Poor Oral Health, That Is, Decreased Frequency of Tooth Brushing, Is Associated With Endothelial Dysfunction

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Background: Poor oral health is an independent predictor of cardiovascular outcome. Endothelial dysfunction is the initial step of atherosclerosis, resulting in cardiovascular outcomes; but there is no information on the association between oral health and endothelial function. The purpose of this study was to determine the relationships between oral health and endothelial function.

Methods and Results: A total of 190 subjects who underwent health examinations (mean age, 57 ± 18 years), including patients with cardiovascular disease, completed a questionnaire on oral health and frequency of tooth brushing, and underwent measurement of vascular function, flow-mediated vasodilation (FMD) and nitroglycerine-induced vasodilation. The subjects were divided into 2 groups according to frequency of tooth brushing ($\geq$ twice/day and $<$ once/day). FMD was significantly lower in the $<$ once/day tooth brushing group as compared to the $\geq$ twice/day tooth brushing group ($3.3 \pm 2.2\%$ vs. $5.0 \pm 3.0\%$, $P < 0.001$). There was no significant difference in nitroglycerine-induced vasodilation between the 2 groups. On multiple logistic regression analysis, tooth brushing $<$ once/day remained independently associated with low FMD tertile.

Conclusions: Poor oral health, that is, decreased frequency of tooth brushing, is associated with endothelial dysfunction. (*Circ J* 2014; **78**: 950–954)

Key Words: Cardiovascular risk factor; Endothelial function; Flow-mediated vasodilation; Tooth brushing

Clinical and epidemiological studies have shown a link between oral health, especially periodontitis, and cardiovascular disease,^{1–5} but controversy remains regarding the independent association between oral health and cardiovascular disease.⁵ Systemic inflammation induced by periodontal disease is thought to affect the development and maintenance of atherosclerosis through activation of a biochemical reaction cascade, initiation and development of plaque formation, and injury of the endothelium.

Endothelium-dependent vasodilation, and nitroglycerine-induced vasodilation as an index of endothelium-independent vasodilation in the brachial artery on high-resolution ultrasound, are useful for assessing vascular function.^{6–13} Endothelial dysfunction is the initial step of atherosclerosis, leading to the maintenance and progression of atherosclerosis, resulting in cardiovascular complications.¹⁴ Assessment of FMD is non-invasive and reflects nitric oxide (NO) production. In addition, previous studies have shown that endothelial function assessed using FMD can serve as an independent predictor of cardiovascular events.^{15–19}

Recently, de Oliveira et al showed that poor oral health is associated with increasing risk of cardiovascular disease.²⁰ Several investigators, including us, have shown that periodontitis

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Flow-mediated vasodilation (FMD) as an index of endothe-

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Table 1. Subject Clinical Characteristics

Variable	Total (n=190)	<Once/day (n=56)	≥Twice/day (n=134)	P-value
Age (years)	57±18	63±14	55±20	0.001
Sex (M/F)	112/78	48/8	64/70	<0.001
BMI (kg/m ²)	23.6±4.0	24.5±4.2	23.1±3.8	0.03
SBP (mmHg)	132±21	135±23	130±20	0.21
DBP (mmHg)	77±14	81±15	75±14	0.02
Heart rate (beats/min)	70±13	70±13	70±13	0.94
Total cholesterol (mg/dl)	188±36	183±30	191±39	0.17
Triglycerides (mg/dl)	134±79	130±87	136±75	0.71
HDL-C (mg/dl)	58±16	54±14	59±16	0.04
LDL-C (mg/dl)	109±32	108±26	110±34	0.66
Glucose (mg/dl)	122±40	115±33	126±44	0.13
hsCRP (mg/dl)	0.11±0.26	0.20±0.41	0.06±0.06	0.03
Medical history				
Hypertension	121 (63.7)	45 (80.4)	76 (56.7)	0.001
Dyslipidemia	101 (53.2)	25 (44.6)	76 (56.7)	0.12
Diabetes mellitus	55 (28.9)	20 (35.7)	35 (26.1)	0.18
Previous CAD	28 (14.7)	8 (14.3)	20 (14.9)	0.90
Previous stroke	8 (4.2)	2 (3.6)	6 (4.5)	0.77
Smoker	31 (16.3)	17 (30.4)	14 (10.5)	0.001
FMD (%)	4.5±2.9	3.3±2.2	5.0±3.0	<0.001
Nitroglycerine-induced vasodilation (%)	11.6±5.5	11.6±5.7	11.6±5.5	0.99

Data given as mean ± SD or n (%).

