Treatment of periodontitis reduces systemic inflammation in type 2 diabetes

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**Word counts:** Abstract: 297 (max 300)

Main text: 3194 (max 3200)

**ABSTRACT**

The bidirectional relationship between periodontitis and diabetes is well established, and inflammation likely underpins the mechanistic links between the two diseases. The aim of this study was to assess the impact of periodontitis and its treatment on systemic inflammation in people with type 2 diabetes. Adults with type 2 diabetes (cases, n=83) and controls who did not have diabetes (n=75) were recruited and participants diagnosed with periodontitis received periodontal treatment and 12 months’ follow-up. Clinical periodontal indices (probing depths, bleeding on probing, gingival inflammation), biomarkers for periodontal inflammation (gingival crevicular fluid (GCF) interleukin (IL)-6, tumour necrosis factor (TNF)-α, IL-1β, interferon (IFN)-γ, matrix metalloproteinase (MMP)-8, MMP-9, adiponectin) and serum markers of inflammation and diabetes control (HbA1c, high sensitivity C-reactive protein (hsCRP), IL-6, TNF-α, IL-1β, IFN-γ, leptin, adiponectin) were measured. Structural equation modelling was used to evaluate periodontal treatment effects on oral and systemic inflammation. Periodontal treatment resulted in significant improvements in periodontal status from baseline to all post-treatment time-points, and significant reductions in GCF biomarkers (IL-6, TNF-α, IL-1β, MMP-8, MMP-9, adiponectin) from baseline to month 12 (all p<0.05). In cases with periodontitis, no significant change in HbA1c was identified from baseline [7.5 (2.3)%] to month 12 [7.1 (0.7%)] (p=0.08). Structural equation modelling identified that, at baseline, individuals with diabetes and periodontitis had significantly higher systemic inflammation than 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 between months 0 and 12 in individuals with diabetes and periodontitis compared to those with periodontitis but not diabetes (Δ=-0.25, p=0.01), whereas there was no significant difference between groups for changes in oral inflammation (Δ=0.06, p=0.53). These data indicate that coincident periodontitis affects systemic inflammation in diabetes, and this is ameliorated by successful treatment of periodontitis.

Keywords: inflammation; periodontitis; diabetes mellitus; diabetes mellitus, type 2

**INTRODUCTION**

Advanced periodontitis is identified as 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, and risk of tooth loss, with concomitant effects on oral function and life quality (Buset et al. 2016). Diabetes is also highly prevalent, with worldwide prevalence of 9% (Internation diabetes federation (idf). Idf diabetes atlas 8th edition, 2017. Available from http://www.Diabetesatlas.Org accessed 28 august 2019). The bidirectional links between periodontitis and type 2 diabetes are clear, with increased risk of periodontitis (2-3 fold) in people with diabetes, and increased risk of diabetes complications in those who also have advanced periodontitis (Preshaw et al. 2012). 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 (Taylor et al. 2013). Systemic inflammation results from the 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 (AGEs) and their receptor contributing to increased susceptibility to periodontitis in people with diabetes (Borgnakke et al. 2013; Chapple et al. 2013). To further investigate the role of inflammation in periodontitis and diabetes, we evaluated the effects of periodontitis treatment on periodontal and systemic inflammation and glycaemic control in individuals with and without diabetes and periodontitis.

**MATERIALS AND METHODS**

**Study design**

This was a case-control study of clinical and biochemical outcomes following periodontal treatment in individuals with diabetes and matched non-diabetic controls. Following written informed consent, adults with type 2 diabetes (cases) 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. Exclusion criteria included immunosuppression, pregnancy, conditions requiring prophylactic antibiotics prior to dental treatment, bleeding disorders, and non-surgical 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 06/Q0904/8).

