Treatment of periodontitis reduces systemic inflammation in type 2 diabetes

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ABSTRACT

Aims: To assess the impact of periodontal treatment on systemic inflammation in type 2 diabetes.

Materials and Methods: Adults with type 2 diabetes (n=83) and without diabetes (controls, n=75) were recruited, and participants with periodontitis received periodontal treatment and 12 months' follow-up. Biomarkers for periodontal inflammation (gingival crevicular fluid interleukin-6, tumour necrosis factor-α, interleukin-1β, interferon-γ, matrix metalloproteinase-8, matrix metalloproteinase-9, adiponectin) and serum markers of inflammation and diabetes control (glycated haemoglobin, high sensitivity C-reactive protein, interleukin-6, tumour necrosis factor-α, interleukin-1β, interferon-γ, leptin, adiponectin) were measured. Structural equation modelling was used to evaluate periodontal treatment effects on oral and systemic inflammation.

Results: Periodontal treatment resulted in significant improvements in clinical status and reductions in gingival crevicular fluid biomarkers from baseline to month 12. Structural equation modelling identified that, at baseline, individuals with diabetes and periodontitis had significantly higher systemic inflammation than non-diabetic controls with periodontitis (Δ=0.20, p=0.002), with no significant differences between groups for oral inflammation. There was a greater reduction in systemic inflammation following periodontal treatment in individuals with diabetes and periodontitis compared to those with periodontitis but not diabetes (Δ=-0.25, p=0.01).

Conclusions: Diabetes and periodontitis together appear to increase systemic inflammation, with evidence of reductions following periodontal treatment.

Keywords (MeSH terms): inflammation; periodontitis; diabetes mellitus; diabetes mellitus, type 2

CLINICAL RELEVANCE

Scientific rationale for the study

The bidirectional association between periodontitis and type 2 diabetes is likely mediated by inflammation, though precise mechanisms are, as yet, unclear.

Principal findings

Individuals with both periodontitis and diabetes have significantly higher systemic inflammation than those with periodontitis but not diabetes. Treatment of periodontitis results in significantly greater reductions in systemic inflammation in people with diabetes and periodontitis compared to those with periodontitis but not diabetes.

Practical implications

Assessment of periodontal status should be routine in diabetes care, and treatment of periodontitis in people with diabetes should be a core component of diabetes management.

Word count: 99 (max 100)

INTRODUCTION

Advanced periodontitis is the 6th most common disease worldwide, with prevalence of 11.2% (Kassebaum et al., 2014). Periodontitis is characterised by inflammation affecting the tooth supporting tissues (gingiva, periodontal ligament, alveolar bone) resulting in progressive tissue breakdown (periodontal attachment loss, alveolar bone resorption), tooth mobility, tooth loss, and concomitant effects on oral function and life quality (Buset et al., 2016). Diabetes is also highly prevalent, with worldwide prevalence of 9% (International Diabetes Federation, 2019). The bidirectional links between periodontitis and type 2 diabetes are clear, with increased risk of periodontitis (2-3 fold) in individuals with diabetes, and poorer glycaemic control and increased risk of diabetes complications in people with diabetes and advanced periodontitis (Borgnakke et al., 2013, Preshaw et al., 2012, Saremi et al., 2005, Shultis et al., 2007). Meta-analyses and Cochrane reviews have consistently identified that periodontitis treatment results in reductions in HbA1c of 3-4 mmol/mol (0.3-0.4%) (Engebretson and Kocher, 2013, Madianos and Koromantzos, 2018, Simpson et al., 2010, Simpson et al., 2015), as well as improvements in atherosclerotic profile and endothelial function (Teeuw et al., 2014, Tonetti et al., 2007).

It is likely that inflammation plays a major role in linking periodontitis and diabetes, though precise mechanisms are not yet clear. Untreated periodontitis contributes to elevated circulating levels of pro-inflammatory mediators such as interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α), as well as C-reactive protein (CRP) and oxygen radicals (Polak and Shapira, 2018). Systemic inflammation can also result from entry of periodontal bacteria and their virulence factors into the circulation, with activation of oxidative-stress mediated pathways and interactions between advanced glycation end-products and their receptor contributing to increased susceptibility to periodontitis in people with diabetes (Borgnakke et al., 2013, Chapple et al., 2013). Whereas many studies have evaluated the role of single, or limited numbers of, inflammatory mediators in linking periodontitis and diabetes, it is clear that a more holistic approach is required (Taylor et al., 2013). To begin to address this issue, we evaluated periodontal and systemic inflammation in patients with and without diabetes according to periodontal status. Furthermore, we investigated the effects of periodontitis treatment on periodontal and systemic inflammation in individuals with and without diabetes.

