Improvement of glycemic control after periodontal treatment by resolving gingival inflammation in type 2 diabetic patients with periodontal disease

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ABSTRACT

Aims/Introduction: Chronic inflammation aggravates glycemic control in patients with type 2 diabetes mellitus. An increase or decrease in the release and activities of various inflammatory mediators, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and C-reactive protein (CRP), are presumed to be responsible for inducing insulin resistance. The purpose of the present study was to examine the effects of non-surgical periodontal treatment incorporating topical antibiotics on glycemic control and serum inflammatory mediators in patients with type 2 diabetes mellitus with periodontitis.

Materials and Methods: Periodontal inflammation and periodontal tissue destruction were evaluated by bleeding on probing (BOP) and the probing pocket depth (PPD), respectively. A total of 41 patients with type 2 diabetes and periodontitis received periodontal treatment with the topical application of antibiotics four times within a 2-month period. A periodontal examination, including PPD and BOP, and venous blood sampling were carried out at baseline and at 2 and 6 months after periodontal treatment. Glycated hemoglobin (HbA_{1c}), and serum levels of high-sensitivity (hs)-CRP, TNF-α and IL-6 were analyzed.

Results: A generalized linear model showed significant associations between the change in the HbA_{1c} values at 6 months after periodontal treatment, and the change in the BOP, baseline TNF-α levels and the baseline mean PPD.

Conclusions: As BOP is a marker of total gingival inflammation, these results suggest that non-surgical periodontal therapy with topical antibiotics in patients with mild periodontitis might improve glycemic control by resolving periodontal inflammation. Such treatments might be insufficient for the amelioration of insulin resistance in type 2 diabetic patients with severe periodontitis. This trial was registered with the University Hospital Medical Information Network (no. UMIN000006693). (J Diabetes Invest, doi: 10.1111/j.2040-1124.2012.00209.x, 2012)

KEY WORDS: Glycated hemoglobin, Periodontal disease, Type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetes is a multifactorial metabolic disease, and recent evidence suggests that chronic subclinical inflammation plays an important role in the development of type 2 diabetes[1]. Periodontal disease is a chronic infection caused by gram-negative periodontal bacteria that affects the supporting structures of the teeth, and leads to the destruction of connective tissue and alveolar bone, eventually resulting in tooth loss[2]. Periodontal infection has been reported to be a risk factor for various systemic diseases, particularly for type 2 diabetes[3].

Recently, several reports have addressed the effects of periodontal treatment on glycemic control. Grossi *et al.*[4] reported that glycated hemoglobin (HbA_{1c}) levels in type 2 diabetes patients who received periodontal treatment with systemic antibiotics were reduced significantly after 3 months, although the HbA_{1c} levels in patients who received periodontal treatment without systemic antibiotics were not significantly reduced. In contrast, Jones *et al.*[5] reported no significant benefit at 4 months after periodontal treatment, but the trends in some studies have favored benefits from periodontal treatment. Some reports have mentioned that periodontal treatment with or without adjunctive antibiotic therapy improves glycemic control after periodontal treatment[4,6–8]. However, other studies found no improvement in glycemic control after periodontal treatment[5,9]. More recently, Teeuw *et al.*[10]

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carried out a meta-analysis of five reliable intervention studies, and concluded that periodontal treatment with or without the use of local or systemic antibiotics leads to a significant improvement of glycemic control in type 2 diabetic patients.

Although an increasing number of studies have suggested a relationship between periodontitis and diabetes, the results remain inconsistent. At present, only a few studies have reported that diabetes and/or glucose intolerance have a significant cause–effect relationship with periodontal disease[11]. The lack of consistency regarding the cause–effect relationship between periodontitis and diabetes is likely due, at least in part, to inappropriate approaches to the treatment of different severities of periodontitis. In this regard, we carried out a study to prove the effect of therapeutic approaches on periodontitis and the mitigation of glycemic control in diabetic patients, which could be considered as direct evidence of its causal nature[11]. The patients were divided into subgroups based on the severity of periodontitis, and the effects of periodontal treatments on the glycemic control in diabetic patients were assessed.

