

Increased Oral *Porphyromonas gingivalis* Prevalence in Cardiovascular Patients with Uncontrolled Diabetes Mellitus

Norio Aoyama,¹ DDS, Jun-ichi Suzuki,^{2,3} MD, Naho Kobayashi,⁴ DDS, Tomoya Hanatani,⁵ DDS, Norihiko Ashigaki,⁶ DDS, Asuka Yoshida,⁴ DDS, Yuka Shiheido,⁴ DDS, Hiroki Sato,⁴ DDS, Yuichi Izumi,⁴ DDS and Mitsuaki Isobe,³ MD

Summary

The aim of this study was to determine the correlation between periodontopathic bacteria and diabetes mellitus (DM) status in cardiovascular disease (CVD) subjects.

DM is associated with the progression of periodontitis. Several epidemiological studies have suggested that periodontitis may be a risk factor for CVD. However, no study has compared the periodontal condition between well-controlled and poorly-controlled DM patients with CVD.

The subjects were well-controlled ($n = 73$) or poorly-controlled ($n = 39$) DM patients with CVD. Blood examinations and dental clinical measurements, including number of teeth, probing pocket depth, bleeding on probing (BOP), and clinical attachment level (CAL) were performed. Periodontopathic bacterial existence was evaluated.

Worsened CAL and BOP rate were detected in the uncontrolled DM group compared to the controlled group. We found increased salivary *Porphyromonas gingivalis* counts in the uncontrolled DM group compared to well-controlled DM subjects.

Specific periodontopathic bacterial infection may affect DM condition in CVD patients.

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Key words: Periodontal diseases, Periodontitis, Hemoglobin A1c

Periodontitis, which is a common chronic oral inflammatory disease, is characterized by the destruction of soft tissue and bone. Diabetes mellitus (DM) is known to promote the progression of periodontitis.^{1,2} Several epidemiological studies have suggested that periodontitis is a risk factor for cardiovascular disease (CVD).³ Some papers have indicated that the treatment of periodontitis improved the condition of DM.⁴⁻⁶ Furthermore, it was also suggested that DM treatment improved the condition of periodontitis.⁷ However, no previous study has compared the periodontal conditions of well-controlled and poorly-controlled DM patients with CVD.

Porphyromonas gingivalis (*P. gingivalis*) is known as a major periodontopathic bacterium. We have reported that *P. gingivalis* deteriorated arterial injury and transverse aortic constriction in animal CVD models.^{8,9} However, no

clinical report has compared the prevalence of periodontal bacteria between well-controlled and poorly-controlled DM in patients with CVD. Thus, the purpose of this cross-sectional study was to examine the correlation between periodontopathic bacteria and DM status in CVD subjects.

Methods

Study population: Male subjects between 66 and 80 years old diagnosed as DM were recruited from among patients of the Department of Cardiovascular Medicine of Tokyo Medical and Dental University Hospital between May 2012 and August 2015. A total of 112 subjects were enrolled in the study. Patients who did not consent to participation or who had a history and/or presence of other

From the ¹Division of Periodontology, Department of Oral Interdisciplinary Medicine, Graduate School of Dentistry, Kanagawa Dental University, Yokosuka, Japan, ²Department of Advanced Clinical Science and Therapeutics, The University of Tokyo, Tokyo, Japan, ³Department of Cardiovascular Medicine, Tokyo Medical and Dental University, Tokyo, Japan, ⁴Department of Periodontology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan, ⁵Department of Periodontology, Kyushu Dental University, Kitakyushu, Japan and ⁶Department of Oral Microbiology, Tsurumi University, Yokohama, Japan.