MATERIALS AND METHODS

Study design

This was a descriptive study of clinical and biochemical outcomes following periodontitis treatment in individuals with diabetes and matched non-diabetic controls. Following written informed consent, adults with type 2 diabetes were recruited from secondary care diabetes clinics in Newcastle-upon-Tyne and Gateshead (UK), and age- and gender-matched adults who did not have diabetes (controls) were recruited from periodontology clinics of the Newcastle-upon-Tyne Dental Hospital. Inclusion criteria were male or female adults, aged 30-55 years, with at least 20 natural teeth. Exclusion criteria included immunosuppression, pregnancy, conditions requiring prophylactic antibiotics prior to dental treatment, bleeding disorders, medical conditions that may affect study participation (e.g. unstable cardiovascular disease), chronic inflammatory conditions linked to periodontitis (e.g. rheumatoid arthritis), periodontal treatment in the past 6 weeks. The study was conducted in accordance with the guiding principles of the Declaration of Helsinki and was approved by the UK National Research Ethics Service (Sunderland Local Research Ethics Committee, reference 6/Q0904/8).

Periodontal examination and therapy

Participants underwent periodontal examination by trained and calibrated examiners at baseline (month 0). Blinding to diabetes/periodontal status was not possible due to limited examiner numbers. Assessments included plaque index (Silness and Loe, 1964), modified gingival index (Lobene et al., 1986), full mouth probing depths and bleeding on probing (BOP) recorded at 6 sites per tooth with a UNC-15 periodontal probe. Mean gingival index, plaque index and probing depths were calculated, together with %BOP and percent sites with probing depth ≥5mm. Participants were assigned a diagnosis of: periodontal health (no probing depths >4mm, BOP <15%, no attachment loss); gingivitis (no probing depths >4mm, BOP ≥15%, no attachment loss); or periodontitis (≥6 sites with probing depth ≥5mm at separate teeth, with attachment loss and radiographic alveolar bone loss), commensurate with a clinical diagnosis of moderate-to-severe chronic periodontitis using the 1999 classification (Armitage, 1999). Individuals with periodontal health or gingivitis participated only at baseline. Those with periodontitis received non-surgical periodontal therapy (root surface debridement of the whole dentition) using manual and ultrasonic instruments with local anesthesia over 1-2 sessions within 1 week, plus oral hygiene instruction. Early periodontal maintenance follow-

up appointments were provided at 3 and 6 weeks, including prophylaxis and reinforcement of oral hygiene as indicated clinically. Periodontal maintenance appointments with oral hygiene instruction and prophylaxis were provided at 3, 6 and 12 months, with recording of periodontal indices as outlined above.

Metabolic and biochemical analyses

Venous blood samples were obtained from all participants at baseline, and from those with periodontitis 3, 6 and 12 months following treatment. Samples were analysed in the Newcastle-upon-Tyne Hospitals Clinical Biochemistry department for HbA1c and high sensitivity CRP (hsCRP). Clotted blood samples were centrifuged (1500g, 15 min, 4^{o}C) for serum separation, and 0.5ml serum aliquots were frozen at -80^{o}C for storage prior to analysis by laboratory staff unaware of the clinical diagnosis. Gingival crevicular fluid (GCF) samples were obtained at baseline (all participants), and at months 3, 6 and 12 (participants with periodontitis) to assess periodontal inflammation. Following isolation and drying of sites, four GCF samples were collected (30 s sampling technique) using Periopaper strips (Oraflow Inc, New York, USA). Periopapers were placed into sterile 0.5 ml micro-tubes (Sarstedt, Leicester, UK) containing 150 µl sterile phosphate buffered saline (PBS), and frozen at -80^{o}C for storage. Before analysis, GCF was eluted from Periopapers by thawing on ice for 15 min and addition of 50 µl 1% bovine serum albumin in PBS, followed by centrifugation (1500g for 60 min, then 13,000g for 2 min, at 4^{o}C) (Wassall and Preshaw, 2016).

IL-6, TNF-α, IL-1β and interferon-γ (IFN-γ) concentrations in serum and pooled GCF samples were determined with multiplex assays (MesoScale Discovery, Maryland, USA) with sensitivities of 0.76 pg/ml, 0.96 pg/ml, 0.78 pg/ml and 1.8 pg/ml, respectively. Leptin and adiponectin in serum, and adiponectin in GCF were determined using commercial ELISA kits (R&D Systems, Abingdon, UK) with sensitivities of 5.6 pg/ml and 1.0 pg/ml, respectively. GCF matrix metalloproteinase-8 (MMP-8) and MMP-9 were measured using commercial ELISA kits (R&D Systems), with sensitivities of 10 pg/ml and 4 pg/ml, respectively.

Statistical analyses

The study was powered to evaluate change in HbA1c following periodontal therapy in people with diabetes and periodontitis. Based on available assumptions, we estimated that 27 individuals with periodontitis would provide 85% power to detect a difference in mean HbA1c of 0.6% (Janket et al., 2005) assuming the SD of change scores was 1.0% (two-sided alpha level 0.05, paired comparisons). Data were analysed using Stata 16 (StataCorp LP, College Station, TX, USA). Descriptive statistics included medians (interquartile range) and frequency data. For demographics, significance of differences between diabetes categories were determined using Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables. Significance of differences between periodontal category groups within the diabetes and no diabetes groups were determined using the Wilcoxon rank-sum test with post-hoc Bonferroni adjustments for multiple comparisons; the adjustment factor was determined by the number of relevant comparisons per response (only certain pairwise comparisons were considered relevant). Significance of differences between parameters at follow-up time points relative to baseline within diabetes groups were determined using Wilcoxon matched-pairs test.