MATERIALS AND METHODS

Study Population

The concept of the current study is briefly summarized elsewhere[11]. A total of 59 diabetic patients were recruited from the diabetes clinic at Tokyo Medical and Dental University Hospital, Faculty of Medicine, and the Department of Diabetes and Metabolic Medicine, National Center for Global Health and Medicine, Tokyo, Japan. At the diabetes clinic, the patients were selected based on the following inclusion criteria: age 35–75 years, HbA_{1c} level 6.2–10.4%, absence of severe diabetic complications, no evidence of systemic disease other than diabetes as a risk factor for periodontitis, no administration of systemic antibiotics during the preceding 3 months, no pregnancy or lactation, no allergy to tetracycline and no modifications to the treatment received for diabetes during the preceding 2 months. One patient suffered from an immune disorder and one patient had type 1 diabetes. The HbA_{1c} levels of two patients were not within the range required by the present study criteria. As a result, four patients were excluded for not meeting the inclusion criteria, and seven patients refused to receive periodontal treatment at the periodontal clinic at the Tokyo Medical and Dental University Hospital, Faculty of Dentistry, Tokyo, Japan. Thereafter, 48 patients were transferred to the periodontal clinic at the Tokyo Medical and Dental University Hospital, Faculty of Dentistry, Tokyo, Japan. The patients included in the present study were then selected based on the following dental condition criteria: ≥10 remaining teeth, at least two sites with a probing pocket depth (PPD) ≥4 mm and no administration of periodontal treatment during the preceding 6 months. Four patients who did not meet these criteria were excluded. A final total of 44 participants fulfilled the criteria and provided written informed consent to participate.

Study Design

All the patients underwent an initial examination including a general medical history and complete full-mouth periapical radiographs (bisecting angle projections). At the second and third visits, we instructed the patients in oral hygiene methods (tooth brushing, interdental brushing and/or dental flossing), and removed supragingival plaque and calculus using an ultrasonic scaler (Varios; Nakanishi Inc., Tochigi, Japan). At the fourth and fifth visits, subgingival scaling and root planing was carried out to remove plaque and calculus using the same device. On the second to fifth visits, 10 mg of local minocycline ointment (Periofil®; Showa Yakuhin, Tokyo, Japan) was applied to each periodontal pocket after treatment. The maximum duration between the second visit and the fifth visit was 2 months.

After the fifth visit, all the participants visited the medical and dental clinics at 2 and 6 months. Supportive periodontal therapy, including instructions for oral hygiene techniques, were given and supra- and subgingival debridement without the topical administration of antibiotics was carried out. The study protocol is shown in Figure 1.

Briefly, during the initial 2 months, patients received intensive periodontal treatment with topical administration of