**Periodontal examination and therapy**

Following screening, each participant underwent full periodontal examination by trained examiners at baseline (month 0). This 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 University of North Carolina-15 periodontal probe. Mean gingival index, plaque index and probing depth values were calculated, together with %BOP and percent of sites with probing depth ≥5mm. Participants were assigned a diagnosis of either periodontal health (no probing depths >4mm, BOP <15%, no periodontal attachment loss), gingivitis (no probing depths >4mm, BOP ≥15%, no attachment loss) or advanced periodontitis (≥6 periodontal sites with probing depth ≥5mm at separate teeth, plus attachment loss and radiographic alveolar bone loss). Individuals with periodontal health or gingivitis participated only at baseline. Those with periodontitis received non-surgical periodontal therapy (full mouth root surface debridement) using manual and ultrasonic instruments with local anesthesia, together with motivation and education in oral hygiene techniques. Early periodontal maintenance follow-up appointments were provided at 3 and 6 weeks, including further prophylaxis and reinforcement of oral hygiene as indicated clinically. Periodontal maintenance appointments with reinforcement of oral hygiene 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 C-reactive protein (hsCRP). Additional blood samples were centrifuged (1500g for 15 min at 4oC) for serum separation, and 0.5 ml serum aliquots were frozen at -80oC for storage prior to analysis by laboratory staff who were unaware of the clinical diagnoses. 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 using Periopaper strips (Oraflow Inc, New York, USA) and a 30 s sampling technique at the mesiobuccal aspect of the four first molars (or adjacent teeth if missing). Periopapers were placed into sterile 0.5 ml micro-tubes (Sarstedt, Leicester, UK) containing 150 µl sterile phosphate buffered saline (PBS), and frozen at -80oC 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 at 1500g for 60 min then 13,000g for 2 min at 4oC (Wassall and Preshaw 2016).

Interleukin-6 (IL-6), tumour necrosis factor (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 concentrations in serum samples, and adiponectin concentrations in GCF samples 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 concentrations were measured using commercial ELISA kits (R&D systems, Abingdon, UK), 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% assuming the SD of change scores was 1.0% (two-sided alpha level 0.05). Data were analysed using Stata 14 (StataCorp LP, College Station, TX, USA). Descriptive statistics included medians (interquartile range) and frequency data. For demographics, significance of differences between cases and controls 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 in cases and controls 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. Significance of differences between parameters at follow-up time points relative to baseline within cases and controls were determined using Wilcoxon matched-pairs test.

For participants with periodontitis, structural equation modelling 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. Structural equation modelling 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 (cases) and 75 adults without diabetes (controls) were recruited. There were no significant differences between cases and controls at baseline for age, gender, ethnicity or smoking, though the median BMI of cases was significantly higher compared to controls (p<0.001). Table 2 shows baseline data for cases and controls according to periodontal status, comprising 6 groups; diabetes/periodontal health, diabetes/gingivitis, diabetes/periodontitis, no diabetes/periodontal health, no diabetes/gingivitis, and no diabetes/periodontitis. Cases (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 with the numbers studied. 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. Results for IL-1β and IFN-γ should be interpreted with caution as their values were close to, or below, assay sensitivities.

When considering clinical periodontal indices at baseline (Table 2), as would be expected, within cases and controls, 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 cases and controls 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 controls, 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 cases and controls. 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 cases and controls, 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 those individuals with periodontitis only. At baseline, 32 cases and 44 controls were identified as having periodontitis, with 27 cases and 36 controls completing the study to month 12 (lost to follow-up: 5 cases, 8 controls). Among the cases with 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 cases with periodontitis, statistically significant reductions in serum TNF-α were noted from baseline to months 3 and 6, but not month 12. Furthermore, among the controls with periodontitis, 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, % of sites with probing depth >5 mm, and %BOP were noted in both cases and controls with periodontitis from baseline to all post-treatment time-points (all p<0.05), representing a good response to periodontal treatment. Analysis of differences in change data between cases and controls 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 the cases and controls; broadly, significant reductions in GCF levels of 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 structural equation modelling. At baseline, cases (with periodontitis) had significantly higher systemic inflammation compared to controls (with periodontitis) as assessed using the latent variable *systemic inflammation* (Δ=0.20, p=0.002), whereas there were no significant differences between cases and controls for *oral inflammation* (panel A2). Additionally, for cases with 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 controls with periodontitis (panel A1). Change data between months 0 and 12 indicated that the cases with periodontitis demonstrated a significantly greater reduction in systemic inflammation compared to the controls with periodontitis (Δ=-0.25, p=0.01), whereas there was no difference in change data between the groups for oral inflammation (Δ=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 cases and controls, we performed additional analyses to investigate whether BMI might be an explanatory variable in the SEM analyses (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 cases and controls, or their average 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**