For participants with periodontitis, structural equation modelling (SEM) was utilised to construct two latent variables (hypothetical constructs) at baseline; *systemic inflammation* and *oral inflammation* (Figure 1) (Kline, 2011). Systemic inflammation comprised 7 serum variables (IL-6, TNF-α, IL-1β, IFN-γ, adiponectin, leptin, hsCRP). Oral inflammation comprised 9 variables, including 7 GCF biomarkers (IL-6, TNF-α, IL-1β, IFN-γ, adiponectin, MMP-8, MMP-9) and 2 clinical variables (%BOP, mean probing depth). For biochemical variables, zero values were replaced with their minimum nonzero value (at month 0) and variables were log-transformed to achieve normality. SEM was also used to evaluate differences between 12 month changes, utilising change scores from baseline to month 12 for each indicator to calculate latent variable values reflecting changes.

RESULTS

Table 1 presents demographic characteristics. 83 adults with type 2 diabetes and 75 adults without diabetes were recruited. There were no significant differences between groups at baseline for age, gender, ethnicity or smoking, though the median body mass index (BMI) of people with diabetes was significantly higher compared to non-diabetic controls ($p<0.001$). Supplementary Table S1 details the

medications used by the patients for their diabetes control. Table 2 shows baseline data for the two groups according to periodontal status, comprising 6 subgroups; diabetes/periodontal health, diabetes/gingivitis, diabetes/periodontitis, no diabetes/periodontal health, no diabetes/gingivitis, and no diabetes/periodontitis. Adults with diabetes showed some evidence of differences in HbA1c between diabetes/periodontal health [45 (34) mmol/mol, 6.3 (3.1)%] [median (interquartile range)], diabetes/gingivitis [53 (20) mmol/mol, 7.0 (1.8)%] and diabetes/periodontitis [59 (25) mmol/mol, 7.5 (2.3)%], though these were not significant. hsCRP levels were significantly higher in the no diabetes/periodontitis group compared to no diabetes/periodontal health ($p<0.05$), though no other significant differences between groups were identified. No significant differences in baseline serum IL-6 or TNF-α concentrations were noted between groups.

When considering clinical periodontal indices at baseline (Table 2), as would be expected, within the diabetes/no diabetes groups, individuals with periodontal health had significantly lower gingival and plaque index scores than those with gingivitis or periodontitis (all $p<0.05$). Gingival and plaque index scores were significantly higher in the diabetes/gingivitis group than in the no diabetes/gingivitis group ($p<0.05$). Mean probing depth and %BOP were highest for individuals with periodontitis, lower for those with gingivitis, and lowest for those who were periodontally healthy, with no significant differences between diabetes groups within periodontal status categories. More sites with probing depth ≥5mm were present in the no diabetes/periodontitis group [19.5 (22.6)%] compared to the diabetes/periodontitis group [11.4 (12.1)%], though this was not statistically significant. In the non-diabetic patients, GCF IL-6, TNF-α and IFN-γ levels were significantly higher in the periodontitis groups compared to the periodontally healthy or gingivitis groups, with no evidence of differences between diabetes groups. GCF IL-1β levels were significantly higher in the diabetes/periodontitis and no diabetes/periodontitis groups, compared to respective health and gingivitis groups (all $p<0.05$). Mostly, GCF MMP-8, MMP-9 and adiponectin levels were significantly higher in individuals with periodontitis compared to those with periodontal health and gingivitis, with no particular evidence of differences between diabetes groups, except for adiponectin which was significantly higher in the no diabetes/periodontitis group compared to the diabetes/periodontitis group.

Table 3 presents 12-month longitudinal data for individuals with periodontitis. At baseline, 32 people with diabetes and 44 non-diabetic controls were identified as having periodontitis; 27 people with diabetes and 36 controls completed the study to month 12 (lost to follow-up, i.e. early drop-outs: 5 people with diabetes, 8 controls). Among those with diabetes and periodontitis, HbA1c at month 12 [54 (19) mmol/mol, 7.1 (1.7%)] was not significantly different from baseline [59 (25) mmol/mol, 7.5 (2.3)%] ($p=0.08$). In this group, statistically significant reductions in serum TNF-α were noted from baseline to months 3 and 6, but not month 12. Furthermore, among non-diabetic controls, statistically significant increases in serum TNF-α were noted from baseline to months 6 and 12 (both $p<0.001$).

