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Effect of periodontal treatment on metabolic control, systemic inflammation and cytokines in patients with type 2 diabetes

Fernanda O. B. Correa[1,2], Daniela Gonçalves[1], Carlos M. S. Figueredo[2,3], Alliny S Bastos[1], Anders Gustafsson[2] and Silvana R. P. Orrico[1]

[1]Department of Diagnosis and Surgery, School of Dentistry, São Paulo State University, Araraquara, SP, Brazil; [2]Division of Periodontology, Institute of Odontology, Karolinska Institutet, Stockholm, Sweden; [3]Department of Periodontology, School of Dentistry, Rio de Janeiro State University, Rio de Janeiro, RJ, Brazil

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Abstract

Objective: The aim of this study was to investigate the effect of periodontal therapy on the circulating concentration of high-sensitivity capsule-reactive protein (hs-CRP), fibrinogen (FIB), interleukin (IL)-4, IL-6, IL-8, IL-10 and tumour necrosis factor-α (TNF-α) and on the metabolic control in type 2 diabetes mellitus (T2DM) patients.

Material and Methods: Twenty-three T2DM patients with chronic periodontitis were enrolled in this study. Periodontal clinical parameters, namely visible plaque index, gingival bleeding index, bleeding on probing, probing depth and clinical attachment levels, were evaluated. Blood samples for plasma were collected and assessed for the levels of hs-CRP, FIB, IL-4, IL-6, IL-8, IL-10 and TNF-α. The glycated haemoglobin (HbA_{1c}) and fasting plasma glucose were also measured. All parameters were evaluated before and 3 months after non-surgical periodontal therapy.

Results: All clinical parameters were significantly improved 3 months after the periodontal therapy. A univariate comparison showed a tendency towards a decrease of the measured biomarkers, most pronounced for TNF-α and FIB, after therapy. Periodontal treatment also reduced HbA_{1c} and hs-CRP levels, albeit not significantly.

Conclusions: The clinically successful non-surgical periodontal therapy tended to reduce systemic inflammation and the concentration of some circulating cytokines.

Key words: diabetes mellitus; fibrinogen; glycaemic control; periodontal disease/therapy; tumour necrosis factor-α

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Periodontitis is an inflammatory disorder characterized by the destruction of periodontal tissues with a subsequent attachment loss (Albandar et al. 1999). The periodontal destruction is host-mediated by locally produced pro-inflammatory cytokines in response to the bacterial flora and its products (Van Dyke & Serhan 2003). It is possible that the production of local cytokine (Prabhu et al. 1996, Gorska et al. 2003) and/or low-level asymptomatic bacteraemia/endotoxaemia (AAP 1998) affects the plasma concentrations of pro-inflammatory biomarkers. Significant differences in the plasma concentrations of such biomarkers have been described previously (Fredriksson et al. 1999, Loos et al. 2000, Noack et al. 2001, Buhlin et al. 2003, Pitiphat et al. 2008).

Periodontitis may have an even greater influence on systemic inflammatory condition in individuals with diabetes. Elevated circulating levels of interleukin (IL)-6, tumour necrosis factor-α (TNF-α) and high-sensitivity capsule-reactive protein (hs-CRP), which can worsen insulin resistance and thereby impair glycaemic control, have been shown in several studies (Taylor et al. 1996, Amar & Han 2003). Thus, periodontal disease may have a significant impact on the metabolic state in diabetes (Mealey & Oates 2006).

The question of whether periodontal disease aggravates systemic inflammation associated with diabetes remains to be answered. In individuals with diabetes, clinical interventional trials

Conflict of interest and source of funding statement

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showed a significant reduction of acute-phase proteins levels, such as CRP (Lalla et al. 2007) and fibrinogen (FIB) (Christgau et al. 1998), following periodontal therapy. TNF-α, an important pro-inflammatory cytokine, has been associated with insulin resistance (Ling et al. 1994, Mishima et al. 2001, King 2008) and type 2 diabetes (Fernandez-Real & Ricart 2003). Previous studies have shown that periodontal treatment associated with local administration of antibiotic (Iwamoto et al. 2001, Iwamoto et al. 2003, Nishimura et al. 2003) reduced circulating TNF-α in type 2 diabetes mellitus (T2DM) subjects with a subsequent reduction in the insulin concentration and glycated haemoglobin levels. On the other hand, some studies (Al-Mubarak et al. 2002, Talbert et al. 2006, Lalla et al. 2007, O'Connell et al. 2008) reported that circulating TNF-α is not reduced following periodontal treatment in subjects with diabetes.