The bidirectional relationship between periodontitis and diabetes is characterized by increased risk of periodontitis in individuals with diabetes, and poorer glycaemic control and increased risk of diabetes complications in people with diabetes who also have advanced periodontitis (Borgnakke et al. 2013; Preshaw et al. 2012; Saremi et al. 2005; Shultis et al. 2007). Periodontitis is frequently found in adults with type 2 diabetes, suggesting a need for periodontal screening of all patients with diabetes (Pumerantz et al. 2017). A review of pathogenic mechanisms that may link periodontitis and diabetes suggested that inflammation is likely to link the two diseases, and that longitudinal clinical studies are required to investigate this further (Taylor et al. 2013). Dysregulation of peripheral cytokine activity and chronic low-grade inflammation have been linked to development of diabetes (Kolb and Mandrup-Poulsen 2010), and periodontitis is associated with elevated levels of circulating inflammatory mediators such as CRP, TNF-α and IL-6 (Bretz et al. 2005; Engebretson et al. 2007; Paraskevas et al. 2008). It has been presumed that reductions in HbA1c following periodontitis treatment result from resolution of periodontal inflammation, leading to reduced inflammation locally in the periodontal tissues and also systemically, though this has not previously been tested beyond studies of single (or low numbers of) inflammatory mediators.

In order to address this issue, structural equation modelling 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 which may occur following therapy. The current 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 cases and controls, we considered that BMI might be a potential confounding factor in the SEM outcomes; additional analyses showed this not to be the case, with no impact of BMI being observed on the reductions in systemic inflammation between case and controls.

Limitations include that this was a case-control study rather than a cohort study or randomized controlled trial. However, strengths include the precise clinical phenotyping with use of robust criteria to define cases of advanced periodontitis to avoid misclassification, a high standard of analysis including quality control assessments, and a wide range of local and systemic inflammatory mediators that were assessed cohesively using structural equation modelling. Whereas many previous studies have assessed only single or few biomarkers, it is recognized that inflammatory mediators effectively function in networks and such a restricted approach only provides limited information (Preshaw and Taylor 2011). Furthermore, variability in experimental designs and mediator levels that are close to the limits of sensitivity of assay systems further contribute to problems inherent in evaluating individual or few mediators. Interestingly, a study of people with diabetes and periodontitis identified no significant individual changes in 8 investigated serum biomarkers over 6-months after periodontal therapy (Geisinger et al. 2016). Indeed, as indicated in Table 3 of the present study, it can be challenging to meaningfully interpret changes occurring in individual biomarkers over time. Structural equation modelling offered an alternative to overcome some of these problems.

Our study was originally powered to identify changes in HbA1c following periodontal therapy that, with consideration of current evidence from systematic reviews, would now be regarded as being overly optimistic. In the cases with periodontitis, there was a non-significant change in HbA1c of around 5 mmol/mol (0.4%), which is similar in magnitude to statistically significant reductions in HbA1c reported in meta-analyses (Engebretson and Kocher 2013; Madianos and Koromantzos 2018; Simpson et al. 2010; Simpson et al. 2015). The outcomes of periodontal treatment in the individuals with periodontitis were consistent with those previously reported (Cobb 2002), indicating a good response to therapy. The cases with periodontitis presented with slightly less advanced disease (fewer sites with probing depth >5mm, non-significant difference) than the controls with periodontitis, whereas the expectation is usually that diabetes increases extent and severity of periodontitis. This finding maybe a manifestation of the recruitment strategy, in that controls were recruited from dental hospital clinics where they had been referred for management of periodontitis, whereas cases were recruited from diabetes clinics with no *a priori* knowledge of their periodontal disease status. When considering the number of sites with probing depth >5mm, this reduced from 11.4% at baseline to 3.1% at month 12 in cases (73% reduction), and from 19.6% to 4.6% in controls (77% reduction), indicating that periodontal treatment was similarly effective in individuals with and without diabetes. Also, within the gingivitis categories (representing a tightly constrained classification of gingival inflammation), gingival and plaque index scores were significantly higher in cases compared to controls, potentially suggesting an increased susceptibility to oral inflammation in individuals with diabetes, a finding that has been reported previously (Lalla et al. 2006).