When considering longitudinal changes in clinical indices (Table 3), statistically significant improvements in mean gingival index, plaque index, probing depth, % sites ≥5mm, and %BOP were noted in patients with periodontitis (both those with diabetes and non-diabetic controls) from baseline to all post-treatment time-points (all $p<0.05$). Analysis of differences in change data from baseline to all post-treatment time-points revealed no significant differences between groups (data not shown), indicating a comparable treatment response in both groups. Reductions in GCF biomarker levels from baseline to all post-treatment time-points were consistent in both groups; broadly, significant reductions in GCF IL-6, TNF-α, IL-1β, MMP-8, MMP-9 and adiponectin were identified from baseline to month 12 (all $p<0.01$), with additionally a significant reduction in GCF IFN-γ levels in the controls from baseline to month 12 ($p<0.001$).

Figure 2 presents results of the SEM analysis. At baseline, individuals with diabetes and periodontitis had significantly higher systemic inflammation compared to non-diabetic controls (with periodontitis) as assessed using the latent variable *systemic inflammation* ($\Delta=0.20$, $p=0.002$), whereas there were no significant differences between these groups for *oral inflammation* (panel A2). Additionally, for individuals with diabetes and periodontitis, a significant, positive correlation ($r=0.58$, $p=0.02$) was observed between oral and systemic inflammation, while there was no significant correlation for non-diabetic controls with periodontitis (panel A1). Change data between months 0 and 12 indicated that the individuals with diabetes and periodontitis demonstrated a significantly greater reduction in systemic inflammation compared to the non-diabetic controls with periodontitis ($\Delta=-0.25$, $p=0.01$), whereas there was no difference in change data between the groups for oral inflammation ($\Delta=0.06$,

p=0.53) (panel B2). To allow interpretation of this difference in change data (Δ=-0.25), its effect size (Δ/SD) relative to the population SD (0.32) was calculated (-0.79). Given the pre-existing differences in BMI between patients with and without diabetes, we performed additional analyses to investigate whether BMI might be an explanatory variable in the SEM (panels A3, B3). Regression by group with an interaction of BMI resulted in no significant effects of BMI on the SEM outcomes, whether evaluating BMI effects separately for individuals with diabetes and controls, or their averaged effect. The regression analyses confirmed the SEM findings, identifying that for change data in systemic inflammation, the effect of diabetes vs. no diabetes was statistically significant, with no impact of BMI on outcomes.

DISCUSSION

A review of evidence regarding pathogenic mechanisms linking periodontitis and diabetes suggested that inflammation is likely to link the two diseases, and that longitudinal studies are required to investigate this further (Taylor et al., 2013). However, many studies have been cross-sectional in design or focused on single, or low numbers of inflammatory mediators, and such a restricted approach only provides limited information. To begin to address this issue, SEM has been applied for the first time in the context of periodontitis and diabetes, to assess the relationship between oral and systemic inflammation. This powerful technique allowed us to represent, holistically within the experimental design, hypothetical constructs representing oral and systemic inflammation, and the changes occurring following therapy. The study showed that while post-treatment changes in oral inflammation were comparable in periodontitis sufferers with and without diabetes, periodontitis treatment resulted in significantly greater reductions in systemic inflammation in individuals with diabetes and periodontitis compared to those with periodontitis alone after 12 months. Additionally, we identified a positive correlation between oral and systemic inflammation that was only present in the diabetes group and not in controls. Given the baseline differences in BMI between individuals with diabetes and non-diabetic controls, we considered that BMI might be a potential confounding factor in the SEM; additional analyses showed this was not the case, with no impact of BMI being observed on the reductions in systemic inflammation between the groups.

Our study was originally powered to identify changes in HbA1c following periodontal therapy (Janket et al., 2005) that, with consideration of current evidence from systematic reviews, would now be regarded as being overly optimistic. In the individuals with diabetes and periodontitis, there was a non-significant change in HbA1c of around 5 mmol/mol (0.4%). Although this is similar in magnitude to reductions in HbA1c reported in meta-analyses (Madianos and Koromantzos, 2018, Simpson et al., 2015), we recognise that from the viewpoint of assessing HbA1c change following periodontal therapy, our study was underpowered. On the other hand, the application of SEM in the analysis of multiple local and systemic biomarkers has enabled us to identify a potential benefit of periodontal treatment in reducing systemic inflammation in people with diabetes.

Considerable variability in local (GCF and saliva) and systemic biomarker data in patients with diabetes and periodontal conditions has been reported previously (Kardesler et al., 2010, Kardesler et al., 2011, Navarro-Sanchez et al., 2007, Santos et al., 2010). Indeed, as indicated in Table 3 of the present study, it can be challenging to interpret changes in individual biomarkers over time. The use of SEM offered an alternative to potentially overcome some of these problems. We consider that our biomarker data (GCF and serum) are consistent with previous reports in respect of both absolute values, and variability of the data (Taylor et al., 2013). We also recognize that some of the mediator levels were close to, or below, the assay limits of sensitivity and should be interpreted with caution. It is clear that selecting any of the mediators in isolation would not necessarily be informative in improving understanding of the links between the two diseases. A similar study of people with diabetes and periodontitis identified no significant individual changes in eight serum biomarkers over 6-months following periodontal therapy (Geisinger et al., 2016). In a randomized controlled trial (RCT) comparing two treatments for periodontitis in patients with type 2 diabetes, whereas a significant difference in TNF-α levels was noted between groups following treatment, no significant differences in other inflammatory biomarkers were observed (D'Aiuto et al., 2018).

