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Short communication

Relationship of *Porphyromonas gingivalis* with glycemic level in patients with type 2 diabetes following periodontal treatment

N. Makiura[1], M. Ojima[2], Y. Kou[1,3], N. Furuta[1], N. Okahashi[1], S. Shizukuishi[2], A. Amano[1]

Departments of [1]Oral Frontier Biology, and [2]Preventive Dentistry, Osaka University Graduate School of Dentistry, Suita-Osaka 565-0871, Japan, [3]Department of Oral Medicine, School of Stomatology, China Medical University, Shenyang-Liaoning, China

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Introduction: The aim of this study was to assess the relationship between serum glycemic levels and subgingival microbial profile alteration following periodontal treatment in patients with type 2 diabetes mellitus.
Methods: We studied 30 periodontitis patients with type 2 diabetes mellitus who received full-mouth subgingival debridement by analyzing their subgingival microbial profiles using a polymerase chain reaction method at baseline and various time-points for 12 months following treatment. Concurrently, probing pocket depth, bleeding on probing, and metabolic parameters, including glycated hemoglobin A1c (HbA1c), blood sugar level, C-reactive proteins, total cholesterol, triglyceride, and high-density and low-density lipoprotein cholesterol, were recorded.
Results: Periodontal conditions were significantly improved after treatment, and the occurrence rates of periodontal bacterial species, including *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Treponema denticola*, and *Prevotella intermedia*, were also reduced. Interestingly, *P. gingivalis* was detected more frequently in subjects with increased HbA1c values after periodontal treatment than in those patients with decreased HbA1c values. Furthermore, *P. gingivalis* with type II fimbriae was detected only in HbA1c-increased subjects, while improvements in HbA1c values were observed only in subjects without type II clones.
Conclusions: These results suggest that glycemic level in diabetes is affected by the persistence of *P. gingivalis*, especially clones with type II fimbriae, in periodontal pockets.

Key words: *fimbriae; glycated hemoglobin A1c; periodontitis; Porphyromonas gingivalis;* type 2 diabetes mellitus

Atsuo Amano, Department of Oral Frontier Biology, Osaka University Graduate School of Dentistry, 1-8 Yamadaoka, Suita-Osaka 565-0871, Japan
Tel.: + 81 6 6879 2976;
fax: + 81 6 6879 2110;
e-mail: amanoa@dent.osaka-u.ac.jp
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Periodontal disease is the sixth most common complication of diabetes mellitus (DM) (12) and the chronic nature of this infection is considered to contribute to a worsening of diabetic status (19), while successful periodontal treatment has been suggested to improve metabolic control in diabetes (5, 8, 9, 11, 15, 17, 22). In contrast, a recent report based on a meta-analysis of data from 10 intervention trials that included 456 patients revealed that the decrease in glycated hemoglobin A1c (HbA1c) following periodontal therapy was not statistically significant (10).

Porphyromonas gingivalis is considered to be a bona fide periodontal pathogen and its fimbriae are classified into six genotypes (types I–V and Ib), based on the nucleotide sequences of the *fimA* genes encoding the fimbrial subunits (1). Our recent cross-sectional study showed that the presence of *P. gingivalis* with type II fimbriae was a critical infectious factor closely associated with the deterioration of periodontitis seen in patients with DM (14). Inflammatory cytokines induced by periodontitis are considered to be related to

the metabolic abnormalities associated with type 2 DM (8). *P. gingivalis* and its components, including fimbriae and lipopolysaccharide, reportedly activate various host cells, resulting in the release of cytokines, such as interleukin-1, -6, and -8, and tumor necrosis factor-α (4). The level of cytokine induction by *P. gingivalis* with type II fimbriae was shown to be greater than that by organisms with type I fimbriae (18), indicating the possibility that possession of type II fimbriae is a factor related to glycemic level in diabetes. Nevertheless, the effects of therapeutic elimination of periodontal pathogens, including *P. gingivalis* with type II fimbriae, on diabetes glycemic levels are unclear. In the present study, we assessed the relationship between diabetes glycemic level and alteration of the subgingival microbial profile after periodontal treatment in patients with type 2 DM.