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Address for correspondence: Norio Aoyama, DDS, Division of Periodontology, Department of Oral Interdisciplinary Medicine, Graduate School of Dentistry, Kanagawa Dental University, 82 Inaokacho, Yokosuka, Kanagawa, 238-8580 Japan. E-mail: aoyama@kdu.ac.jp

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Table I. Characteristics of the Subjects

	Controlled DM ($< 7.0\%$ HbA1c)	Uncontrolled DM ($\geq 7.0\%$ HbA1c)	<i>P</i>
Number	73	39	
Age	72.7 ± 3.8	73.5 ± 3.5	NS
Smoker (%)	52	67	NS
Hypertension (%)	84	72	NS
Dyslipidemia (%)	53	59	NS
CRP [mg/dL]	0.48 ± 1.37	0.86 ± 2.64	NS
LDL-c [mg/dL]	96.3 ± 27.6	90.6 ± 22.6	NS
HbA1c [%]	6.4 ± 0.4	7.6 ± 0.5	< 0.0001

DM indicates diabetes mellitus; CRP, C-reactive protein; LDL-c, low density lipoprotein; and HbA1c, hemoglobin A1c.

infections were excluded. The Ethics Committees of the School of Medicine and the School of Dentistry, Tokyo Medical and Dental University approved the protocol. The protocol conformed to the Helsinki Declaration of 1975, as revised in 2013. Written informed consent was provided by each subject.

Medical examination: A medical history was recorded and a physical examination was performed. Subjects who were diagnosed and treated as having hypertension (HT) and dyslipidemia (DL) in the department were recorded. Cigarette smoking history and habits were obtained by interview. Peripheral blood samples were collected, centrifuged at 1,500 g for 20 minutes, and then stored at -20°C until analysis. Laboratory parameters determined from serum samples included concentrations of low density lipoprotein (LDL-c), hemoglobin A1c (HbA1c), and C-reactive protein (CRP). We divided the subjects into two groups according to the level of HbA1c and classified subjects with a 7.0% or higher HbA1c level as uncontrolled DM patients and the others (less than 7.0% of HbA1c) as controlled DM patients. The type of DM was not determined, however, most DM patients were diagnosed as type 2 DM.

Clinical periodontal examination: Trained periodontists counted the number of teeth and recorded probing pocket depth (PPD), clinical attachment level (CAL), and bleeding on probing (BOP) at 6 points (buccal-mesial, mid-buccal, buccal-distal, lingual-mesial, mid-lingual, and lingual-distal) on a right upper molar, an upper incisor, a left upper molar, a right lower molar, a lower incisor, and a left lower molar with a manual probe (PCP-UNC 15, Hu-Friedy, Chicago, IL, USA). An adjacent tooth was used if the representative tooth was missing.

Bacterial identification: Unstimulated saliva was obtained. Bacterial DNA was extracted from 200 μL of saliva using a DNeasy Blood and Tissue kit (Qiagen, Tokyo) according to the manufacturer's protocol. A real-time polymerase chain reaction (PCR) method was used to detect the periodontopathic bacteria *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*). The real-time PCR was performed as described in a previous report.¹⁰ Specific primers for each bacterium were used as previously described.¹¹

Data analysis: Numerical data are presented as the mean \pm standard deviation (SD). Student's *t*-test was performed to compare age, CRP, LDL-c, HbA1c, the number of

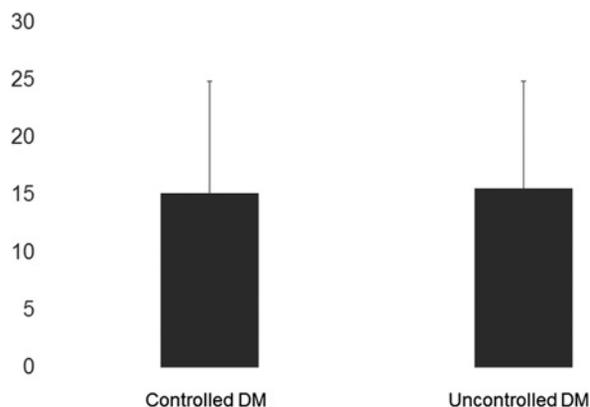


Figure 1. Number of missing teeth in the two groups is shown. Data is presented as the mean \pm SD.