Recently, comprehensive and high resolution studies of systemic inflammation in periodontitis using mass cytometry immunoassays have revealed substantial ‘immune system dysfunctions’ in patients with periodontitis as compared to periodontally healthy individuals (Gaudilliere et al., 2019). Intriguingly, the differences lie with innate immune signalling and responses rather than in the

proportions of cellular elements; this endorses the value of candidate studies of key inflammatory mediators in investigating the influence of oral inflammation on systemic health. Significantly, systemic parameters of inflammation reverted to values more closely associated with periodontal health after periodontal treatment, providing strong evidence that systemic immune processes are indeed influenced by oral inflammation (Gaudilliere et al., 2019). It will be interesting to apply these novel analytical and bioinformatic approaches to studies of diabetes and periodontitis.

Limitations include that this was an observational, descriptive study rather than a RCT, in which we selected the mediators of interest based on our existing knowledge of candidate biomarkers (whereas there may be other pro- and anti-inflammatory mediators that are relevant in linking diabetes and periodontitis). Furthermore, within the study design, we could not specify any impact of smoking on the outcomes, and future studies may address separately the role of smoking (and potential effects on systemic inflammation) in relation to periodontitis and diabetes. However, strengths include the precise clinical phenotyping, a high standard of technical analysis including quality control assessments, and a range of local and systemic inflammatory mediators that were assessed cohesively using a multivariate statistical technique.

The outcomes of treatment in the individuals with periodontitis were consistent with those previously reported (Cobb, 2002), indicating a good response to therapy. The patients with diabetes and periodontitis presented with less advanced disease (fewer sites with probing depth ≥5mm, non-significant difference) than the non-diabetic controls with periodontitis, whereas the expectation is usually that diabetes increases extent and severity of periodontitis. This finding may be a manifestation of the recruitment strategy, in that non-diabetic controls were recruited from dental hospital clinics where they had been referred for management of periodontitis, whereas people with diabetes were recruited from diabetes clinics with no *a priori* knowledge of their periodontal status. Ideally, future studies should attempt to match groups for periodontitis extent and severity. When considering the number of sites with probing depth ≥5mm, this reduced from 11.4% at baseline to 3.1% at month 12 in the patients with periodontitis and diabetes (73% reduction), and from 19.6% to 4.6% in the controls (77% reduction), suggesting that periodontal treatment was similarly effective in individuals with and without diabetes. It is noteworthy that although there were no statistically

significant differences in %BOP between diabetes and control groups in any comparisons, %BOP was higher in the diabetes/gingivitis group than the no diabetes/gingivitis group at baseline (Table 2, non-significant difference), as well as at month 12 in the diabetes/periodontitis group compared to controls (Table 3, non-significant difference). Also, within the gingivitis categories (representing a tightly constrained classification of gingival inflammation), gingival and plaque index scores at baseline were significantly higher in individuals with diabetes compared to controls. These observations potentially suggest an increased susceptibility to oral inflammation in individuals with diabetes, a finding reported previously (Lalla et al., 2006) and which merits further investigation.

Recently, the European Federation of Periodontology and International Diabetes Federation established a workshop on periodontitis and diabetes (Sanz et al., 2018a, Sanz et al., 2018b). The workshop concluded periodontitis has a significant impact on diabetes control, incidence and complications (Graziani et al., 2018), as well as confirming the reductions in HbA1c that result from periodontitis treatment in people with diabetes (3-4 mmol/mol, 0.3-0.4%) (Madianos and Koromantzos, 2018). Pathogenic mechanisms linking the diseases were also reviewed, with the authors calling for studies to investigate further the impact of the systematic inflammatory burden of periodontitis on diabetes (Polak and Shapira, 2018), a call which we consider we have begun to address by the current research.

In conclusion, using SEM, we identified that periodontitis and diabetes together increase systemic inflammation. Furthermore, we identified evidence to support that treatment of periodontitis resulted in significantly greater reductions in systemic inflammation in people with diabetes and periodontitis compared to those with periodontitis but not diabetes.

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Table 1 Demographic characteristics of study population at month 0: individuals with diabetes and non-diabetic controls

	Diabetes (n=83)	**No diabetes** (n=75)
Age (years)	49.0 (9.0)	47.0 (12.0)
Gender (N, %)		
Male	55 (66.3%)	45 (60.0%)
Female	28 (33.7%)	30 (40.0%)
UK ethnic group (N, %)		
White	78 (94.0%)	75 (100.0%)
Black	1 (1.2%)	-
Asian	4 (4.8%)	-
Smoking status (N, %)		
Current smoker	6 (7.2%)	8 (10.7%)
Former smoker	29 (34.9%)	24 (32.0%)
Never smoker	48 (57.8%)	43 (57.3%)
BMI (kg/m^2)	32.7 (6.4)***	27.2 (4.6)

Data for continuous variables presented as median (interquartile range).
P values determined using Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables.
***p<0.001 compared to controls.