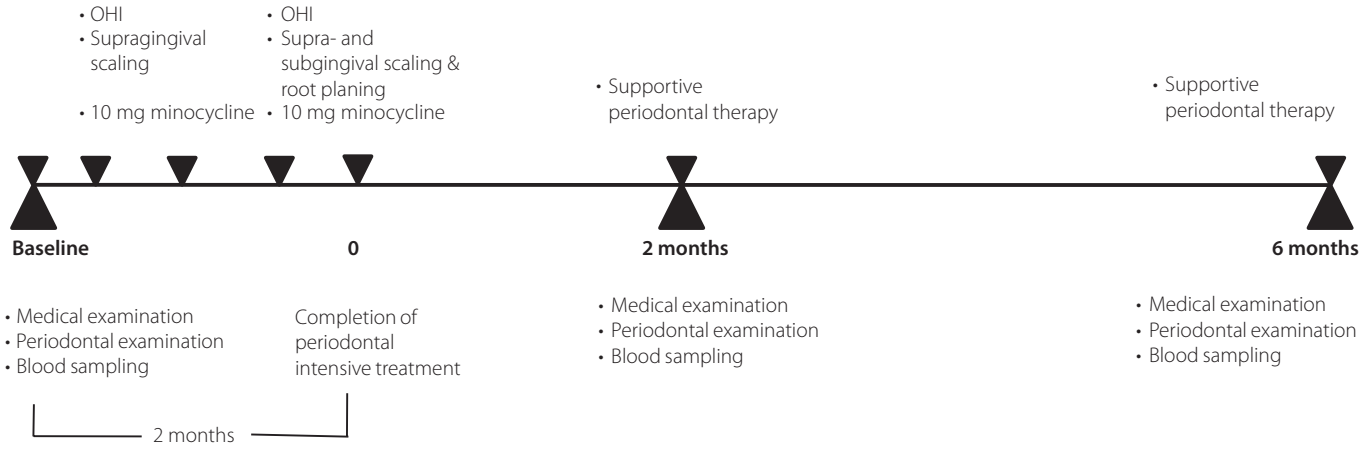

Figure 1 | Protocol of the present intervention study. OHI, oral hygiene instruction.

antimicrobial agents four times every other week, followed by 2 and 6 months' observation with additional supportive periodontal treatments. Periodontal examination and blood sampling were carried out at baseline, and at 2 and 6 months after the intensive phase of the periodontal treatment.

The doses and the kinds of antidiabetic drugs, including oral hypoglycemic drugs and insulin injections, and the patients' diets and exercise levels remained unchanged during the course of the study.

The patients were divided into two groups based on the decrease in the bleeding on probing (BOP) or the mean PPD. Patients showing a decrease of BOP by more than 50% compared with the baseline were included in the BOP-D group, whereas patients who did not show a decrease of BOP by more than 50% compared with baseline were included in the BOP-ND group. The patients whose baseline mean PPD was less than 2.5 mm were grouped in the PPD-L group, whereas patients whose baseline mean PPD was more than 2.5 mm were grouped in the PPD-H group.

The study protocol was approved by the Ethics Committees at both of the University Hospitals and was carried out in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Periodontal Examination

All the participants underwent a full-mouth clinical periodontal examination at the dental clinic. The periodontal parameters were recorded at baseline and at 2 and 6 months. Full-mouth clinical measurements of the PPD and BOP were made at the buccal, lingual, mesiobuccal, mesiolingual, distobuccal and distolingual surfaces of the teeth using a manual probe (PCP-UNC 15; Hu-Friedy Manufacturing, Chicago, IL, USA).

Detection of Markers of Glycemic Control and Inflammatory Mediators

At the diabetes clinic, venous blood samples were obtained from each patient, and the levels of HbA_{1c}, high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor (TNF)-α and interleukin (IL)-6 were measured. The HbA_{1c} levels were determined using high-performance liquid chromatography (Kyotokagaku, Kyoto, Japan). The value for HbA_{1c} (%) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula HbA_{1c} (%) = HbA_{1c} (Japan Diabetes Society; JDS) (%) + 0.4%, considering the relational expression of HbA_{1c} (JDS) (%) measured by the previous Japanese standard substance and measurement methods, and HbA_{1c} (NGSP)[12]. Serum was stored at –70°C for other measurements. The serum hs-CRP level was measured using a latex particle-enhanced immunonephelometric method. The TNF-α and IL-6 levels in the serum were determined with appropriate enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA).

Statistical Analysis

The statistical analysis was carried out using JMP® software (SAS Institute, Cary, NC, USA). Changes in the parameters from baseline to 2 and 6 months after the completion of periodontal therapy were compared using the Wilcoxon signed-rank test. The Mann–Whitney *U*-test was used to compare changes in parameters between the groups of patients. The correlations between various sets of parameters were examined with the Spearman's rank correlation coefficient. A multiple logistic regression analysis was carried out to determine the odds ratio for the reduction of HbA_{1c} values. Before the analysis, distribution of the data was assessed with the Shapiro–Wilk test. Because the variables did not show normal distribution, variables were categorized into four groups; the cut-off points of the categories were upper quartiles. The explanatory variables used in the study were age, sex, body mass index (BMI), baseline HbA_{1c}, baseline PPD, change in PPD at 6 months, baseline BOP, change in BOP at 6 months, baseline hs-CRP, change in hs-CRP at 6 months, baseline TNF-α, change in TNF-α at 6 months, baseline IL-6 and change in IL-6 at 6 months. Variables of the significant level <0.25 were entered into the regression model in a stepwise approach. Values of $P < 0.05$ were considered significant.