Because of the contradicting findings in the literature, we wanted to confirm the hypothesis that non-surgical periodontal therapy can influence the levels of systemic pro- and anti-inflammatory cytokines (IL-6, IL-8 and TNF-α, IL-4 and IL-10), and the levels of acute-phase proteins (hs-CRP and FIB) in T2DM patients. Furthermore, another objective was to evaluate the influence of non-surgical periodontal therapy on the metabolic control of diabetes.

Materials and Methods

Sample population and experimental design

Patients were recruited from the Clinic of Periodontics for Diabetic Patients of the Department of Diagnosis and Surgery of the Araraquara School of Dentistry, São Paulo State University (UNESP), Brazil, between January 2005 and October 2006. This study was conducted in full accordance with the applicable ethical principles, including the World Medical Association Declaration of Helsinki, and was independently reviewed and approved by the Ethics in Human Research Committees of the Araraquara School of Dentistry (UNESP, Araraquara, Brazil; Protocol number 60/04) and Huddinge Hospital (Karolinska Institutet, Huddinge, Sweden; Protocol number 2007/384-31/2). All volunteers were informed about the aims and methods of this study, and gave their written consent to participate.

As inclusion criteria, all subjects who participated in this study had to have type 2 diabetes and chronic periodontitis (AAP 1999), present a minimum of 15 natural teeth and present at least four teeth with one or more sites with probing depth (PD) $\geqslant 5$ mm, clinical attachment level (CAL) $\geqslant 4$ mm and bleeding on probing (BOP). The following exclusion criteria were considered: a history of antibiotic therapy within the previous 6 months and anti-inflammatory drugs within the previous 3 months, pregnancy or use of contraceptives or any other form of hormone, current smoker or former smoker for <5 years and periodontal treatment within the previous 12 months.

The sample size was calculated based on previous information from a pilot study recently conducted by our research group, using data relative to the mean difference and standard deviation (SD) between the experimental periods (baseline and 3 months after periodontal treatment) for the clinical and immunological parameters of the type 2 diabetes patients (unpublished data). It was estimated that with a minimum of 13 patients with type 2 diabetes, significant differences in the clinical parameters, circulating TNF-α, hs-CRP and FIB would be detected between the study periods (baseline and 3 months after non-surgical periodontal therapy), with 90% statistical power and a 95% confidence interval.

The sample was composed of 23 T2DM subjects (ADA 2008). All patients were taking prescribed oral hypoglycaemic agents and/or insulin for the treatment of their diabetes. They were under the supervision of an endocrinologist, with no alteration in the diabetes treatment in the last year before the study and were authorized by their physician to undergo periodontal treatment. The body mass index (BMI) was estimated by dividing the body weight (in kilograms) by the square of the height (in metres).

All patients were subjected to a periodontal clinical examination performed in six sites per tooth (excluding third molars) by a single trained calibrated examiner ($\kappa = 0.93$, data not shown). The presence of supragingival biofilm and marginal gingival bleeding was recorded, respectively, with the visible plaque index (VPI) (Ainamo & Bay 1975) and the gingival bleeding index (GBI) (Ainamo & Bay 1975). PD, CAL and BOP were also evaluated using a University of North Carolina probe (Hu-Friedy, Chicago, IL, USA).