Table 2 Clinical characteristics of study population (individuals with diabetes and non-diabetic controls) at month 0 according to periodontal diagnosis of health, gingivitis or periodontitis

	DIABETES (n = 83)			NO DIABETES (n = 75)		
	Periodontal health (n=14)	Gingivitis (n=37)	Periodontitis (n=32)	Periodontal health (n=16)	Gingivitis (n=15)	Periodontitis (n=44)
Age (years)	49.5 (10.0)	48.0 (7.0)	50.0 (7.0)	48.5 (11.5)	44.0 (17.0)	47.5 (8.0)
BMI (kg/m^2)	31.2 (9.6)	32.9 (5.5)§§	33.6 (7.1)§§	24.7 (4.6)	27.3 (4.5)	27.6 (6.4)
Years since diabetes diagnosis	2.5 (4.0)	6.0 (10.0)*	6.5 (6.5)*	N/A	N/A	N/A
HbA1c (mmol/mol)	45 (34)	53 (20)	59 (25)	37 (4)	39 (7)	37 (4)
HbA1c (%)	6.3 (3.1) §§	7.0 (1.8) §§	7.5 (2.3) §§	5.5 (0.3)	5.7 (0.6)	5.5 (0.3)
hsCRP (mg/l)	2.4 (3.2)	2.4 (3.6)	2.6 (4.2)	0.6 (1.5)	2.1 (3.1)	2.0 (3.1)*
Serum IL-6 (pg/ml)	0.5 (0.3)	1.4 (2.3)	0.5 (1.1)	0.7 (0.7)	0.8 (0.9)	0.6 (0.5)
Serum TNF-α (pg/ml)	8.0 (2.4)	7.7 (3.5)	7.1 (6.2)	6.3 (4.4)	6.9 (3.0)	3.4 (4.8)
Serum IL-1β (pg/ml)	0.3 (0.3)	0.2 (0.3)	0.1 (0.3)§	0.1 (0.1)	0.1 (0.2)	0.0 (0.1)***††
Serum IFN-γ (pg/ml)	1.2 (0.7)	2.0 (5.2)	1.2 (2.0)	1.8 (3.0)	0.9 (2.1)	0.6 (1.0)*
Serum leptin (ng/ml)	17.6 (31.2)	18.8 (21.2)	16.4 (17.2)	10.0 (10.7)	10.6 (10.8)	7.5 (13.9)
Serum adiponectin (µg/ml)	2.5 (1.0)	2.7 (1.5)	2.3 (1.3)	3.8 (1.9)	2.7 (1.3)	2.8 (1.2)
Teeth present (N)	25.0 (4.5)	26.0 (5.0)	27.0 (5.8)	27.0 (3.5)	26.0 (3.0)	26.0 (4.0)
Mean gingival index	0.8 (1.0)	1.9 (1.1)**§	1.9 (1.2)**	0.5 (0.6)	1.4 (0.8)**	2.4 (0.6)***†††
Mean plaque index	0.4 (0.5)	0.9 (0.3)**§	0.7 (0.4)*	0.2 (0.4)	0.6 (0.3)**	0.6 (0.4)***
Mean probing depth (mm)	1.7 (0.2)	2.1 (0.2)***	2.9 (0.8)***†††	1.6 (0.2)	1.9 (0.2)***	3.0 (1.0)***†††
% sites probing depth ≥ 5mm	-	-	11.4 (12.1)	-	-	19.5 (22.6)
% BOP	4.5 (12.3)	35.1 (19.6)***	47.1 (33.8)***	0.6 (2.5)	20.5 (14.6)***	43.0 (25.0)***††
GCF IL-6 (pg/ml)	0.8 (0.6)	1.1 (2.2)	1.4 (3.9)	0.7 (1.0)	0.4 (0.8)	1.8 (1.9)**††
GCF TNF-α (pg/ml)	1.2 (5.6)	1.8 (1.5)	3.4 (4.2)†	1.5 (2.0)	1.6 (1.2)	4.3 (4.5)**†
GCF IL-1β (pg/ml)	90.1 (78.1)	109.7 (132.3)	208.3 (391.4)*†	36.4 (58.5)	72.4 (75.9)	343.8 (438.5)***††
GCF IFN-γ (pg/ml)	0.5 (5.4)	1.1 (1.7)	1.9 (2.9)	0.7 (1.0)	1.5 (1.3)	3.7 (4.6)***††
GCF MMP-8 (ng/ml)	11.0 (8.9)	24.7 (29.1)*	48.7 (60.7)***††	14.4 (13.4)	18.9 (23.3)	69.6 (97.6)***†††