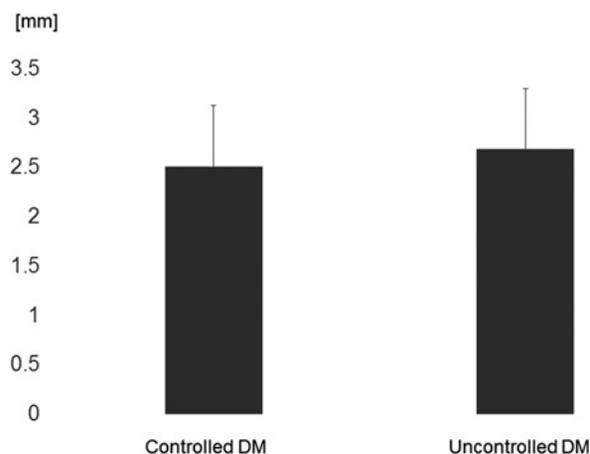


Figure 2. Mean probing pocket depth (PPD). Data is presented as the mean \pm SD.

missing teeth, PPD, CAL, and BOP. The chi-square test was performed to compare the smoker rate and presence of HT and DL. The Wilcoxon test was used to compare bacterial counts. JMP 9.0.3 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. Values of $P < 0.05$ were considered significant.

Results

Patient characteristics: The characteristics of the subjects and blood data are shown in Table I. There were no statistically significant differences in age, smoker rate, HT, or DL between the controlled and uncontrolled DM groups. CRP and LDL-c levels were comparable between the groups.

Oral and periodontal conditions: The oral and periodontal conditions of the subjects are shown in Figure 1-4. The CAL (Figure 3A) and BOP rate (Figure 4A) were worse in the uncontrolled DM group compared to the controlled group. There were no statistically significant differences in the number of missing teeth (Figure 1) and PPD (Figure 2) between the groups. We also analyzed the direct relationship between HbA1c level and clinical periodontal pa-

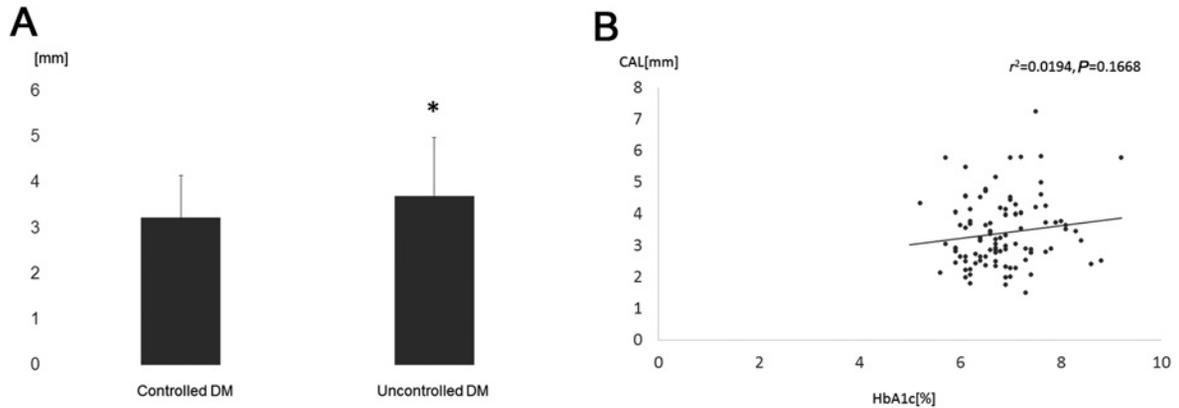


Figure 3. **A:** Mean clinical attachment level (CAL). Data is presented as the mean \pm SD. * $P < 0.05$ between groups. **B:** Association between CAL and hemoglobin A1c level.