Inflammatory biomarker assays

Blood samples were collected after a minimum of 8 h of overnight fasting for all individuals. FIB (mg/dl) was recorded by the Clauss method using a commercial kit (Wiener Laboratorios A.S.I.C., Rosário, Argentina), and hs-CRP (mg/l) were analysed immunologically using a commercial kit (DADE Behring, Deerfield, IL, USA) in accordance with the manufacturer's instructions.

The plasma concentrations of IL-4, IL-6, IL-8, IL-10 and TNF-α were analysed using the multiplex bead technique (Bio-Plex system, Bio-Rad Laboratories, Hercules, CA, USA.) using commercially available high-sensitivity human cytokine kit (Linco Research Inc., St. Charles, MO, USA), following the manufacturer's instructions. The results were calculated using a specific software (Bio-Plex system, Bio-Rad Laboratories).

Metabolic control

To evaluate the metabolic control, the concentration of glycated haemoglobin (HbA_{1c}) (%) was measured by high-performance liquid chromatography (DiaSTAT Haemoglobin A_{1c} Analyser System, BioRad Laboratories). Fasting plasma glucose (FPG) (mg/dl) was determined using the glucose oxidase method (Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil).

Periodontal treatment

The patients received non-surgical periodontal treatment, comprising oral hygiene instructions, and scaling and root planing under local anaesthesia. The treatment took, on average, four sessions within 1 month using manual instruments (Gracey and McCall Curettes, Hirschfield Files, Trinity Periodontia, São Paulo, Brazil). After the periodontal treatment, a professional plaque control programme was performed twice a month for 3 months (six sessions), consisting of supragingival plaque removal and reinstruction of oral hygiene procedures.

Clinical, inflammatory biomarkers and metabolic outcomes as well as the measurement of BMI were assessed at baseline and at 3 months after comple-

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tion of periodontal therapy. During the experimental period, the anamnesis was updated and patients were questioned about changes in medications related to diabetes therapy, use of anti-inflammatory or antibiotic and alteration of lifestyle, including exercise and diet. All participants completed the study.

Statistical analysis

For VPI, GBI and BOP, the percentage of positive sites was obtained per patient, and thereafter a mean value was calculated for the group. For PD and CAL, measured in millimetres, metabolic control and BMI, a mean value of each individual was first obtained and thereafter a mean value was calculated for the group. For analysis of inflammatory biomarkers, a value of each individual was first obtained and then a median value was calculated for the group

The changes in the clinical and metabolic data were presented as mean and SD, and the changes in inflammatory biomarker data were presented as median values with variability measures (25% and 75% quartiles). The differences between periods from clinical, metabolic and immunological data were analysed using non-parametric statistical method (Wilcoxon's matched pairs test and signed-rank test). A Bonferroni's correction was used to adjust for multiple comparisons.

The correlation was calculated by the coefficient of Spearman's correlation between clinical (BOP, PD or CAL) and metabolic (HbA1c) variables, clinical and immunological (IL-4, IL-6, IL-8, IL-10 or TNF-α) variables and metabolic and immunological variables.

The BMI values were presented as mean and SD, and the difference between periods was calculated by Student's *t*-test, as the data were normally distributed. The Statistica 7.0 software program (StatSoft Inc., Tulsa, OK, USA) was used to analyse the data.

Results

Sample population

The sample was composed of nine men and 14 women, 14 being white and nine black, mean age 47.5 ± 7.2 years (range 32–60 years old) and mean diabetes duration of 10.0 ± 6.8 years (range 1–20 years).

In all clinical sessions, the patients did not report changes in lifestyle and medications related to diabetes therapy. Besides, none of the patients took anti-inflammatory and/or antibiotic medications during the experimental period.

Thirty nine per cent of the sample had at least one diabetes-related complication, retinopathy and nephropathy being the most common complications associated with diabetes. Fifty-two per cent were receiving oral hypoglycaemic agents or dietary control only, 22% were undergoing insulin treatment and 26% were being treated with a combination of insulin and oral hypoglycaemic agents.

The mean (± SD) of BMI was 30.6 (± 4.8) at baseline and 30.0 (± 4.6) after treatment. There was no significant change following therapy ($p = 0.681$).

