
2. Receiving immunosuppression
3. Received specialist periodontal treatment in the previous 1 year



	STUDY PERIOD								
	Enrolment	Allocation	Post-allocation						Close-out
TIMEPOINT**	$-t_1$	0	P_i	t_1	t_2	t_3	t_4	P_d	t_5	t_6
ENROLMENT:										
Eligibility screen	X									
Informed consent	X									
Allocation		X								
INTERVENTIONS:										
Immediate treatment			X							
Delayed treatment								X		
ASSESSMENTS:										
Screening periodontal examination	X									
Demographic, anthropomorphic, cardiovascular data, sample collection, QoL		X		X	X	X	X		X	X

P_i- Immediate periodontal treatment; P_d- Immediate periodontal treatment

0-Baseline assessment; t_1-3 month recall, t_2- 6 month recall, t_3-9 month recall, t_4-12 month recall, t_5-15 month recall, t_6-18 month recall

Fig. 3 Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) Figure for the INSPIRED trial

4. Not amenable to periodontal treatment, e.g. severe bleeding disorders that contraindicate periodontal treatment.

Interventions

Immediate-treatment arm

This will consist of patients with CKD and periodontitis who are randomised to the immediate-treatment arm (30 patients). The patients will receive intensive periodontal treatment including oral hygiene instruction, supra and subgingival scaling and non-surgical root surface debridement followed by periodontal maintenance therapy. This will be provided by a qualified, experienced dental hygienist at the Birmingham Dental Hospital, UK. The root surface debridement will be performed on an outpatient basis under local anaesthesia over three or four visits (approximately 45 min each) approximately 1 week apart. Patients will be supported by a maintenance programme, for the duration of the trial, which will include a detailed periodontal examination to identify any recurrent disease early and facilitate any remedial treatment, if indicated.

Delayed-treatment arm

This will consist of patients with CKD and periodontitis who are randomised to the delayed-treatment arm (30 patients). Patients allocated to the delayed-treatment arm will still be eligible to receive the standard of care within the NHS, which is standard community level of periodontal care, as they would if they had not participated in the study [25]. Patients in this arm will have their oral and systemic health closely monitored at 3-monthly intervals for 12 months. After the 12-month review, patients in the control arm will be offered identical intensive periodontal treatment to those in the

immediate-treatment arm. This will be done to facilitate recruitment and retention through assuring patients in the control arm that they will have future access to the same intensive treatment as patients in the intervention arm.

As chronic periodontitis is slowly progressing, usually over decades, there is no provision for modifying the allocation of patients from the delayed-treatment arm to the immediate-treatment arm

Data and sample collection

Data will be collected on the periodontal status of all teeth present using a UNC-15 periodontal probe. The probing pocket depth (PPD) will be measured to the nearest millimetre from the base of the periodontal pocket to the gingival margin and recession will be measured to the nearest millimetre from the cement-enamel junction (CEJ) to the gingival margin (Fig. 1). For each tooth, PPD and recession will be measured at six sites – the mesial, mid and distal aspects of the buccal and palatal/lingual surfaces. The total clinical attachment loss (CAL) will be recorded as the sum of the probing depths and recession. Bleeding on probing (BOP) will be recorded dichotomously (present or absent) for each site probed. A marginal bleeding score will be calculated using bleeding from the gingival margin. A plaque score will be calculated dichotomously as the presence or absence of plaque on four surfaces of each tooth present.

Trial participants will have saliva, subgingival plaque and gingival crevicular fluid (GCF) samples collected and analysed. Collection of saliva and GCF samples will allow assessment of inflammatory and oxidative stress markers in the oral environment. Plaque samples will be collected to gauge changes in the periodontal microbiome over time and following periodontal therapy.

Collection of blood samples from trial participants will allow the assessment markers of renal health, glycated haemoglobin levels, and inflammatory and oxidative stress markers.

Renal health will be measured using the eGFR formula as calculated by the four-variable modification of diet in renal disease (MDRD) equation [26] with serum creatinine that is IDMS (isotope dilution mass spectrometry) traceable. We will also calculate eGFR using the CKD-Epidemiology Collaboration creatinine-cystatin C equation [27] as a sensitivity analysis. As local clinical laboratories are reporting based on MDRD GFR at the time of protocol development, approval and commencement we will use this equation for assessment of the CKD 4 threshold (eGFR < 30 ml/min/1.73 m^2) and report any variances with the CKD-EPI equation.