RESULTS

All Participants

Among the 44 patients, three patients stopped visiting the hospitals during the active periodontal treatment and were excluded from the analysis, resulting in a final total of 41 patients (23 men, 18 women) who were analyzed. The mean age of the patients was 63.3 ± 9.9 years. A total of 13 of the 41 patients were using insulin injections, 28 patients were using oral hypoglycemic drugs, and none was being treated with diet alone. The mean BMI was 24.6 ± 3.6 kg/m^2 at baseline, showing that the study group had a moderately high BMI. The mean number of present teeth was 21.7 ± 6.5.

All the clinical periodontal parameters, such as the PPD and the BOP, showed significant improvement from the baseline to 2 months after periodontal treatment, and these significant improvements were maintained during the study period (Table 1).

Table 1 | Periodontal parameters and systemic markers involved in glycemic control during baseline and follow-up periods in all participants

	Baseline	2 months	6 months
HbA_{1c} (%)	7.3 ± 0.8	7.2 ± 0.7	7.1 ± 0.6
PPD (mm)	2.7 ± 0.7	2.2 ± 0.5*	2.1 ± 0.5*
BOP (%)	46.6 ± 30.0	25.2 ± 29.0*	24.7 ± 27.6*
hs-CRP (ng/mL)	1179 ± 1750	1307 ± 1991	932 ± 1003
TNF-α (pg/mL)	0.7 ± 0.4	0.9 ± 0.5	0.8 ± 0.6
IL-6 (pg/mL)	1.2 ± 1.0	0.9 ± 0.6	1.1 ± 0.6

Values are given as mean ± SD. *Significant decrease compared with baseline ($P < 0.05$). BOP, bleeding on probing; HbA_{1c}, glucated hemoglobin; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleakin-6; PPD, probing pocket depth; TNF-α, tumor necrosis factor-α.

The HbA_{1c} level was reduced from 7.3 ± 0.8% to 7.2 ± 0.7% at 2 months and 7.1 ± 0.6% at 6 months after periodontal treatment. The value at 6 months was slightly lower than that at baseline, although the difference was not significant (Table 1). No significant differences were observed in the hs-CRP, TNF-α or IL-6 levels (Table 1).

Effect of Improvement of BOP on HbA_{1c}

To clarify the effect of the improvement of the BOP on glycemic control, patients were divided into two groups based on the ratio of change in the BOP at 6 months after periodontal treatment. Out of the 41 patients, the BOP at 6 months had decreased by more than 50% compared with the baseline value in 25 patients (BOP-D group), whereas the decrease in the BOP at 6 months was <50% compared with the baseline value in the other 16 patients (BOP-ND group). We further compared the results between the BOP-D and BOP-ND groups. As shown in Tables 2 and 3, no differences in the BOP, age, sex, BMI, method of diabetic treatment, number of teeth, HbA_{1c}, hs-CRP, TNF-α or IL-6 values at baseline were observed between the BOP-D and BOP-ND groups. The PPD was found to be significantly deeper in the BOP-D group than in the BOP-ND group at baseline, but this significance in the differences in the PPD between the two groups disappeared at 2 and 6 months after periodontal treatment. The PPD and BOP decreased significantly after periodontal treatment throughout the study period in the BOP-D group, and although the PPD and the BOP in the BOP-ND group showed a significant reduction at 2 months, no significant difference was observed at 6 months. The BOP in the BOP-D group was significantly lower than that in the BOP-ND group at 2 and 6 months after periodontal treatment.

The HbA_{1c} level at baseline was not different between the BOP-D and the BOP-ND groups. The HbA_{1c} level in the BOP-D group significantly decreased at 6 months, but it remained unchanged in the BOP-ND group during the follow-up period. No significant differences in the hs-CRP, TNF-α and IL-6 levels were observed in either the BOP-D or the BOP-ND group between the baseline and the follow-up periods.