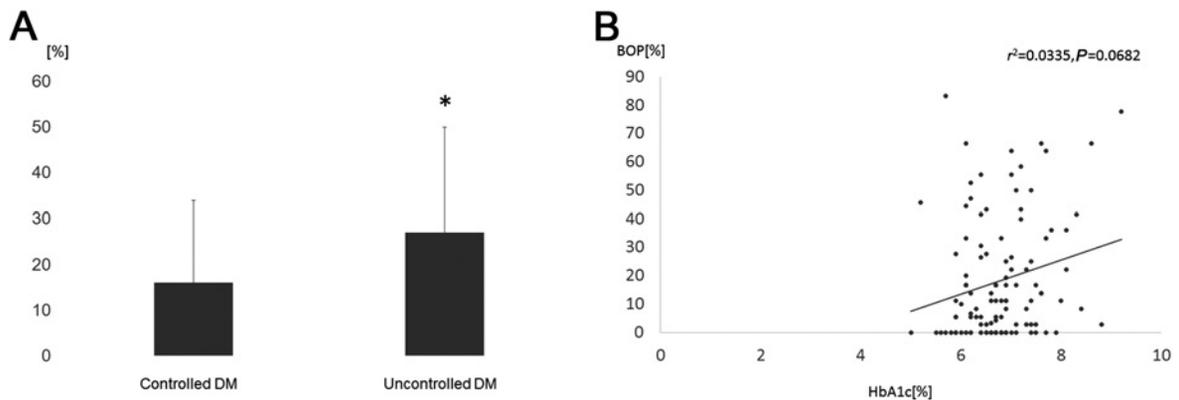


Figure 4. **A:** Mean bleeding on probing (BOP) rate. Data is presented as the mean \pm SD. * $P < 0.05$ between groups. **B:** Association between BOP and hemoglobin A1c level.

Table II. Bacterial Counts in Saliva

	Controlled DM ($< 7.0\%$ HbA1c)	Uncontrolled DM ($\geq 7.0\%$ HbA1c)	<i>P</i>
<i>P. gingivalis</i> in saliva [counts/mL]	1.7×10^4 (2.7×10^2 , 2.5×10^5)	8.9×10^4 (9.5×10^2 , 5.6×10^6)	0.0444
<i>A. actinomycetemcomitans</i> in saliva [counts/mL]	0 (0, 0)	0 (0, 0)	NS

Data are shown as the median (first and third quartile). DM indicates diabetes mellitus; *P. gingivalis*, *Porphyromonas gingivalis*; and *A. actinomycetemcomitans*, *Aggregatibacter actinomycetemcomitans*.

rameters such as CAL (Figure 3B) and BOP (Figure 4B). There were positive trends of the association, however, no statistical relationships were detected.

Oral bacterial counts: Bacterial counts are shown in Table II. We found increased salivary *P. gingivalis* in the uncontrolled DM group. *A. actinomycetemcomitans* was comparable between the groups. We calculated the association between bacterial counts and HbA1c level (Figure 5). There was no statistical relationship between bacteria and HbA1c level. We also analyzed the association between bacterial counts and clinical parameters such as CAL and BOP (Figure 6). *P. gingivalis* counts were related with both CAL (Figure 6A) and BOP (Figure 6C), while *A. actinomycetemcomitans* had no statistical relationship with clinical parameters.

Discussion

In this paper, we, for the first time, have demonstrated that deteriorated periodontitis with increased salivary *P. gingivalis* was observed in a group of patients with uncontrolled DM compared to a group with well-controlled DM.

Periodontitis and DM in CVD patients: Periodontitis, a common chronic oral inflammatory disease, is characterized by destruction of soft tissue and bone. Modulation of host factors, such as DM, is important for the progression of periodontitis.^{1,2)} Although the pathogenesis of periodontal disease in DM remains unclear, most evidence suggests that prolonged exposure to hyperglycemia results in acquired immune deficiency.¹²⁻¹⁴⁾ Several epidemiological studies have suggested that periodontal infection is a risk