For assessing the rate of decline of kidney function, each participant will have at least four eGFR results available during the follow-up period to allow an accurate assessment of the rate of change of eGFR with time in patients who have accelerated progression of kidney disease [28]. This approach has now been validated by a number of studies [29, 30]. However, we recognise that this approach may not have the sensitivity to detect decline in kidney function in patients who are sustaining a lower rate of decline of eGFR (e.g. < 2 ml/min/year) which may still have long-term clinical significance, in particular for patients with an eGFR < 30 ml/min. Urine samples will be collected to assess urinary markers of renal disease including ACR.

Cardiovascular health will be measured by measuring blood pressure, the carotid to femoral PWV, a surrogate marker of arterial stiffness [31], and surrogate markers of inflammation (serum CRP, IL-6) and oxidative stress.

Patient-reported outcomes will be collected by conducting an interview with patients before and after periodontal therapy to assess the patient's perception of any benefit. The OHIP-14 questionnaire is a validated short version (14 questions) of the original OHIP-49 questionnaire and will be used to measure the patient perspective of oral health. The questionnaire has good reliability, validity and precision [32].

Other details of participants such as anthropomorphic (height, weight and body measurements), demographic and socioeconomic data will also be assessed.

The planned measurements and samples will allow assessment of their potential as primary or secondary outcome measures in a definitive trial as well as evaluating the collection and processing protocols of such data for a definitive study.

Measurements will take place at the Birmingham Dental Hospital, UK, at baseline and during follow-up appointments as detailed below, for participants in both arms of this trial and will be performed by qualified members of the research team.

Follow-up measurements

Periodontal and cardiovascular measures and biological samples (blood, saliva, urine, subgingival plaque and GCF) will be taken at baseline, prior to initiation of periodontal treatment, and then repeated at 3, 6, 9, 12, 15 and 18 months. Patient questionnaires will also be administered at the same time points.

Review appointments will be timed to allow assessment of the response to periodontal treatment and also allow assessment of the need for reinforcement of oral hygiene instructions and any further periodontal treatment required, in the immediate-treatment arm. Treatment will be performed to a clinical endpoint of periodontal stability, defined as:

1. Ninety percent of patients will have fewer than five sites or fewer than two teeth with PPD ≥ 5 mm at the end of therapy that bleed on probing

2. In 90% of patients, plaque scores will be ≤ 20% at 6 months
3. In 90% of patients, bleeding from the pocket base will be ≤ 10% at 6 months

For patients in the control arm, review appointments will allow for careful monitoring of oral and systemic health. At the 12-month time point, patients in the control arm will be offered intensive periodontal treatment allowing for assessment of their treatment response and reinforcement of oral hygiene instructions at the 15- and 18-month review appointments. Therefore, measurements at the 15- and 18-month time points represent post-treatment follow-up measurements for both arms.

Sample size

Due to a lack of previous research to indicate a reliable primary clinical endpoint, a 'conventional' sample size for a pilot study was chosen to obtain meaningful estimates of effect sizes for the various outcome measures. This sample size is also informed by the prevalence of periodontitis in an existing cohort of patients with high-risk CKD with the same recruitment criteria as employed in this trial [18].

Randomisation

The randomisation uses permuted block-randomisation, with variable block size, stratified by CKD stage (stages 1–3 vs. stages 4–5) and smoking status (never vs. ever). The randomisation code will be held securely at the Birmingham Clinical Trials Unit (BCTU) at the University of Birmingham. After obtaining patients' informed consent and completion of the baseline assessments participants can be randomised into the INSPIRED trial.

Allocation concealment

Allocation concealment of the randomisation of participants in the INSPIRED trial to the immediate- or delayed-treatment arms will be ensured by using a centralised, telephone randomisation service provided by the BCTU. The random sequence will be generated by staff within BCTU and independently of the clinical trial staff. Research nurses involved in the trial will telephone the BCTU and will be informed of the treatment allocation of the patients.

Blinding

The assessor of periodontal health within this study will be blinded to the treatment allocation of the participants as the periodontal care will be provided by an independent operator. Blinding of patients or operator (dental hygienist) is not possible within this interventional trial. Blinding of the assessor of general health will not be possible for logistic reasons. The measurements taken by the assessor of general health, such as BP, PWV, body measurements, etc., are objective and hence will not to be influenced by knowledge of treatment arm. The medical assessor being unblinded negates the need for unblinding in the duration of the trial.