Recently, the European Federation of Periodontology and International Diabetes Federation established a workshop on the subject of periodontitis and diabetes, with outputs published simultaneously in the *Journal of Clinical Periodontology* and *Diabetes Research and Clinical Practice* to help to improve awareness of both medical and dental professionals on this topic (Sanz et al. 2018a; 2018b). The workshop concluded that 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). The pathogenic mechanisms linking the two 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 structural equation modelling, we identified that periodontitis and diabetes appear to act synergistically in increasing systemic inflammation, and showed 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.

**AUTHOR CONTRIBUTIONS:**

P.M. Preshaw, J.J. Taylor, J. Weaver, R. Taylor and R.R. Wassall contributed to conception, design, data acquisition and interpretation, drafted and critically revised the manuscript. K.M. Jaedicke, M. De Jager contributed to design, data acquisition and interpretation, drafted and critically revised the manuscript. S.M. Bissett, K.M. Whall, R. van de Merwe, A. Areibi, P. Jitprasertwong and R. Al-Shahwani contributed to design, data acquisition and interpretation, and critically revised the manuscript. J.W. Bikker and W. Selten contributed to design, data acquisition and interpretation, performed all statistical analyses, drafted and critically revised the manuscript. All authors gave their final approval and agree to be accountable for all aspects of the work.

**ACKNOWLEDGEMENTS:**

The research was supported by the Newcastle Dental Clinical Research Facility based at Newcastle upon Tyne Hospitals NHS Foundation Trust, and by the Gateshead Health NHS Foundation Trust and Newcastle University (UK). Funding for this study was received from the UK National Institute for Health Research (NIHR) via a Clinician Scientist Fellowship awarded to P.M. Preshaw (ref. DHCS/03/G121/46), and research grants from the Dunhill Medical Trust (ref. R63/1107) and a PhD studentship grant from Philips Research (ref. DRC-0417). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the UK Department of Health. The study sponsor was the Newcastle upon Tyne Hospitals NHS Foundation Trust. Philips Research provided funding for the commercial statistical consultants (CQM, The Netherlands) to assist with the analysis of the data. P.M. Preshaw and J.J. Taylor have received research grants from Philips for the submitted work, and P.M. Preshaw has received research funding, honoraria and travel grants from Philips Research and from Colgate. M. De Jager is an employee of Philips Research.

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**Table 1** Demographic characteristics of cases (diabetes) and controls (no diabetes) at month 0

|  |  |  |
| --- | --- | --- |
|  | **Cases: diabetes**  (n=83) | **Controls: no diabetes**  (n=75) |
| Age (years) | 49.0 (9.0) | 47.0 (12.0) |
| Gender (N, %)  Male  Female | 55 (66.3%)  28 (33.7%) | 45 (60.0%)  30 (40.0%) |
| UK ethnic group (N, %)  White  Black  Asian | 78 (94.0%)  1 (1.2%)  4 (4.8%) | 75 (100.0%)  -  - |
| Smoking status (N, %)  Current smoker  Former smoker  Never smoker | 6 (7.2%)  29 (34.9%)  48 (57.8%) | 8 (10.7%)  24 (32.0%)  43 (57.3%) |
| BMI (kg/m2) | 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 cases (diabetes) and controls (no diabetes) at month 0 according to periodontal diagnosis of health, gingivitis or periodontitis

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **CASES: DIABETES (n = 83)** | | | **CONTROLS: 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/m2) | 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) |
| 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 ≥ 5 mm | - | - | 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 case or control groups.

†p<0.05, ††p<0.01, †††p<0.001 indicates statistically significant differences compared to gingivitis within case or control groups.

§p<0.05, §§p<0.01 indicates statistically significant differences compared to corresponding periodontal status between cases and controls.

Table 3 Longitudinal characteristics of the cases (diabetes) and controls (no diabetes) with periodontitis over the 12 month study duration

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Cases (diabetes)** | | | | **Controls (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 ≥ 5 mm | 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 case and control groups (p values calculated using Wilcoxon matched pairs test).

**FIGURE LEGENDS**

**Figure 1:** Structural equation modelling 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 case and 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 data at baseline (A1-A3) and over 12 months (B1-B3) for individuals with periodontitis in the diabetes (cases) and no diabetes (controls) 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 cases (individuals with diabetes and periodontitis) had significantly higher systemic inflammation compared to the 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 cases (individuals with periodontitis and diabetes) showed a significantly greater reduction in systemic inflammation compared to 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