Table 2 | Demographic characteristics at baseline in the decrease of bleeding on probing and no decrease of bleeding on probing groups

Group	BOP-D group	BOP-ND group
	n = 25	*n* = 16
Age (years)	63.2 ± 9.8	63.6 ± 10.2
Sex (male/female)	13/12	10/6
BMI	24.8 ± 3.9	24.3 ± 3.3
Diabetic treatment (insulin/oral hypoglycemic)	7/18	6/10
No. present teeth	21.8 ± 6.4	21.5 ± 6.7

There were no significant differences between the decrease of bleeding on probing (BOP-D) and no decrease of bleeding on probing (BOP-ND) groups. BMI, body mass index.

Table 3 | Periodontal parameters and systemic markers involved in glycemic control during baseline and follow-up periods in the decrease of bleeding on probing and no decrease of bleeding on probing groups

	Baseline	2 months	6 months
BOP-D group			
HbA_{1c} (%)	7.5 ± 0.8	7.3 ± 0.8	7.2 ± 0.6*
PPD (mm)	2.8 ± 0.7†	2.2 ± 0.4‡	2.0 ± 0.4‡
BOP (%)	44.4 ± 25.3	15.0 ± 16.9†‡	8.9 ± 5.6‡§
hs-CRP (ng/mL)	1173 ± 1872	1135 ± 1585	853 ± 676
TNF-α (pg/mL)	0.8 ± 0.4	0.9 ± 0.5	0.7 ± 0.4
IL-6 (pg/mL)	1.2 ± 1.0	0.9 ± 0.5	1.1 ± 0.6
BOP-ND group			
HbA_{1c} (%)	7.0 ± 0.6	7.0 ± 0.6	7.1 ± 0.6
PPD (mm)	2.4 ± 0.7†	2.2 ± 0.6*	2.2 ± 0.6
BOP (%)	50.0 ± 36.5	40.2 ± 36.4*†	43.3 ± 31.5§
hs-CRP (ng/mL)	1187 ± 1626	1565 ± 2516	1046 ± 1363
TNF-α (pg/mL)	0.6 ± 0.5	0.8 ± 0.5	0.9 ± 0.8
IL-6 (pg/mL)	1.3 ± 0.9	1.0 ± 0.8	1.1 ± 0.5

Values are given as mean ± SD.
*Statistically significant decrease compared with baseline ($P < 0.05$).
†Statistically significant difference between the decrease of bleeding on probing (BOP-D) and no decrease of bleeding on probing (BOP-ND) groups ($P < 0.05$).
‡Statistically significant decrease compared with baseline ($P < 0.01$).
§Statistically significant difference between the BOP-D and BOP-ND groups ($P < 0.01$).
BOP, bleeding on probing; HbA_{1c}, glycated hemoglobin; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; PPD, probing pocket depth; TNF-α, tumor necrosis factor-α.

Effect of Baseline PPD Severity on HbA_{1c}

To clarify the effect of the baseline severity of periodontitis on glycemic control, the patients were divided into two groups based on the baseline PPD. Out of the 41 patients, the mean PPD at baseline was <2.5 mm in 21 patients (PPD-L group), whereas the mean PPD at baseline was more than 2.5 mm in the other 20 patients (PPD-H group). We analyzed the differences in the glycemic control after periodontal therapy between the PPD-L and PPD-H groups. As shown in Tables 4 and 5, there were no differences in age, sex, BMI, method of diabetic treatment, number of teeth, HbA_{1c}, hs-CRP, TNF-α or IL-6 levels at baseline between the PPD-L and PPD-H groups. The PPD and BOP were significantly lower in the PPD-L group than in the PPD-H group at baseline. The BOP decreased significantly both in the PPD-L and the PPD-H groups 2 and 6 months after periodontal treatment. The PPD significantly decreased in both the PPD-L and PPD-H groups at 2 and 6 months. The significant differences in the PPD between the PPD-L and PPD-H groups persisted from the baseline until the end of the study, showing that deep periodontal pockets were not eliminated in the PPD-H group.