Anticipated compliance issues

The successful maintenance of periodontal health relies heavily upon patients improving and maintaining their oral hygiene. There can be compliance issues associated with attaining and maintaining an adequate level of oral hygiene and home care on the part of the patient. Patients in either arm, following periodontal intervention, will be supported with this through reinforcement of oral hygiene instructions during treatment and follow-up visits along with maintenance periodontal therapy being provided as required.

Compliance with meticulous oral hygiene will be assessed using plaque scores, a dichotomous measure of the presence or absence of supragingival plaque, and bleeding scores, a dichotomous measure of the presence or absence of bleeding on periodontal probing. These scores will be used in individualised biofeedback as patient motivation tools.

With regard to compliance in maintaining appointments, patients will routinely be sent letters and text messages reminding them of their appointments. This will be reinforced by telephone calls to patients. We anticipate a retention rate of over 90% based on other RCTs carried out in the Periodontal Department of the Birmingham Dental Hospital [32]. This rate of retention is also an indicator of good compliance within research participants [33].

Statistical analysis plan

Recruitment and retention

Data on the patients approached to enter the study will be analysed descriptively in terms of number of patients approached, number eligible and number randomised. Reasons for non-entry into the trial will be assessed, particularly in relation to patient eligibility criteria and reasons for patient refusal. Data on patients who do not complete the trial (e.g. withdrawals and those lost to follow-up) will also be collected throughout the study to allow assessment of patient retention rates, and reasons for non-completion of the trial.

Dropouts will be analysed as the number and proportion of patients who did not complete the trial overall, and by trial arm. Reasons for non-completion will be analysed descriptively.

Outcome data

Outcome data collected will be summarised using summary statistics and an exploratory analysis will be performed using an intention-to-treat approach. Continuous

variables will be summarised using means and standard deviations and categorical variables will be summarised using frequency tables. Appropriate graphical methods will be used in conjunction with these.

The differences between the arms in the means and mean change from baseline to each time point will be calculated, along with the 95% confidence intervals. For dichotomous variables, changes in proportions, instead of means, will be analysed over time. This will help to determine the sensitivity of the outcome measures, such as eGFR, ACR, PWV and measures of inflammation or oxidative stress to change following periodontal therapy.

Frequency of analyses

Analyses will be carried out using data from 3, 6, 9, 12, 15 and 18-month review appointments when patients retained in the trial have reached the 18-month review appointment. This will be done at the end of the trial and no formal interim analyses are planned as part of this pilot study.

Dissemination policy

The results from this pilot study will be disseminated via oral and poster presentations in national and international conferences in the dental and medical (renal) disciplines. If applicable, results will also be published as open-access publications in peer-reviewed journals in both the renal and dental communities.

If appropriate, the wider dissemination of these results might take the form of a website for patients and practitioners to access. The European Federation of Periodontology (EFP) website will be utilised, in keeping with the EFP Manifesto (http://www.efp.org/efp-manifesto/manifesto.html), alongside media releases to a reporter with longstanding interest in periodontal and systemic health at Bloomberg News Centre and also via media outputs from the British Society of Periodontology (BSP). The dissemination amongst the renal community will be sought in conjunction with the British Renal Society (BRS).

Discussion

Patients with existing CKD are at an increased risk of progression and mortality, arising primarily from adverse cardiovascular events [5]. The elevated risk is associated with an increase in the systemic inflammatory and/or oxidative stress burden [7, 6], which may be elevated by periodontitis as the treatment of periodontitis has been shown to reduce these systemic markers of inflammation in patients with and without CKD [13, 17].

The present pilot study aims to assess the feasibility of undertaking a larger scale study investigating the effects of successful periodontal treatment and maintenance of periodontal health on cardio-renal function, and ultimately on survival of patients with CKD. If this proves to be beneficial, then periodontal health may be an important factor in the management of patients with CKD.