Oral clinical findings

Table 1 shows the values of clinical parameters at baseline and a 3-month follow-up examinations. After the non-surgical periodontal treatment, there were improvements in all of the monitored clinical parameters ($p < 0.05$). The periodontal treatment had no adverse effect in the patients.

Inflammatory biomarkers and metabolic findings in plasma

A univariate analysis showed that the non-surgical periodontal treatment tended to reduce the levels of all cytokines measured, although for most of them the change did not reach significance. However, the reduction in TNF-α levels was statistically significant 3 months after non-surgical periodontal therapy ($p = 0.014$). The treatment also reduced IL-4, from 4.0 to 0.3 pg/ml, but not significantly ($p = 0.211$) (Table 2). There was a large variation in the IL-4 response; 11 patients out of 23 patients showed lower levels of IL-4 after treatment, six patients had more IL-4 after the treatment compared with the base-

Table 1. Mean values (± SD) for visible plaque index (VPI), gingival bleeding index (GBI) and bleeding on probing (BOP)

Clinical parameters	BL	3 months	Δ 3 months to BL†	*p*-value
VPI (% sites)	84.5 (± 11.1)	18.2 (± 13.8)	− 79.1 (± 13.9)	<0.001*
GBI (% sites)	46.8 (± 22.0)	11.4 (± 10.8)	− 73.5 (± 19.5)	<0.001*
BOP (% sites)	90.4 (± 10.5)	28.9 (± 16.5)	− 68.5 (± 15.9)	<0.001*
Mean PD (mm)	4.2 (± 1.0)	2.8 (± 0.4)	− 1.5 (± 0.7)	<0.001*
Mean CAL (mm)	5.4 (± 1.3)	4.6 (± 1.1)	− 0.8 (± 0.6)	<0.001*
Deep sites (% sites)	38.0 (± 22.8)	7.5 (± 6.8)	− 80.7 (± 13.7)	<0.001*
Deep sites (*n*)	51.3 (± 34.9)	9.6 (± 10.5)	− 41.7 (± 27.9)	<0.001*
Teeth with deep sites (*n*)	15.5 (± 6.3)	4.9 (± 3.7)	− 10.7 (± 4.5)	<0.001*

Mean number and percentage of deep sites (PD ⩾ 5 mm), mean number of teeth with deep sites, mean probing depth (PD) and clinical attachment level (CAL), measured at all sites at baseline (BL) and 3 months after treatment in patients with type 2 diabetes ($n = 23$).

*Significant difference comparing baseline and 3 months after treatment (Wilcoxon's signed rank test; $\alpha = 5\%$).

†Changes from baseline to 3 months.

Table 2. Median values (25th/75th percentiles) of glycated haemoglobin (HbA_{1c}), fasting plasma glucose (FPG), high-sensitivity C-reactive protein (hs-CRP), fibrinogen (FIB), tumour necrosis factor-α (TNF-α) and interleukin (IL): IL-4, IL-6, IL-8 and IL-10 at baseline and 3 months after periodontal therapy in patients with type 2 diabetes ($n = 23$)

Markers in plasma	Baseline	3 months	*p*-value
HbA_{1c} (%)	9.1 (7.9/11.0)	8.7 (7.7/11.3)	0.871
FPG (mg/dl)	241.0 (163.5/283.5)	195.0 (154.0/259.5)	0.533
hs-CRP (mg/l)	6.3 (4.0/10.6)	5.4 (2.1/9.1)	0.212
FIB (mg/dl)	368.0 (310.0/420.0)	329.5 (296.5/378.5)	0.037*
TNF-α (pg/ml)	5.6 (4.3/7.4)	4.8 (2.7/6.7)	0.014*
IL-4 (pg/ml)	4.0 (0.0/12.3)	0.3 (0.0/4.9)	0.211
IL-6 (pg/ml)	3.1 (2.1/4.2)	2.3 (1.2/4.5)	0.627
IL-8 (pg/ml)	3.0 (2.1/4.4)	2.4 (1.6/4.7)	0.144
IL-10 (pg/ml)	11.9 (2.9/22.9)	8.6 (4.1/12.7)	0.244

*Significant difference comparing baseline and 3 months after treatment (Wilcoxon's matched pairs test $\alpha = 5\%$).

