【 ORIGINAL ARTICLE 】

Japanese Cardiovascular Disease Patients with Diabetes Mellitus Suffer Increased Tooth Loss in Comparison to Those without Diabetes Mellitus—A Cross-sectional Study

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Abstract:

Objective Tooth loss is an irreversible condition that reflects the end-stage of oral diseases, including periodontitis. Although periodontitis is a major factor in the progression of diabetes mellitus (DM) and cardiovascular disease (CVD), no previous studies have compared tooth loss in CVD patients with and without DM.

Methods The subjects included CVD patients with (n=94) and without (n=145) DM who attended Tokyo Medical and Dental University Hospital. Blood examinations and periodontal measurements were performed.

Results The oral and periodontal examinations revealed that the numbers of missing teeth in the DM group were increased in comparison to the non-DM group. There was no significant difference between the groups with regard to the incidence of edentulism, the probing pocket depth, the clinical attachment level or the incidence of bleeding on probing.

Conclusion We showed that the numbers of missing teeth among CVD patients with DM was significantly higher than that among CVD patients without DM.

Key words: periodontal disease, bacteria, diabetes mellitus, hemoglobin A1c

Introduction

Periodontitis is a common oral inflammatory disease and is characterized by the destruction of alveolar bone and supporting connective tissue (1). The modulation of host factors, such as diabetes mellitus (DM), has been shown to be highly associated with the progression of periodontitis (2, 3). Although the pathogenesis of periodontitis in DM remains unclear, evidence suggests that periodontal destruction may result from prolonged hyperglycemia and that it

may occur with high frequency and severity in patients with poor glycemic control (4-6). Several studies have also suggested that periodontal infection may be a risk factor for cardiovascular disease (CVD) (7, 8). Tooth loss is a marker for an increased risk of CVD and all-cause mortality (9). Even a few missing teeth may indicate an increased risk of CVD, DM or all-cause mortality (10). Tooth loss is an irreversible condition that reflects the end-stage of oral diseases such as periodontitis (11).

DM patients with severe periodontal disease may be particularly susceptible to microvascular and macrovascular

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complications, which are primarily responsible for the increased morbidity and mortality associated with DM. However, there have been few reports on the association between tooth loss or periodontitis and DM among CVD patients. We hypothesized that the presence of DM among CVD patients affected the periodontal condition and that periodontal destruction led to tooth loss. The purpose of the present study was to compare the numbers of lost teeth and the periodontal condition between CVD patients with and without DM.

Materials and Methods

Study population

Male subjects between 69 and 80 years of age were recruited from patients of the Department of Cardiovascular Medicine in Tokyo Medical and Dental University Hospital from May 2012 to August 2015. Only male patients were recruited in order to prevent gender differences from affecting results. Two hundred thirty-nine subjects were enrolled in this study. Patients who did not provide their consent for participation in this study or who had a history of other infections (or current infections) were excluded. The Ethics Committee of the School of Medicine and the School of Dentistry, Tokyo Medical and Dental University approved the protocol of the present study, and the protocol conformed to the Helsinki Declaration of 1975, as revised in 2013. Written informed consent was obtained from each subject.

Medical examination

In each case, the patient's medical history was recorded and a physical examination was performed. The subjects who were diagnosed and treated in the department for DM, hypertension (HT) and dyslipidemia (DL) were recorded. The history of cigarette smoking was obtained from an interview. Peripheral blood samples were collected, centrifuged at 1,500×g for 20 minutes and then stored until use at -20℃. The concentrations of low density lipoprotein (LDL-c), high density lipoprotein (HDL-c), triglyceride (TG), hemoglobin A1c (HbA1c) and C-reactive protein (CRP) were measured.

DM was defined based on the following conditions: (i) fasting blood sugar >120 mg/dL, (ii) 75 g oral glucose tolerance test >200 mg/dL (2h), (iii) blood sugar at any time point >200 mg/dL or (iv) HbA1c >6.5% (National Glycohemoglobin Standardization Program: NGSP). Systemic HT was defined by a systolic blood pressure of >140 mmHg and/or a diastolic blood pressure of >90 mmHg. DL was defined as (i) LDL-c >140 mg/dL, (ii) HDL-c <40 mg/dL, or (iii) TG >150 mg/dL. Obesity was defined as a body mass index of >25 kg/m^2.