The HbA_{1c} level at baseline was not significantly different between the PPD-L and PPD-H groups. The HbA_{1c} level in the PPD-L group significantly decreased at 6 months, but it remained unchanged in the PPD-H group during the course of

Table 4 | Demographic characteristics at baseline in the probing pocket depth less than 2.5 mm group and the probing pocket depth more than 2.5 mm group

Group	PPD-L group	PPD-H group
	n = 21	*n* = 20
Age (years)	63.0 ± 7.9	63.7 ± 11.8
Sex (male/female)	12/9	11/9
BMI	24.4 ± 4.0	24.8 ± 3.3
Diabetic treatment (insulin/oral hypoglycemic)	8/13	5/15
No. present teeth	22.3 ± 5.4	21.1 ± 7.5

There were no significant differences between the probing pocket depth less than 2.5 mm (PPD-L) and probing pocket depth more than 2.5 mm (PPD-H) groups. BMI, body mass index.

Table 5 | Periodontal parameters and systemic markers involved in glycemic control during baseline and follow-up periods in the probing pocket depth <2.5 mm group and the probing pocket depth more than 2.5 mm group

	Baseline	2 months	6 months
PPD-L group			
HbA_{1c} (%)	7.2 ± 0.6	7.0 ± 0.6	7.0 ± 0.6*
PPD (mm)	2.1 ± 0.2†	1.9 ± 0.2§†	1.9 ± 0.3*†
BOP (%)	37.3 ± 29.1‡	21.2 ± 26.1§	23.6 ± 24.6§
hs-CRP (ng/mL)	806 ± 724	1656 ± 2385	901 ± 730
TNF-α (pg/mL)	0.6 ± 0.4	1.0 ± 0.6	0.6 ± 0.4
IL-6 (pg/mL)	1.1 ± 1.0	1.1 ± 0.7	1.1 ± 0.6
PPD-H group			
HbA_{1c} (%)	7.4 ± 0.9	7.3 ± 0.8	7.3 ± 0.6
PPD (mm)	3.3 ± 0.6†	2.5 ± 0.5§†	2.4 ± 0.5§†
BOP (%)	56.9 ± 28.3‡	29.1 ± 7.2§	25.9 ± 31.0§
hs-CRP (ng/mL)	1552 ± 2339	958 ± 1479	965 ± 1250
TNF-α (pg/mL)	0.8 ± 0.5	0.8 ± 0.3	0.9 ± 0.8
IL-6 (pg/mL)	1.3 ± 0.9	0.8 ± 0.3	1.1 ± 0.5

Values are given as mean ± SD.
*Statistically significant decrease compared with baseline ($P < 0.05$).
†Statistically significant difference between the PPD-L and PPD-H groups ($P < 0.01$).
‡Statistically significant difference between the probing pocket depth <2.5 mm (PPD-L) and probing pocket depth more than 2.5 mm (PPD-H groups; $P < 0.05$).
§Statistically significant decrease compared with baseline ($P < 0.01$).
BOP, bleeding on probing; HbA_{1c}, glycated hemoglobin; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; PPD, probing pocket depth; TNF-α, tumor necrosis factor-α.

the study. No significant differences in the hs-CRP, TNF-α or IL-6 levels were observed between the baseline and the follow-up periods in either the PPD-L or the PPD-H group (Table 5).

Correlations Between Periodontal Parameters and TNF-α

The change in the mean PPD at 6 months was significantly and positively correlated with the change in the BOP at 6 months ($P < 0.01$, $\rho = 0.57$) and the change in the TNF-α level at 6 months ($P < 0.01$, $\rho = 0.45$; Figure 2). The change in the TNF-α level was also significantly and positively correlated with the change in the HbA_{1c} level at 6 months ($P < 0.05$, $\rho = 0.36$).

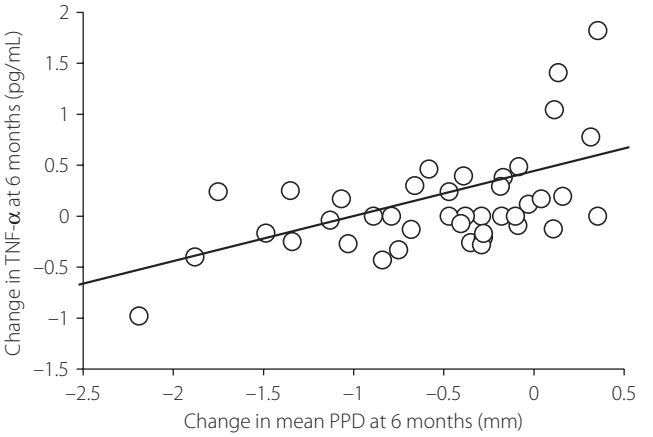

Figure 2 | Relationship between changes in the mean probing pocket depth (PPD) at 6 months and changes in tumor necrosis factor (TNF)-α at 6 months. Regression lines were calculated and drawn.