A total of 30 Japanese adults (14 male and 16 female) suffering from type 2 DM and with adult periodontitis were recruited according to a protocol approved by the Ethics Committee of Osaka Police Hospital, after fulfilling the entrance criteria and the submission of a signed informed consent form. The ages of the subjects ranged from 41 to 80 years (mean 63.9 ± 9.4 years) and duration of DM was between 3 and 13 years (8.5 ± 5.6 years), with HbA1c values ranging from 6.0% to 10% (7.11 ± 1.16%). The criteria used for inclusion in the study were: (i) no major diabetic complications; (ii) no history of systemic antibiotic drug administration within the previous 3 months; (iii) no periodontal treatment for at least 6 months prior to the study; (iv) no evidence of current acute illness; and (v) more than 18 functional teeth remaining, without dentures. Peripheral blood samples were analyzed for levels of C-reactive protein, blood sugar level, HbA1c, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol, and triglyceride by an outside laboratory (SRL Inc., Tokyo, Japan).

All subjects underwent a periodontal examination conducted by a single skilled operator (N. M.), which included determinations of bleeding on probing and probing pocket depth, measured to the nearest millimeter at six points, as described previously (14). The ratio (%) of bleeding sites was assessed by gentle probing of the bottom of the pockets using a periodontal probe. The periodontal parameters for representative teeth where subgingival plaque samples were collected were used for analysis. The subjects received oral hygiene instructions, as well as full-mouth scaling and root planing performed under local anesthesia.

Subgingival plaque samples were obtained at the baseline and during follow-up examinations from the mesiolingual surface of the left mandibular anterior tooth, and the rearmost posterior teeth in the left maxilla and right mandible (three samples per subject) using sterile curettes. Bacterial occurrence within subgingival plaque samples was analyzed using a polymerase chain reaction method, with bacterial species-specific and universal primer sets (2, 3). The target microorganisms were *P. gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythensis*, *Treponema denticola*, and *Prevotella intermedia.* The sampling technique and sample processing utilized have been described in detail previously (2). Genotyping of *P. gingivalis* fimbriae was also assessed using a polymerase chain reaction, as described previously (3, 13). When the three plaque samples from a single subject showed different detection profiles, a sample with positive occurrence was used as the representative microbial profile. All analyses were performed using DR SPSS FOR WINDOWS (SPSS Inc., Chicago, IL), with the level of significance set at 5%.

First, the effects of treatments on periodontal status and diabetes metabolic control were analyzed. Figure 1 summarizes the periodontal and metabolic parameters of the subjects at baseline, 2 and 4 weeks, and 3, 6, and 12 months after the non-surgical periodontal treatment. Changes in clinical parameters and biomarkers from the baseline to the measurement time-points were verified utilizing a paired *t*-test. The periodontal parameters probing pocket depth and bleeding on probing each demonstrated a significant improvement in periodontal condition after treatment. Although there was a slight trend toward reduction in some DM metabolic parameters, including C-reactive