Clinical periodontal examination

A clinical periodontal examination was performed by three periodontists (N. Ao., N. K. and T. H) who were certified by the Japanese Society of Periodontology. The number of teeth was counted and the probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP) were recorded at six points (buccal-mesial, mid-buccal, buccal-distal, lingual-mesial, mid-lingual, 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, USA). When the representative tooth was missing, the next tooth was used.

Bacterial detection

Unstimulated saliva was obtained. Bacterial DNA was extracted from 200 μL saliva using a DNeasy Blood and Tissue kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. A real-time polymerase chain reaction (PCR) was performed to detect three periodontopathic bacteria: *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia*. The real-time PCR was performed according to previously described methods (12). The specific primers for each bacterium have been described previously (13). The bacterial counts in saliva were shown as count/mL.

Antibody measurement

Serum samples were analyzed to detect IgG antibodies against three major periodontal pathogens, *P. gingivalis*, *A. actinomycetemcomitans* and *P. intermedia*, using an enzyme-linked immunosorbent assay as previously described (14). The absorbance of wells was read using a microplate reader at 450 nm with a 650 nm reference wavelength. Individual serum antibody levels were calculated from the standard curve obtained from the gradual dilutions of the reference and were expressed as Units/mL.

Data analysis

Numerical data were presented as the mean ± standard deviation (SD). Student's *t*-test was performed to compare age, body mass index, CRP, LDL-c, HDL-c, TG, HbA1c, the number of missing teeth, PPD, CAL and BOP. The chi-squared test was performed to compare the rate of smoking, presence of HT, DL and obesity, and the rate edentulism. Bacterial counts and antibody levels were presented as the median and 25th and 75th percentiles. The Wilcoxon test was used to compare the bacterial count and the presence of bacterial antibodies. The JMP software program (version 9.0.3; SAS Institute, Cary, USA) was used for all of the statistical analyses. p values of <0.05 were considered to indicate statistical significance.

Results

The characteristics of the study population

The characteristics of the patients in the present study are

Table 1.   The Characteristics of the Subjects.

Group	DM	Non-DM	p
Number	94	145	
Age	74.1+/-2.9	73.7+/-3.5	0.3407
Smoker [%]	55	57	0.8513
Hypertension [%]	78	73	0.4252
Dyslipidemia [%]	54	40	0.0306
Obesity [%]	23	15	0.1119
Body mass index [kg/m²]	24.2+/-3.5	23.5+/-2.6	0.0808
CRP [mg/dL]	0.67+/-2.08	0.33+/-0.66	0.0637
LDL-c [mg/dL]	93.2+/-25.4	102.3+/-26.5	0.0102
HDL-c [mg/dL]	51.4+/-14.4	57.1+/-16.8	0.0082
TG [mg/dL]	135.0+/-68.6	132.3+/-91.0	0.8129
HbA1c [%]	6.8+/-0.8	5.7+/-0.4	<0.0001

DM: diabetes mellitus, CRP: C-reactive protein, LDL-c: low density lipoprotein, HDL-c: high density lipoprotein, TG: triglyceride, HbA1c: hemoglobin A1c

Table 2.   The Oral Condition of the Subjects.

Group	DM	Non-DM	p
Missing Teeth	16.1+/-9.6	13.6+/-9.3	0.0492
Edentulous [%]	12	8	0.3845
PPD [mm]	2.59+/-0.65	2.51+/-0.61	0.4184
CAL [mm]	3.43+/-1.11	3.34+/-1.14	0.5926
BOP [%]	21+/-22	17+/-20	0.2736

DM: diabetes mellitus, PPD: probing pocket depth, CAL: clinical attachment level, BOP: bleeding on probing

shown in Table 1. There were no significant differences in the age, the rates of smoking, HT, obesity, or the body mass index values of the DM and non-DM groups. The rate of DL was higher and the HDL-c levels were lower in the DM group than they were in the non-DM group. The level of LDL-c in the DM group was lower than that in the non-DM group. The CRP levels of the two groups were comparable.