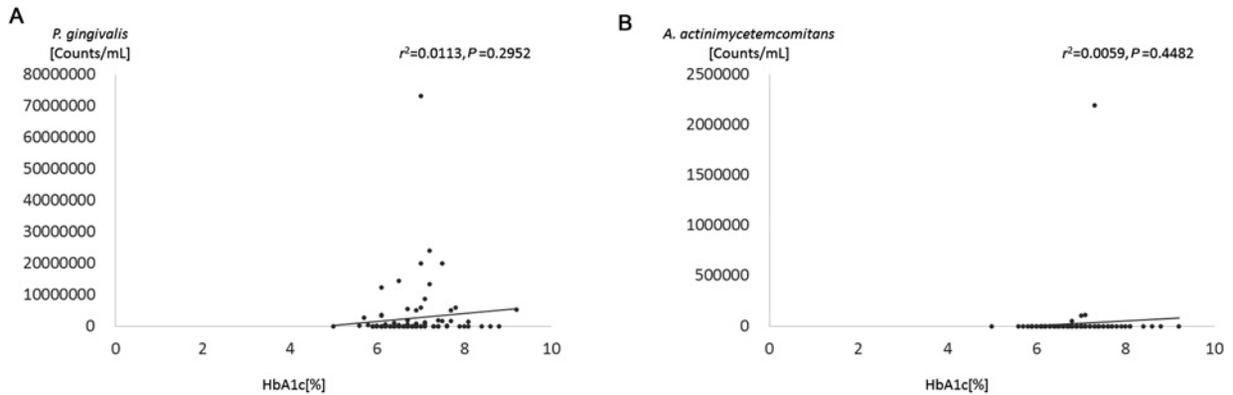


Figure 5. Associations between hemoglobin A1c level and *Porphyromonas gingivalis* (A) and *Aggregatibacter actinomycetemcomitans* (B) in saliva.