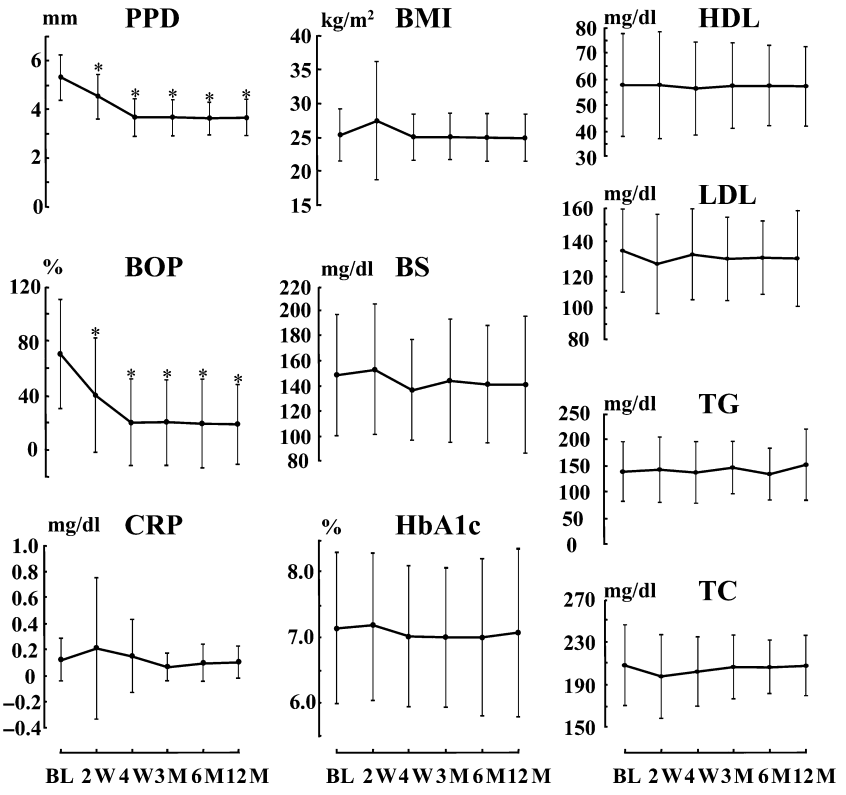

Fig 1. Effects of periodontal treatment on periodontal condition and diabetes mellitus (DM) metabolic control. Periodontal and metabolic parameters of type 2 DM patients were recorded at the baseline (BL) and at various time-points (2 and 4 weeks, 3, 6, and 12 months) following non-surgical periodontal treatment. Periodontal parameters; probing pocket depth (PPD) and bleeding on probing (BOP) for representative teeth from which subgingival plaque samples were collected. DM metabolic parameters: C-reactive protein (CRP), body mass index (BMI), blood sugar level (BS), glycated hemoglobin A1c (HbA1c), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), and total cholesterol (TC); $*P < 0.05$.

Table 1. Temporal changes of subgingival bacterial profile in patients with type 2 diabetes mellitus

	Bacterial detection rate before and after treatment (%)					
Bacterium	Baseline	2 weeks	4 weeks	3 months	6 months	12 months
P. gingivalis	53.3	56.7	33.3*	23.3*	16.7*	20.0*
Type I fimbria clone	0.0	6.7	6.7	3.3	3.3	3.3
Ib	10.0	13.3	0.0*	3.3*	0.0*	0.0*
II	33.3	13.3*	13.3*	6.7*	3.3*	6.7*
III	6.7	13.3	6.7	6.7	6.7	6.7
IV	3.3	6.7	3.3	3.3	0.0	3.3
V	0.0	3.3	3.3	0.0	3.3	0.0
A. actinomycetemcomitans	13.3	10.0	0.0*	10.0	16.7	20.0
T. forsythensis	86.7	86.7	50.0*	36.7*	30.0*	50.0*
P. intermedia	33.3	16.7*	6.7*	3.3*	6.7*	13.3*
T. denticola	63.3	60.0	26.7*	26.7*	23.3*	23.3*

*Significant difference ($P < 0.05$) as compared to baseline.

protein, HbA1c, blood sugar level, and low-density lipoprotein, in association with periodontal improvement, no significant reductions were observed after treatment. These results suggest that the effects of therapeutic periodontal improvement on diabetes metabolic control are limited.

Next, subgingival microbial profiles were determined at the baseline and at the time-points following periodontal treatment (Table 1). *T. forsythensis* was the most prevalent at the baseline ($n = 26$, 86.7%), followed by *T. denticola*. Among *P. gingivalis*-positive subjects ($n = 16$, 53.3%) the majority ($n = 10$) were found to be infected with type II fimbria clones at the baseline. The occurrence of *P. gingivalis*, *T. forsythensis*, *P. intermedia*, and *T. denticola* was significantly reduced to non-detectable levels following periodontal treatment. Furthermore, the detection rates of *P. gingivalis* clones with types Ib and II fimbriae were also significantly decreased, whereas those of the other clones varied among the different measurement times. The occurrence of *A. actinomycetemcomitans* seemed to not be influenced by periodontal treatment. The elimination or persistence of the above bacterial species was not statistically related to improvement in periodontal condition for this group of patients.

Finally, the relationships of the microbial profiles with the metabolic parameters in diabetes at various time-points were analyzed. The patients were categorized into two groups based on changes in their HbA1c levels, those with a decrease in HbA1c from the baseline to each measurement and those with an increase. Differences in the occurrence of *P. gingivalis* and its type II fimbria clones between these two groups were evaluated using a chi-squared test. Clear tendencies were observed only for the relationship of increase or decrease of HbA1c with the presence of *P. gingivalis* at each measurement time-point. In subjects with increased HbA1c values (+ 0.5 ± 0.4%) after periodontal treatment, *P. gingivalis* was detected more frequently than in those patients with decreased HbA1c values (– 0.6 ± 0.6%) (Table 2) with significant differences observed at 3 and 6 months after treatment. In addition, *P. gingivalis* with type II fimbriae was detected only in subjects with an HbA1c increase with significant differences at 2 and 4 weeks; improvements in HbA1c values were observed only in subjects without type II clones. Such relationships were not seen among the other bacterial species and *P. gingivalis* with other fimbria types.