Multiple Logistic Regression Analysis

To further analyze the effects of periodontal treatment on glycemic control, the associations between the change in the HbA_{1c} level at 6 months and various explanatory variables in all the patients were assessed with a multiple logistic regression analysis. Almost all variables (except two variables: baseline TNF-α and change in PPD at 6 months), failed to show normal distribution, so all variables were categorized respectively into four groups (Table 6). Three variables were selected by the stepwise approach, and the multiple regression analysis showed that baseline mean PPD < 2.5 mm, change in TNF-α ≤ 0 at 6 months and baseline TNF-α significantly correlated with the reduction of the HbA_{1c} level at 6 months (Table 7).

DISCUSSION

In the present study, the multiple logistic regression analysis showed that a baseline mean PPD <2.5 mm, a change in TNF-α ≤0 at 6 months and baseline TNF-α significantly correlated with the reduction of the HbA_{1c} level at 6 months (Table 7). These results suggested that periodontal treatment with the topical application of antibiotics might be effective against mild periodontitis with high systemic inflammation (high TNF-α). BOP is a clinical marker for net gingival inflammation in periodontitis patients[13], and the HbA_{1c} level was significantly reduced in the BOP-D group. These results suggest that the resolution of periodontal inflammation is closely related to an improvement in glycemic control in diabetes patients. Inflammatory cytokines, including TNF-α and IL-6, are thought to induce insulin tolerance[14–16], and the serum TNF-α, IL-6 and CRP levels are used as surrogate markers for systemic inflammation. Elevated

Table 6 | Categories used in multivariable analysis†

	Categories			
	0	1	2	3
Age (years)	≥72	71–67	66–57	≥56
BMI	>26.2	26.2≥, >23.8	23.8≥, >22.4	22.4≥
Baseline HbA_{1c} (%)	≥7.7	7.6–7.3	7.2–6.9	6.8≥
Baseline PPD (mm)	>3.0	3≥, ≥2.5	2.5>, >2.1	2.1≥
Change in PPD (mm)	>−0.1	−0.1≥, >−0.4	−0.4≥, >−1.0	−1.0≥
Baseline BOP (%)	>64.1	64.1≥, >40	40≥, >20.4	20.4≥
Change in BOP (%)	>−4.2	−4.2≥, >−17.7	−17.7≥, >−34.9	−34.9≥
Baseline hs-CRP (ng/mL)	>1150	1150≥, >640	640≥, >230	230≥
Change in hs-CRP (ng/mL)	>100	105≥, >0	0≥, >−323	−300≥
Baseline TNF-α (pg/mL)	>1.0	1≥, >0.7	0.7≥, >0.4	0.4≥
Change in TNF-α (pg/mL)	>0.24	0.24≥, >0	0≥, >−0.21	−0.21≥
Baseline IL-6 (pg/mL)	>1.5	1.5≥, >1.0	1.0≥, >0.5	0.5≥
Change in IL-6 (pg/mL)	>0.3	0.3≥, >0	0≥, >−0.3	−0.3≥

†Cut-off points of each category were quartiles. BOP, bleeding on probing; HbA_{1c}, glycated hemoglobin; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; PPD, probing pocket depth; TNF-α, tumor necrosis factor-α.