This study is underway and challenges in recruitment and retention have already informed the management of the trial. Initially, this study was designed to employ a combination of a 'classical' parallel group RCT design and a cohort multiple randomised controlled trial (cmRCT) design [34], with the aim of recruiting patients from an on-going longitudinal cohort study [18] as a pool of eligible patients and controls. However, a greater than anticipated number of medical events, unrelated to periodontal treatment have been occurring in the longitudinal cohort study, resulting in insufficient patient recruitment. In addition to sourcing patients who fit the inclusion criteria from different sites, we also needed to relax the inclusion criteria from a "periodontal health" point of view. Initially, our data suggested sufficient patients would have disease that was sufficiently severe to allow for a threshold of cumulative probing depth of 40mm. This was relaxed to a cumulative probing depth of 30mm to allow for more patients to be eligible. The disadvantage of lowering the threshold is that, if a dose-dependent relationship exists between extent and severity of periodontitis and systemic ill-health, the treatment of periodontitis in such patients is likely to produce a lower magnitude of improvement in outcome measures than patients with more severe periodontitis. A change was also made in the total number of participants in each arm of the study. Initially, our data indicated 50 patients per arm would be achievable. This was scaled back to 30 patients per arm in response to the lack of eligible patients in the longitudinal cohort study. This will still be sufficient to inform a power calculation for a larger, multi-centre trial if such a trial appears to required.

Trial status

As of September 2017, this trial is recruiting participants.

Abbreviations

ACR: Albumin:creatinine ratio; BCTU: Birmingham Clinical Trials Unit; BOP: Bleeding on probing; CAL: Clinical attachment loss; CEJ: Cemento-enamel junction; CKD: Chronic kidney disease; CONSORT: Consolidated Standards of Reporting Trials; CRP: C-reactive protein; CVD: Cardiovascular disease; eGFR: Estimated glomerular filtration rate; ESRD: End-stage renal disease; GCF: Gingival crevicular fluid; IDMS: Isotope dilution mass spectrometry; IL-6: Interleukin-6; INSPIRED: INfluence of Successful Periodontal Intervention on REnal Disease; NHS: National Health Service; OHIP: Oral Health Impact Profile; PPD: Probing pocket depth; RCT: Randomised controlled trial; REC: Research Ethics Committee; RRT: Renal replacement therapy

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Availability of data and materials
Not applicable.

Authors' contributions
All authors were involved in the trial design and have read and approved the final manuscript. PS is supervised as a doctoral research fellow in this project by PC, TD, NI, CF and ILC.

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Professor Iain Chapple is the Head of the School of Dentistry and Head of the Periodontology Department at the University of Birmingham School of Dentistry.

Ethics approval and consent to participate
This trial was reviewed and given a favourable opinion by West Midlands – The Black Country Research Ethics Committee (REC) (REC reference: 15/WM/0006). Participants will sign an Informed Consent Form prior to participation.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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To whom it may concern:

The following are statements of Mr Praveen Sharma's specific contribution, **in addition to manuscript preparation and acting as corresponding author**, to the following manuscripts:

1. Dietrich T, Sharma P, Walter C, Weston P, Beck J. The epidemiological evidence behind the association between periodontitis and incident atherosclerotic cardiovascular disease. Journal of Clinical Periodontology. 2013;40:S70-S84.

PS was responsible for the search strategy, reviewing of titles and abstracts. In addition, PS was responsible for reviewing the full texts, generation and piloting of data extraction form and data extraction.

2. Sharma P, Busby M, Chapple L, Matthews R, Chapple I. The relationship between general health and lifestyle factors and oral health outcomes. British dental journal. 2016;221(2):65-9.

PS was responsible for data management and statistical analysis.

3. Sharma P, Yonel Z, Busby M, Chapple I, Dietrich T. Association between periodontal health status and patient reported outcomes in patients managed in a non-specialist, general dental practice. Journal of Clinical Periodontology. 2018.

PS was responsible for data management, statistical analysis plan and statistical analysis.

4. Sharma P, Dietrich T, Ferro CJ, Cockwell P, Chapple ILC. Association between periodontitis and mortality in stages 3-5 chronic kidney disease: NHANES III and linked mortality study. Journal of Clinical Periodontology. 2016;43(2):104-13.

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PS was responsible for data collection, inputting, management and statistical analysis.

6. Sharma P, Fenton A, Dias IHK, Heaton B, Sidhu A, Rahman M, et al. Periodontal Inflammation Influences Renal Function in Patients With Chronic Kidney Disease. (Manuscript in Preparation). 2018.

PS was responsible for data management, statistical analysis plan and statistical analysis.

7. Sharma P, Cockwell P, Dietrich T, Ferro C, Ives N, Chapple ILC. INfluence of Successful Periodontal Intervention in REnal Disease (INSPIRED): study protocol for a randomised controlled pilot clinical trial. Trials. 2017;18(1):535-45.

PS was responsible for trial design and is currently the principal investigator on this trial.

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