P. gingivalis has been shown to be the most frequently detected microorganism in periodontal pockets of patients with type 2 DM (20, 23). We previously reported that *P. gingivalis* with type II fimbriae had a significant association with deterioration of periodontitis in DM patients as well as systemically healthy subjects, whereas type I and IV fimbria clones were not related to periodontitis progression (14). The present results suggest that the presence of *P. gingivalis* is related to changes in HbA1c values in patients with type 2 DM. In addition, *P. gingivalis* with type II fimbriae was only detected in subjects with increased HbA1c, which suggests the involvement of type II clones with the deterioration of glycemic control as well as periodontal destruction in type 2 DM patients. *P. gingivalis* was previously reported to reinfect or recolonize periodontal pockets within 6 months after mechanical debridement (6). We followed the effects of periodontal treatment in the present subjects for 12 months, which was considered sufficient to support the above speculation.

Patients with DM appear to respond to bacterial challenge in an exaggerated manner compared with non-diabetic patients, because of their impaired immune response, and develop more severe forms of inflammatory periodontal diseases (7). Various opportunistic bacterial species may therefore be related to the condition of those patients, although only *P. gingivalis* had a statistically significant effect in the present study. A major portion of the bacterial lifestyle is harbored within the complex multi-species biofilm (16) and it was recently reported that biofilm production by *Candida* species might be involved in systemic disorders, including DM (21). Thus, qualitative and quantitative analyses of biofilms in DM patients are important for future studies.

Table 2. Relationship between persistence of *Porphyromonas gingivalis* and change in glycated hemoglobin A1c (HbA1c) level after treatment

	Number of subjects with improved or deteriorated HbA1c level				
	2 weeks	4 weeks	3 months	6 months	12 months
HbA1c-deteriorated group (ΔHbA1c > 0)	12	13	13	11	14
HbA1c-improved group (ΔHbA1c ≤ 0)	18	17	17	19	16
	Persistence of *P. gingivalis* in HbA1c-deteriorated or improved subjects (%)				
Persistence of *P. gingivalis*					
ΔHbA1c > 0	66.7 (8)	46.2 (6)	46.2 (6)	45.5 (5)	28.6 (4)
ΔHbA1c ≤ 0	50.0 (9)	23.5 (4)	5.9 (1)	0 (0)	6.3 (1)
p-value	ns	ns	0.01	0.001	ns
Persistence of *P. gingivalis* with type II fimbriae					
ΔHbA1c > 0	33.3 (4)	30.8 (4)	15.4 (2)	0 (0)	14.3 (2)
ΔHbA1c ≤ 0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
P-value	0.01	0.014	ns	ns	ns

Δ, change in level of HbA1c (%) at each measurement time from the baseline value.
The numbers of *P. gingivalis*-positive subjects among HbA1c-deteriorated or HbA1c-improved group are shown in parentheses.
The *P*-values indicate a significant difference between HbA1c-deteriorated and HbA1c-improved subjects; ns, not significant.

In addition to fimbriae, *P. gingivalis* has a number of other potential virulence factors, such as gingipains and capsules containing lipopolysaccharide, which are involved in the pathogenesis of periodontitis (4). Those other virulence factors may also contribute to the varying pathogenicity among type II fimbria clones and it is unclear if strong virulence is uniformly conserved among clones with type II fimbriae. Additional investigations are necessary to elucidate the exact relationship between type II *P. gingivalis* and DM.

The present mechanical periodontal treatments were significantly effective for improvement of periodontal conditions as well as the subgingival microbial profile of subjects with DM. The present findings also showed that periodontitis in DM responded to the therapy, we therefore propose that therapeutic elimination/suppression of *P. gingivalis* is an important factor closely associated with improvement of glycemic control.