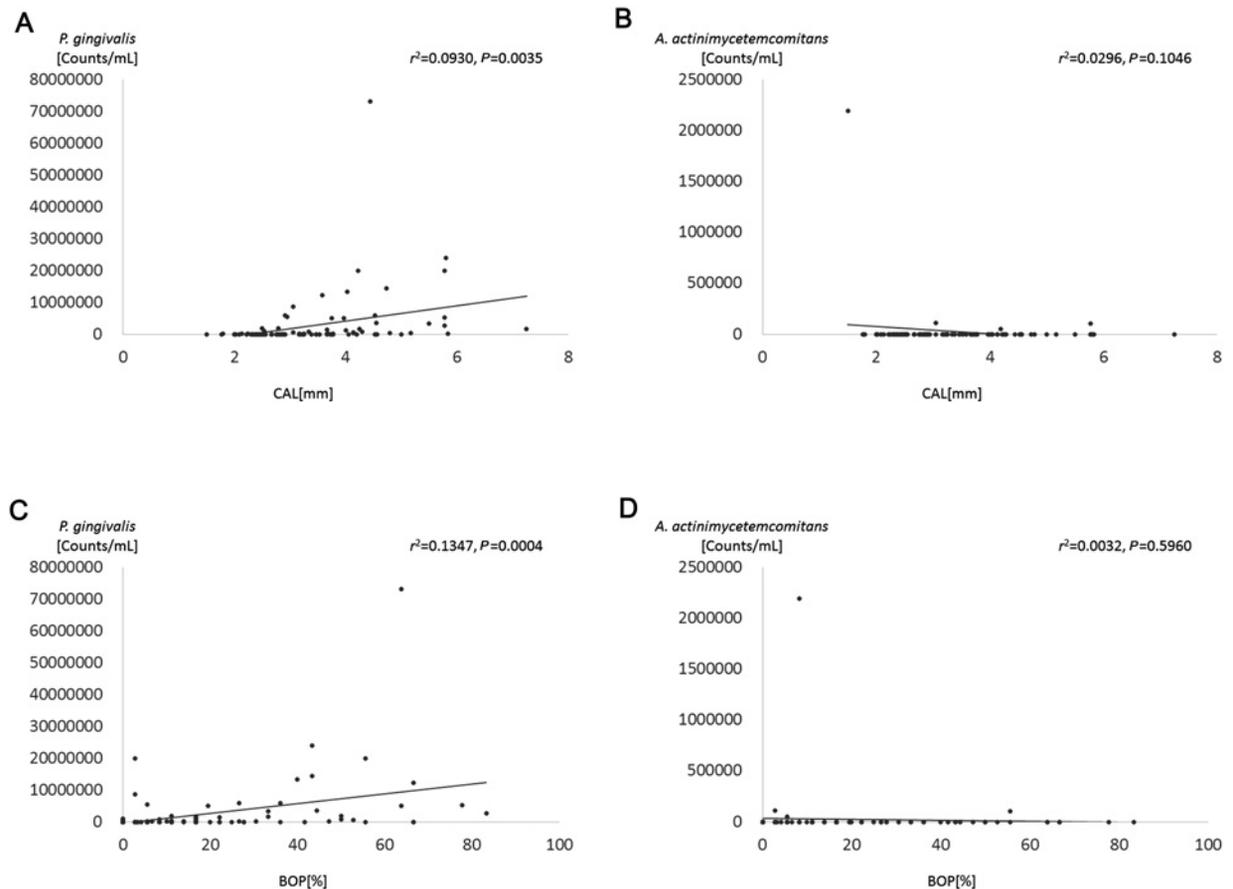


Figure 6. Associations between clinical attachment level (CAL) (A, B) and bleeding on probing (BOP) rate (C, D) and *Porphyromonas gingivalis* (A, C) or *Aggregatibacter actinomycetemcomitans* (B, D) in saliva

factor for CVD.^{3,15}) It is widely known that DM is a major risk factor of CVDs.¹⁶) Diabetics with severe periodontal disease may be particularly susceptible to vascular complications, which are primarily responsible for the increased morbidity and mortality caused by DM. However, no previous study has examined different periodontal conditions and bacterial prevalence between well-controlled and

poorly-controlled DM in CVD patients.

HbA1c and systemic inflammation: HbA1c reflects serum glucose levels during the 120-day life of a red blood cell, and is a measure of glycaemic control. This has been frequently used for treatment decision-making in clinical medicine and is known to be linked to vascular diabetes complications.¹⁷) Patients with diabetes have a high risk of

developing chronic periodontitis, and those with elevated HbA1c have a significantly higher prevalence of periodontitis.¹⁸⁾ It is well known that infections may adversely influence DM control and HbA1c.¹⁹⁾ In this context, a biological link between DM control and periodontitis has been investigated. Several reviews have been conducted to assess the evidence that periodontal treatment influences DM control.^{20,21)}

***P. gingivalis* may affect DM:** It is well known that periodontal disease and DM have a two-way relationship.²²⁾ It was also clarified that periodontal disease was associated with HbA1c progression in a cohort study with 2973 participants.²³⁾ The association between periodontitis and diabetes was strong in people with increased serum CRP and antibody levels against *P. gingivalis*.²⁴⁾ The glycemic levels in patients with DM were affected by the persistence of *P. gingivalis*, especially specific clones.²⁵⁾ The authors suggested that therapeutic suppression of *P. gingivalis* is an important factor for improvement of glycemic control.

Recent papers have suggested possible mechanisms of glycemic control are affected by *P. gingivalis* infection. Hyperglycemic patients with diabetes may run the risk of generating macrophages that are not able to elicit proper inflammatory mediators and cellular activation when faced with a periodontal infection.²⁶⁾ A high glucose level appeared to enhance the inflammatory response induced by *P. gingivalis* lipopolysaccharide (LPS).²⁷⁾ *P. gingivalis* LPS induced a pro-inflammatory adipokine secretory profile and oxidative stress in adipocytes, which may lead to obesity-related insulin resistance.²⁸⁾ Baht, *et al* demonstrated that *P. gingivalis* LPS stimulates insulin secretion by pancreatic beta cells.²⁹⁾ It was also reported that *P. gingivalis* translocation from the oral cavity to the liver may contribute to the progress of DM by influencing hepatic glycogenesis in mice.³⁰⁾

Because the underlying mechanism between periodontal infection and DM control has not been fully identified, further analyses regarding the interactions of periodontal disease, DM, and CVD are required.

Disclosures

Conflicts of interest: None declared.

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