GCF MMP-9 (ng/ml)	61.4 (32.6)	133.8 (100.4)	189.8 (155.0)***	60.2 (36.2)	84.3 (75.8)	264.2 (330.4)***†††
GCF adiponectin (µg/ml)	0.4 (0.2)	0.9 (0.9)*	1.1 (1.0)** §§	0.7 (0.6)	0.9 (0.5)	2.0 (1.7)***††

Data presented as median (interquartile range). p values calculated with Wilcoxon rank-sum test with post-hoc Bonferroni adjustment.
*p<0.05, **p<0.01, ***p<0.001 indicates statistically significant differences compared to health within diabetes or no diabetes groups.
†p<0.05, ††p<0.01, †††p<0.001 indicates statistically significant differences compared to gingivitis within diabetes or no diabetes groups.
§p<0.05, §§p<0.01 indicates statistically significant differences compared to corresponding periodontal status between individuals with and without diabetes.

Table 3 Longitudinal characteristics of study population (individuals with diabetes and non-diabetic controls) with periodontitis over the 12 month study duration

	DIABETES				NO DIABETES			
	Month 0 (n=32)	Month 3 (n=32)	Month 6 (n=32)	Month 12 (n=27)	Month 0 (n=44)	Month 3 (n=41)	Month 6 (n=39)	Month 12 (n=36)
HbA1c (mmol/mol)	59 (25)	53 (32)	53 (28)	54 (19)	37 (4)	38 (4)	36 (2)	37 (4)
HbA1c (%)	7.5 (2.3)	7.0 (2.9)	7.0 (2.5)	7.1 (1.7)	5.5 (0.3)	5.6 (0.3)	5.4 (0.2)	5.5 (0.3)
hsCRP (mg/l)	2.6 (4.2)	2.2 (4.3)	1.8 (3.8)	1.6 (3.0)	2.1 (3.1)	1.5 (2.1)	1.6 (2.3)	1.6 (2.1)
Serum IL-6 (pg/ml)	0.5 (1.1)	0.5 (0.9)	0.5 (1.3)	0.9 (0.8)	0.6 (0.5)	0.7 (0.8)*	0.5 (0.5)	0.6 (0.5)
Serum TNF-α (pg/ml)	7.1 (6.2)	3.5 (4.9)**	4.0 (5.3)*	8.2 (4.4)	3.4 (4.8)	6.4 (5.4)	7.0 (3.1)***	7.6 (3.4)***
Serum IL-1β (pg/ml)	0.1 (0.3)	0.0 (0.2)**	0.0 (0.2)*	0.0 (0.1)*	0.0 (0.1)	0.0 (0.1)*	0.0 (0.1)	0.0 (0.0)**
Serum IFN-γ (pg/ml)	1.2 (2.0)	0.7 (1.0)***	1.0 (1.2)**	1.1 (0.6)	0.6 (1.0)	1.0 (1.4)	1.1 (1.2)	1.1 (0.8)*
Serum leptin (ng/ml)	16.4 (17.2)	16.4 (13.5)	16.6 (14.3)	16.3 (30.2)	7.5 (13.9)	7.7 (14.2)	6.9 (10.5)	3.5 (6.9)***
Serum adiponectin (μg/ml)	2.3 (1.3)	2.5 (1.2)	2.6 (1.8)	2.4 (1.2)	2.8 (1.2)	2.9 (1.3)	3.2 (2.0)***	3.5 (1.7)**
Mean gingival index	1.9 (1.2)	1.4 (1.3)***	1.5 (1.1)***	1.3 (0.9)***	2.4 (0.6)	1.6 (1.3)***	1.5 (1.3)***	1.4 (1.4)***
Mean plaque index	0.7 (0.4)	0.6 (0.4)**	0.6 (0.4)***	0.5 (0.4)**	0.6 (0.4)	0.3 (0.5)***	0.3 (0.5)***	0.4 (0.5)*
Mean probing depth (mm)	2.9 (0.8)	2.4 (0.7)***	2.3 (0.7)***	2.2 (0.9)***	3.0 (1.0)	2.6 (0.6)***	2.6 (0.8)***	2.3 (0.8)***
% sites probing depth ≥ 5mm	11.4 (12.1)	5.5 (8.5)***	5.3 (6.8)***	3.1 (8.1)***	19.6 (22.7)	10.3 (12.6)***	9.6 (16.7)***	4.6 (12.1)***
% BOP	47.1 (33.9)	18.8 (27.7)***	15.0 (29.4)***	17.6 (17.4)***	43.0 (25.0)	14.7 (16.3)***	14.7 (18.3)***	10.0 (19.1)***
GCF IL-6 (pg/ml)	1.4 (3.9)	0.8 (1.9)*	0.5 (1.3)***	0.6 (1.2)**	1.8 (1.9)	1.7 (1.9)	1.2 (1.2)*	0.7 (1.6)*
GCF TNF-α (pg/ml)	3.4 (4.2)	3.3 (5.9)	1.4 (2.5)***	1.2 (1.9)***	4.3 (4.5)	4.3 (4.7)	3.9 (5.1)	1.7 (1.8)***
GCF IL-1β (pg/ml)	208.3 (391.5)	164.3 (253.8)	79.6 (154.5)***	110.7 (231.7)**	343.8 (438.5)	118.1 (195.1)***	99.6 (192.0)***	95.6 (128.7)***
GCF IFN-γ (pg/ml)	1.9 (2.9)	1.2 (1.9)	0.6 (1.5)**	0.8 (2.6)	3.7 (4.6)	1.9 (3.0)*	1.9 (2.4)*	1.0 (1.2)***
GCF MMP-8 (ng/ml)	48.7 (60.7)	26.3 (26.6)***	25.5 (32.7)***	26.4 (44.1)**	69.6 (97.6)	36.9 (37.0)***	27.6 (40.0)***	30.5 (51.6)***
GCF MMP-9 (ng/ml)	189.8 (155.0)	122.8 (155.1)***	90.9 (102.6)***	95.1 (111.8)**	264.2 (330.4)	141.7 (160.5)***	138.3 (140.2)***	144.3 (194.1)***
GCF adiponectin (μg/ml)	1.1 (1.0)	0.7 (0.7)*	0.8 (0.9)*	0.6 (0.6)**	2.0 (1.7)	1.1 (1.3)***	0.9 (1.1)***	0.8 (0.9)***