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line values and six had levels below the detection limit both before and after treatment. At baseline, there was a significant negative correlation between IL-4 and BMI, $r = -0.48$, $p = 0.020$ (Spearman rank correlation). The change in IL-4 did not correlate with BMI.

Regarding the acute-phase proteins, a univariate comparison showed that the reduction in FIB levels after treatment was statistically significant ($p = 0.037$), whereas the reduction of hs-CRP did not reach significance.

After adjustment for multiple comparisons, the treatment effect on TNF-α and FIB did not reach significance.

At baseline, the HbA_{1c} ranged from 7.0% to 12.5% and FPG values ranged from 100 to 424 mg/dl. The mean (± SD) values of HbA_{1c} at baseline and 3 months after treatment were, respectively, 9.5% (± 1.7) *versus* 9.2% (± 2.0). In addition, the mean (± SD) values of FPG at baseline and after therapy were, respectively, 232.5 mg/dl (± 85.4) *versus* 213.0 mg/dl (± 74.0). The reductions in both parameters were not statistically significant.

No correlation between immunological and clinical parameters of periodontitis was found among the individuals of this study.

Discussion

The clinically successful non-surgical treatment tended to reduce the markers of systemic inflammation and the cytokines measured. Most of the differences did not reach statistical significance but some of the findings could be of relevance. TNF-α has been reported to play a key role in the pathogenesis of type 2 diabetes (Fernandez-Real & Ricart 2003), and the correlation of this cytokine with insulin resistance has also been shown in the metabolic syndrome (Ingelsson et al. 2008, King 2008). The effect of TNF-α on insulin resistance is believed to be due to its ability to inhibit insulin-dependent autophosphorylation of the insulin receptor and the phosphorylation of insulin receptor substrate-1, the major substrate of the insulin receptor in vivo (Nilsson et al. 1998).

The main finding of this prospective study was that the satisfactory clinical response to non-surgical periodontal therapy was followed by a reduction of circulating TNF-α concentration in T2DM patients. This is in line with studies that reported that mechanical periodontal therapy in association with local antibiotic delivery (Iwamoto et al. 2001) or not (Dag et al. 2009) reduced circulating TNF-α. In contrast, some studies did not show changes in the TNF-α level in patients with diabetes following periodontal therapy (Al-Mubarak et al. 2002, Talbert et al. 2006, Lalla et al. 2007, O'Connell et al. 2008). Factors such as periodontal status, type of diabetes and the use of systemic antibiotics could probably explain some of these differences.

The other circulating cytokines investigated in this study (IL-4, IL-6, IL-8 and IL-10) were also higher at baseline than after the periodontal treatment. However, due to the high individual variability in cytokine expressions, the reductions did not reach significance. This inter-patient variability in response to periodontal therapy was also reported by Behle et al. (2009). In the present study, a reduction of IL-4 was observed after treatment, which decreased more than 90%, but it did not reach significance. It is known that not all patients respond similarly to therapy, leading to a variability in responses among the subjects. In this study, there was a large variation in the IL-4 response and although the reason for this variation is unclear, a possible hypothesis is that adipose tissue, represented by the high BMI, may impair the increase of IL-4. In our sample, the IL-4 values at baseline showed a negative correlation with BMI but the change in IL-4 did not correlate with BMI.