The cardiovascular diseases that were diagnosed in the two groups included: myocardial infarction and/or angina pectoris (n=131) (DM group, n=68; non-DM group, n=63), arrhythmia, n=130; (DM group, n=40; non-DM group, n=90); heart failure, n=37 (DM group, n=14; non-DM group, n=23), and 34 valvular disease (DM group, n=8; non-DM group, n=26). In cases in which a subject had multiple cardiovascular diseases, each disease was counted.

Oral and periodontal conditions

The oral and periodontal conditions of the subjects in each group are shown in Table 2. The number of missing teeth in the DM group was increased in comparison to the non-DM group. There were no significant differences in the rates of edentulism, PPD, CAL or BOP between the groups.

We also measured the bacterial counts in saliva and serum antibody levels against *P. gingivalis*, *A. actinomycetemcomitans* and *P. intermedia*. There were no significant differences between the groups with regard to any of the salivary bacterial counts or serum antibody levels (Table 3). We per-

formed a sub-analysis of the bacteria and antibody levels in edentulous patients. No significant difference was observed in the salivary bacterial counts or the serum antibody levels of the edentulous participants (Table 4).

Table 5 shows the results of the multiple logistic regression analyses to assess the associations between DM and obesity and tooth loss. We found that many of the patients with DM had lost more than 10 teeth. After adjustment for obesity, the significant relationship remained.

Discussion

Tooth loss represents an irreversible end-stage oral condition

This cross-sectional study was conducted to reveal the relationship between DM and periodontitis in patients with CVD. Our hypothesis was that periodontitis and diabetes mellitus affect each other and influence the development of CVD. A strong relationship between periodontitis and DM has been proven (3-6) and both diseases are associated with CVD (8, 10). However, we had no information on whether the oral condition and periodontitis of CVD patients with DM patients was worse in comparison to patients with CVD alone. Thus, we conducted the present cross-sectional study.

In this study, we demonstrated that the number of lost teeth in the DM group was increased in comparison to the non-DM group among patients with CVD. Periodontitis is known to be the most critical cause of tooth loss in the Japanese adult population. Although there are other periodontal measurements (e.g. PPD, CAL and BOP), these measurements reflect ongoing and reversible oral conditions. In contrast, the number of missing teeth indicates irreversible end-stage oral conditions, including periodontitis. Thus, the number of missing teeth can be a hard endpoint to use in the assessment of the oral status (11). The observation in the present study indicated that DM patients with CVD had irreversible end-stage periodontitis.

Table 3.   The Detected Bacteria and Antibody Levels.

Group	DM	Non-DM	p
P. gingivalis count [counts/mL]	2.0×10^4 (5.2×10^2, 1.2×10^6)	7.5×10^4 (3.2×10^2, 1.3×10^6)	0.6503
A. actinomycetemcomitans count [counts/mL]	0 (0, 0)	0 (0, 0)	0.8877
P. intermedia count [counts/mL]	0 (0, 0)	0 (0, 4.4×10^2)	0.3269
P. gingivalis antibody [Units/mL]	156 (85, 331)	157 (64, 417)	0.6989
A. actinomycetemcomitans antibody [Units/mL]	62 (25, 102)	59 (30, 135)	0.5218
P. intermedia antibody [Units/mL]	401 (175, 777)	434 (259, 928)	0.1975

Data was presented as median with 25 and 75 percentiles. DM: diabetes mellitus, *P. gingivalis*: *Porphyromonas gingivalis*, *A. actinomycetemcomitans*: *Aggregatibacter actinomycetemcomitans*, *P. intermedia*: *Prevotella intermedia*

Table 4.   The Detected Bacteria and Antibody Levels in the Edentulous Patients.