References

1. Amano A. Disruption of epithelial barrier and impairment of cellular function by *Porphyromonas gingivalis*. Front Biosci 2007: **12**: 3965–3974.
2. Amano A, Kishima T, Akiyama S, Nakagawa I, Hamada S, Morisaki I. Relationship of periodontopathic bacteria with early-onset periodontitis in Down's syndrome. J Periodontol 2001: **72**: 368–373.
3. Amano A, Kuboniwa M, Nakagawa I, Akiyama S, Morisaki I, Hamada S. Prevalence of specific genotypes of *Porphyromonas gingivalis fimA* and periodontal health status. J Dent Res 2000: **79**: 1664–1668.
4. Amano A, Nakagawa I, Okahashi N, Hamada N. Variations of *Porphyromonas gingivalis* fimbriae in relation to microbial pathogenesis. J Periodontal Res 2004: **39**: 136–142.
5. Christgau M, Palitzsch KD, Schmalz G, Kreiner U. Healing response to nonsurgical periodontal therapy in patients with diabetes mellitus: clinical, microbiological, and immunologic results. J Clin Periodontol 1998: **25**: 112–124.
6. Fujise O, Miura M, Hamachi T, Maeda K. Risk of *Porphyromonas gingivalis* recolonization during the early period of periodontal maintenance in initially severe periodontitis sites. J Periodontol 2006: **77**: 1333–1339.
7. Genco RJ. Current view of risk factors for periodontal diseases. J Periodontol 1996: **67**: 1041–1049.
8. Grossi SG. Treatment of periodontal disease and control of diabetes: an assessment of the evidence and need for future research. Ann Periodontol 2001: **6**: 138–145.
9. Iwamoto Y, Nishimura F, Nakagawa M et al. The effect of antimicrobial periodontal treatment on circulating tumor necrosis factor-alpha and glycated hemoglobin level in patients with type 2 diabetes. J Periodontol 2001: **72**: 774–778.
10. Janket SJ, Wightman A, Baird AE, Van Dyke TE, Jones JA. Does periodontal treatment improve glycemic control in diabetic patients? A meta-analysis of intervention studies J Dent Res 2005: **84**: 1154–1159.
11. Kiran M, Arpak N, Unsal E, Erdogan MF. The effect of improved periodontal health on metabolic control in type 2 diabetes mellitus. J Clin Periodontol 2005: **32**: 266–272.
12. Löe H. Periodontal disease. The sixth complication of diabetes mellitus. Diabetes Care 1993: **16**: 329–334.
13. Nakagawa I, Amano A, Ohara-Nemoto Y et al. Identification of a new variant of *fimA* gene of *Porphyromonas gingivalis* and its distribution in adults and disabled populations with periodontitis. J Periodontal Res 2002: **37**: 425–432.
14. Ojima M, Takeda M, Yoshioka H et al. Relationship of periodontal bacterium genotypic variations with periodontitis in type 2 diabetic patients. Diabetes Care 2005: **28**: 433–434.
15. Rodrigues DC, Taba MJ, Novaes AB, Souza SL, Grisi MF. Effect of non-surgical periodontal therapy on glycemic control in patients with type 2 diabetes mellitus. J Periodontol 2003: **74**: 1361–1367.
16. Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. Periodontol 2000 2002: **28**: 12–55.
17. Stewart JE, Wager KA, Friedlander AH, Zadeh HH. The effect of periodontal treatment on glycemic control in patients with type 2 diabetes mellitus. J Clin Periodontol 2001: **28**: 306–310.
18. Sugano N, Ikeda K, Oshikawa M, Sawamoto Y, Tanaka H, Ito K. Differential cytokine induction by two types of *Porphyromonas gingivalis*. Oral Microbiol Immunol 2004: **19**: 121–123.
19. Taylor GW. Bidirectional interrelationships between diabetes and periodontal diseases: an epidemiologic perspective. Ann Periodontol 2001: **6**: 99–112.
20. Tervonen T, Oliver RC, Wolff LF, Bereuter J, Anderson LA, Aeppli DM. Prevalence of periodontal pathogens with varying metabolic control of diabetes mellitus. J Clin Periodontol 1994: **21**: 375–379.
21. Tumbarello M, Posteraro B, Trecarichi EM et al. Biofilm production by *Candida* species and inadequate antifungal therapy as predictors of mortality for patients with candidemia. J Clin Microbiol 2007: **45**: 1843–1850.
22. Westfelt E, Rylander H, Blohme G, Jonasson P, Lindhe J. The effect of periodontal therapy in diabetics. Results after 5 years. J Clin Periodontol 1996: **23**: 92–100.
23. Yuan K, Chang CJ, Hsu PC, Sun HS, Tseng CC, Wang JR. Detection of putative periodontal pathogens in non-insulin-dependent diabetes mellitus and non-diabetes mellitus by polymerase chain reaction. J Peridontal Res 2001: **36**: 18–24.