Data presented as median (interquartile range). ND: not done

*p<0.05, **p<0.01, ***p<0.001 for comparisons between month 3, month 6 or month 12 values to month 0, within the diabetes and no diabetes groups (p values calculated using Wilcoxon matched pairs test).

FIGURE LEGENDS

Figure 1: Structural equation modelling (SEM) for creation of latent variables systemic and oral inflammation

Each part of the model can be regarded as a factor analysis for systemic (left side) and oral (right side) inflammation. Rectangles represent the observed values (indicators) used to assess various facets of the latent variables (represented by the ovals). Each indicator has two incoming arrows, representing them as having two causes: a single factor that the indicator is supposed to measure (the latent factor variable) and all the other sources of influence represented by the error term (ε), which can include random error as well as all other sources of systemic variance not due to the factors (Kline, 2011). For simplicity, we chose the error terms to be all independent so that only the latent variables explain dependence in the observed variables. The centre part of the model contains the latent variables which are the main objects of this study. The latent variables have separate distributions and correlations for the diabetes and non-diabetic control groups, whereas the parts of the model linking latent to measured variables are group invariant. The curved middle arrow represents the correlation between both latent factors which is of primary interest as it represents the association between oral and systemic inflammation. The final model can be viewed as integrating the factor analyses and the structural part consisting of the group-dependent bivariate distribution of the latent variables. The model is estimated using the maximum likelihood missing value estimation method.

ε: error term.

Figure 2: Structural equation modelling (SEM) data at baseline (A1-A3) and over 12 months (B1-B3) for individuals with periodontitis in the diabetes and no diabetes (control) groups

Panel A1: scatter plots with correlation contours showing a moderate positive correlation between oral and systemic inflammation for the diabetes group (r=0.58, p=0.02) but none for the control group (r=0.01, p=0.95). Panel B1: scatter plots with correlation contours showing a slight positive (but non-significant) correlation between changes in oral and systemic inflammation for the diabetes group (r=0.35, p=0.15) but none for the control group (r=0.11, p=0.65). Panel A2: the control group (individuals with periodontitis but not diabetes) is taken as the reference group, and mean values for the latent variables (oral inflammation and systemic inflammation) are set at 0 (note: medians are presented as the measure of central tendency in the box plots). For oral inflammation, there were no significant differences between the groups at baseline. However, the individuals with diabetes and periodontitis had significantly higher systemic inflammation compared to the non-diabetic controls (individuals with periodontitis but not diabetes) (Δ=0.20, p=0.0019). Panel B2: differences in calculated latent variables between month 12 and month 0 are presented. Again, the control group is taken as the reference group, and the mean change values for the latent variables are set at 0. There was no difference between the groups with respect to change in oral inflammation over the 12 months. However, the individuals with periodontitis and diabetes showed a significantly greater reduction in systemic inflammation compared to non-diabetic controls (individuals with periodontitis but not diabetes) over the 12 months following periodontal treatment (Δ=-0.25, p=0.013). Note that the change scores are not directly comparable to the baseline scores since they originate from separate analyses. Panel A3: Scatterplots comparing the groups as in panel A2, and relation to the possible confounder BMI, for both oral and systemic inflammation. The lines indicate regression fits for each of the control and diabetes groups. Each of the regression slopes is near horizontal, and slopes individually or averaged over both groups were not statistically different from zero (all p>0.20), indicating that group differences remain intact even when controlling for BMI. Panel B3: Scatterplots comparing the groups as in panel B2, and relation to the possible confounder BMI. As in panel A3, there is no large or significant effect of BMI (all p>0.20).

*p<0.05; **p<0.01