FPG levels did not change significantly following therapy in the present study in agreement with past studies (Iwamoto et al. 2001, Rodrigues et al. 2003, Faria-Almeida et al. 2006, O'Connell et al. 2008). In this study, there was a decrease in the HbA_{1c} levels after non-surgical periodontal therapy, but this reduction did not reach significance. This is in line with past studies (for a review, see Jones et al. 2007). Conversely, others suggest that the control of periodontal infection improves metabolic control (Iwamoto et al. 2001, Kiran et al. 2005, Navarro-Sanchez et al. 2007, O'Connell et al. 2008); however, in some of those studies, the periodontal therapy was associated with local (Iwamoto et al. 2001) or systemic (O'Connell et al. 2008) antibiotic administration. In this regard, it is important to highlight the fact that tetracycline and its derivates might act directly in the insulin production (Qin et al. 2002) and some of the reduction of HbA_{1c} following therapy would be due to the antibiotic per se and not only due to the improvement of periodontal infection. The participants in this study were considered, an average, as obese (mean BMI of 30.6 kg/m^2 at baseline) and they did not show significant change in level 3 months after periodontal therapy. An increased BMI is associated with an increase in the number and size of adipocytes, which are cells with high metabolic activity that produce large quantities of TNF-α and IL-6, which can worsen insulin resistance and thereby aggravate metabolic control (Mealey & Oates 2006).

FIB, an acute-phase protein produced by the liver, has been identified as a marker of inflammation (Sakkinen et al. 2001). In the present study, the reduction of FIB levels following periodontal therapy was significant, similar to the previously reported by Christgau et al. (1998), who showed significantly lower levels of FIB 4 months after non-surgical periodontal therapy in patients with type 1 and 2 diabetes. In contrast, Lalla et al. (2007) did not report a significant difference in FIB levels following periodontal therapy and justified their results as the diabetes itself causes an elevated pro-inflammatory state.

CRP is elicited by inflammatory stimulus and mediated through a complex network of cytokines (Ablij & Meinders 2002). In this study, the periodontal treatment tended to reduce the CRP level, but it did not reach significance, in agreement with previous findings in diabetic patients (Christgau et al. 1998). A possible explanation for the relatively small reduction of CRP level is that the present sample remained obese during the experimental period and it is known that the level of circulating the CRP is induced not only by periodontal disease but also by other systemic conditions such as hyperglycaemia and obesity (Ingelsson et al. 2008). Previous studies have reported a positive association of BMI and CRP concentrations in T2DM patients (Leinonen et al. 2003).

One limitation of the present study was the absence of a group with T2DM patients who were not undergoing periodontal treatment because it is not known how these patients would progress. Moreover, the small sample size is due to the use of restricted inclusion and

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exclusion criteria in an attempt to minimize the occurrence of confounding factors. Although the power calculation estimated a lower number of participants to reach significant results, the higher variability in responses among the subjects in this study compared with the pilot study caused the discrepancy. Considering the number of comparisons made and the high biological variance among the subjects, it is clear that a larger study population would be needed to draw any final conclusions regarding the effect of periodontal treatment on biomarkers. Hence, further research should be conducted in larger samples, adding a control group, in order to elucidate the effect of periodontal therapy on metabolic control and on parameters of systemic inflammation in T2DM patients.

Within the limitations of this study, it may be concluded that the clinically successful non-surgical periodontal therapy tended to reduce systemic inflammation and the concentration of some circulating cytokines, which could be important for T2DM patients.

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Supporting Information

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

Table S1. Supporting information in accordance with the CONSORT Statement 2001 checklist used in reporting randomized trials.

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Address:
Silvana Regina Perez Orrico
Depto. de Diagnóstico e Cirurgia
Faculdade de Odontologia de Araraquara – UNESP
Rua Humaitá
1680 Araraquara, SP
Brazil
E-mail: s-orrico@foar.unesp.br

Clinical Relevance

Scientific rationale for the study: Periodontal disease may alter the expression of some systemic inflammatory biomarkers. However, there is limited information about the effect of periodontal therapy on systemic inflammatory biomarkers from patients with diabetes. This study investigated the effect of periodontal therapy on markers of systemic inflammation in T2DM mellitus patients.

Principal findings: Periodontal therapy was effective not only in reducing local infection but tend also in reducing systemic inflammation.

Practical implications: Diabetics patients may benefit from periodontal therapy through the reduction of systemic inflammatory biomarkers, particularly TNF-α, a cytokine directly related to insulin resistance.

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