BMI, body mass index; CAD, coronary artery disease; DBP, diastolic blood pressure; FMD, flow-mediated vasodilation; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure.

is associated with endothelial dysfunction and that treatment of periodontitis improves endothelial function in patients with atherosclerosis as well as in healthy subjects.^{21–25} There is no information, however, on the association between oral health and endothelial function. We therefore investigated the relationship between oral health, frequency of tooth brushing, and vascular function in a general population of subjects who underwent a health-screening examination, including healthy subjects and patients with cardiovascular disease.

Methods

Subjects

Between March 2012 and June 2013, a total of 190 consecutive subjects (mean age, 57±18 years) were recruited from people who underwent health-screening examinations at Hiroshima University Hospital. Hypertension was defined as systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg, in a sitting position, on at least 3 different occasions. Diabetes mellitus was defined according to the American Diabetes Association.²⁶ Dyslipidemia was defined according to the third report of the National Cholesterol Education Program.²⁷ The ethical committees in the present institutions approved the study protocol. Written informed consent for participation in the study was obtained from all subjects.

Study Protocol

We had a simple questionnaire about frequency of tooth brushing (<once/day and ≥twice/day).

Subjects fasted the previous night for at least 12 h. The study began at 08.30 A.M. The subjects were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature, 22–25°C) throughout the study. A 23-gauge polyethylene cath-

eter was inserted into the left deep antecubital vein to obtain blood samples. Thirty minutes after maintaining the supine position, FMD and nitroglycerine-induced vasodilation were measured. The observers were blind to the form of examination.

Measurement of FMD

The subjects remained supine throughout the study. The vascular response to reactive hyperemia in the brachial artery was used for assessment of endothelium-dependent FMD. A high-resolution linear artery transducer was coupled to computer-assisted analysis software (UNEXEF18G; UNEX Co, Nagoya, Japan) that used an automated edge detection system for measurement of brachial artery diameter. A blood pressure cuff was placed around the forearm. The brachial artery was scanned longitudinally 5–10 cm above the elbow. When the clearest B-mode image of the anterior and posterior intimal interfaces between the lumen and vessel wall was obtained, the transducer was held at the same point throughout the scan by a special probe holder (UNEX Co) to ensure consistency of the image. Depth and gain setting were set to optimize the images of the arterial lumen wall interface. When the tracking gate was placed on the intima, the artery diameter was automatically tracked, and the waveform of diameter changes over the cardiac cycle was displayed in real time using the FMD mode of the tracking system. This allowed the ultrasound images to be optimized at the start of the scan and the transducer position to be adjusted immediately for optimal tracking performance throughout the scan. Pulsed Doppler flow was assessed at baseline and during peak hyperemic flow, which was confirmed to occur within 15 s after cuff deflation. Blood flow velocity was calculated from the Doppler data and was displayed as a waveform in real time. The baseline longitudinal image of the artery was acquired for 30 s, and then the blood pressure cuff was inflated to

Table 2. Factors Associated With Low FMD Tertile (<2.9%)

Parameters	OR (95% CI)	P-value
Intercept		<0.001
Age (1-year change)	1.03 (1.01–1.05)	0.01
Men	1.39 (0.67–2.93)	0.38
Diabetes mellitus (Yes)	2.65 (1.27–5.65)	0.01
<Once/day (Yes)	2.26 (1.07–4.81)	0.03

Output of a logistic regression model with FMD >2.9% taken as the reference. Adjusted r^2 of the model was 0.14. Initial factors included in the model were age, sex, dyslipidemia, diabetes mellitus, <once/day tooth brushing. CI, confidence interval; OR, odds ratio. Other abbreviation as in Table 1.