Group	DM	Non-DM	p
P. gingivalis count [counts/mL]	8.3×10^2 (1.2×10^2, 4.7×10^3)	2.7×10^2 (0, 3.4×10^2)	0.0928
A. actinomycetemcomitans count [counts/mL]	0 (0, 6.1×10^2)	0 (0, 0)	0.4198
P. intermedia count [counts/mL]	0 (0, 0)	0 (0, 0)	1.0000
P. gingivalis antibody [Units/mL]	133 (40, 321)	54 (37, 142)	0.2545
A. actinomycetemcomitans antibody [Units/mL]	45 (20, 136)	36 (27, 60)	0.5949
P. intermedia antibody [Units/mL]	277 (155, 686)	385 (282, 629)	0.3233

Data was presented as median with 25 and 75 percentiles. DM: diabetes mellitus, *P. gingivalis*: *Porphyromonas gingivalis*, *A. actinomycetemcomitans*: *Aggregatibacter actinomycetemcomitans*, *P. intermedia*: *Prevotella intermedia*

Table 5.   The Relationship between Diabetes Mellitus and the Number of Patients who Lost More than 10 Teeth.

Group	DM	Non-DM	p
Crude ORs	1.82 (1.07-3.11)	1 (Ref)	0.0261
*Adjusted ORs	1.78 (1.05-3.07)	1 (Ref)	0.0337

*Adjusted for obesity. DM: diabetes mellitus, OR: odds ratio

DM and tooth loss

Many reports have shown a relationship between DM and periodontal disease (15, 16). It is widely known that DM patients develop periodontitis at a higher rate than subjects without DM (3) and that periodontal disease is an independent predictor of incidental DM (17). Because periodontitis is one of the main causes of tooth loss, it makes sense that DM patients are at a higher risk of tooth loss and edentulism than adults without DM (18). It is widely accepted that patients with DM have considerable cardiovascular risk (19)

and studies have supported an association between periodontal disease and CVD, independent of known confounders, including DM (8). Thus, DM and periodontal disease can interact adversely and influence the development of CVD events. We assessed the oral and periodontal condition of DM and non-DM subjects in this study. We found that CVD patients with DM had an increased number of lost teeth in comparison to patients with CVD alone. A multiple logistic regression analysis was performed to assess the interaction between DM and obesity with regard to tooth loss. We found that a large number of DM patients had lost more than 10 teeth regardless of the presence of obesity (Table 5). The bacterial counts in the saliva and the levels of serum antibodies against periodontal bacteria were measured. However, we did not detect any significant difference in the salivary bacterial counts and serum antibody levels. Although the bacterial counts and antibody levels among edentulous patients were similar in both DM and non-DM patients, the antibody levels against *P. gingivalis* were quite high in edentulous patients with DM. Most edentulous patients must

have suffered from periodontal infection for a long period and might have lost their teeth due to periodontitis. Further longitudinal clinical studies of large populations and experiments using animal models will be needed to clarify the interactive effects of DM and periodontal disease on CVD.

Study limitations

We showed that CVD patients with DM lost more teeth in comparison to CVD patients without DM. Although periodontal disease is a major reason for tooth extraction in adults, the reasons for tooth loss are not limited to periodontitis. Other conditions, including caries and fracture may result in tooth extraction in the clinical setting. As shown in Table 2, in addition to the number of missing teeth, the average PPD and CAL tended to be increased in the DM group in the present study. However, we have to consider that the number of missing teeth does not clearly reflect periodontal disease alone.

In the present study, we showed a significant difference in the incidence of DL. The incidence of DL among DM was higher in comparison to patients without DM; however, the LDL-c levels of the DM group were lower than those of the non-DM group. This suggested that DL was appropriately treated in the DM group. Thus, periodontitis may not affect DM via DL. The study was also associated with other limitations, including the small number of participants, the single cross-sectional study design, the limited generalizability and the limited information on other important confounding factors. A further analysis is needed to clarify the causal relationships among the factors.

Conclusion

We revealed that CVD patients with DM lost more teeth in comparison to CVD patients without DM. Further investigations are needed to reveal the detailed causal relationship between DM and periodontitis in the near future.

The authors state that they have no Conflict of Interest (COI).

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