Table 7 | Multiple logistic regression model for the reduction in glycted hemoglobin levels between baseline and 6 months

	Estimated odds ratio	95% Confidence interval
Change in TNF-α ≤ 0 (pg/mL)	4.0	1.3–19.1
Baseline PPD < 2.5 (mm)	6.2	1.9–39.4
Baseline TNF-α > 0.7 (pg/mL)	7.1	2.1–42.1

Model $r^2 = 0.42$. PPD, probing pocket depth; TNF-α, tumor necrosis factor-α.

circulating levels of CRP and IL-6 have been reported to be significant risk indicators for the development or progression of type 2 diabetes[17]. Some intervention studies have suggested that successful anti-infective periodontal treatment can reduce the CRP, TNF-α and IL-6 levels in systemically healthy subjects[18–21]. Although the improvements in the clinical parameters significantly correlated with the TNF-α (Figure 2), the present study failed to detect a reduction in the TNF-α, IL-6 and hs-CRP levels after periodontal treatment. In agreement with the present results, Yamazaki *et al.*[22] also reported that the hs-CRP, IL-6 and TNF-α levels were not significantly changed after periodontal treatment. The changes in the TNF-α level at 6 months significantly correlated with an improvement in the periodontal conditions, suggesting that periodontal treatment might ameliorate the systemic inflammation that induces insulin tolerance. However, reduction of the HbA_{1c} values was not always observed equally in all patients, and the multiple logistic regression analysis suggested that the reduction in HbA_{1c} values might be most effective in patients with mild periodontitis and high TNF-α levels, as mild periodontitis can be effectively treated with non-surgical periodontal therapy combined with topical antibiotics.

Periodontal disease is caused by the accumulation of bacterial plaque biofilm on tooth surfaces[23]. The bacterial plaque induces gingivitis within a few days after accumulation[24], and gingivitis eventually progresses to periodontitis in susceptible individuals if the accumulated plaque is not removed effectively within a certain period of time. The severity of periodontitis differs among individuals, and diabetes is an important risk factor for the development of periodontitis[25]. The application of local antibiotics combined with mechanical debridement of the infected root results in significant adjunctive improvement of the periodontal tissue, compared with debridement alone[26], and intensive periodontal treatment, including non-surgical mechanical debridement and local antibiotics, is frequently applied in intervention studies to evaluate the effects of periodontal treatments on systemic diseases, such as type 2 diabetes and cardiovascular diseases[8,27,28]. In the present study, periodontitis patients were divided into two groups (PPD-H and L) based on the median of the mean PPD (PPD 2.5 mm). The severity of the periodontal condition of the PPD-H group corresponded approximately with severe periodontitis in previous reports[29].

In the PPD-L group, the HbA_{1c} level had significantly reduced at the end of the study, but it did not decrease in the PPD-H group. Considering that the bacterial plaque and calculus in the deep periodontal pockets could not be completely removed by the non-surgical treatments[30,31], the lack of improvement in the HbA_{1c} level in the PPD-H group in the present study might be explained by the incomplete resolution of the periodontal infection by the intensive periodontal treatment. In patients with severe periodontitis, surgical intervention, including periodontal surgery and tooth extraction[32], might be necessary to eliminate periodontal inflammation. It should also be noted that invasive periodontal treatments might transiently increase the risk for vascular events[33], and the risks and benefits of surgical periodontal treatment should be carefully evaluated in patients with diabetes.

The present study suggests that the improvement of periodontal inflammation might be important to improve glycemic control in type 2 diabetes patients. The result of the multiple logistic regression analysis suggests that the reduction of glycemic control in type 2 diabetes patients might be mainly ($r^2 = 0.42$, Table 7) explained by the improvement of inflammation (change in TNF-α ≤ 0) in cases with mild periodontitis (mean PPD < 2.5 mm) with a high serum TNF-α level at baseline.

The limitations of the present study included a small sample size, selection bias, short study period, and the lack of adequate data regarding monitoring the lifestyle and dietary habits of patients during the study period. All these factors could have affected the results of the present study. Prospective studies excluding these limitations are required to clarify the

mechanisms responsible for insulin resistance in diabetic patients with periodontitis, emphasizing serum cytokine levels and the periodontal condition as suggested by the results in the present study.

Non-surgical periodontal treatment with topical antibiotics improved glycemic control in type 2 diabetic patients with mild periodontitis, but the treatment might be insufficient for the amelioration of insulin resistance in type 2 diabetic patients with moderate to severe periodontitis. Periodontitis and diabetes are related to each other, and this relationship might produce a vicious circle[11]. The treatment of mild periodontitis might alleviate this downward spiral, but further studies are required to determine the effects of periodontal treatment on glycemic control in diabetic patients with severe periodontitis.

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