50 mmHg above systolic pressure for 5 min. The longitudinal image of the artery was recorded continuously until 5 min after cuff deflation. Pulsed Doppler velocity signals were obtained for 20 s at baseline and for 10 s immediately after cuff deflation. Changes in brachial artery diameter were immediately expressed as a percentage change relative to the vessel diameter before cuff inflation. FMD was automatically calculated as the percentage change in peak vessel diameter from baseline. Percentage of FMD (peak diameter–baseline diameter/baseline diameter) was used for analysis. Blood flow volume was calculated by multiplying the Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area (πr^2). Reactive hyperemia was calculated as the maximum percentage increase in flow after cuff deflation compared with baseline flow.

The response to nitroglycerine was used for assessment of endothelium-independent vasodilation. After acquiring baseline rest image for 30 s, a sublingual tablet (nitroglycerine 75 μ g) was given and imaging of the artery was done continuously for

5 min. Nitroglycerine-induced vasodilation was automatically calculated as a percentage change in peak vessel diameter from baseline. Nitroglycerine percentage (peak diameter–baseline diameter/baseline diameter) was used for analysis. Inter- and intra-coefficients of variation for the brachial artery diameter were 1.6% and 1.4%, respectively.

Statistical Analysis

Results are presented as mean $\pm$ SD for continuous variables and as percentage for categorical variables. Statistical significance was set at $P < 0.05$. Comparison of continuous variables between 2 groups was performed using the Mann-Whitney U-test or the chi-squared test for categorical data. Stepwise multiple regression analysis was performed to find independent predictors of low FMD tertile (<2.9%) among potential confounders ($P < 0.2$) on univariate analysis. Using variables associated with low FMD tertile on stepwise multiple regression analysis, logistic regression analysis was then done. The data were processed using Stata version 9 (Stata, College Station, TX, USA).

Results

Clinical Characteristics

The baseline characteristics of the 190 subjects are listed in Table 1. Of the 190 subjects, 112 (58.9%) were men and 78 (41.1%) were women. One hundred and twenty-one (63.7%) had hypertension, 101 (53.2%) had dyslipidemia, 55 (28.9%) had diabetes mellitus, and 31 (16.3%) were current smokers. Of the 162 subjects who were evaluated, 28 (14.7%) had known coronary artery disease and 8 (4.2%) had known stroke.

Vascular Function and Oral Health

We divided the subjects into 2 groups according to frequency of tooth brushing (Table 1). There were significant differences

between the 2 groups in age, sex, diastolic blood pressure, high-density lipoprotein cholesterol, high-sensitivity C-reactive protein (hsCRP), prevalence of hypertension, and smoking. FMD was significantly lower in the <once/day tooth brushing group than in the $\geq$ twice/day tooth brushing group ($3.3 \pm 2.2\%$ vs. $5.0 \pm 3.0\%$, $P < 0.001$; **Figure**). There was no significant difference in nitroglycerine-induced vasodilation between the 2 groups. On multiple logistic regression analysis for low FMD tertile, age, diabetes mellitus and <once/day tooth brushing remained independently associated with low FMD tertile (**Table 2**).

Discussion

In the present study, we showed that poor oral health, that is, decreased frequency of tooth brushing is associated with endothelial dysfunction. These harmful effects on the vasculature may contribute to an increase in the prevalence of cardiovascular events in subjects with poor oral health. Tooth brushing $\geq$ twice/day is beneficial for not only oral health but also endothelial function, leading to improvement in public health and savings in health-care costs.

Cumulative cardiovascular risk contributes to the development of cardiovascular and cerebrovascular events. Hypertension, dyslipidemia, diabetes mellitus, aging, smoking, and obesity are contributing risk factors. In the present study, there were significant differences in cardiovascular risk factors, including age, prevalence of hypertension, and smoking status between, the low-frequency and high-frequency tooth brushing groups. After adjustment of cardiovascular risk factors, low frequency of tooth brushing remained an independent predictor of low FMD tertile. Therefore, low frequency of tooth brushing may simultaneously or independently affect the maintenance and progression of atherosclerosis. To our knowledge, this is the first report on the effect of oral health, that is, frequency of tooth brushing, on vascular function.

It is well known that there is a relationship between inflammation and oral health. In the present study, we confirmed results of previous studies showing that the inflammation marker CRP was significantly associated with poor oral health.^{24,25} An association between inflammation and endothelial dysfunction has been suggested in many studies.^{21–25} We previously showed that chronic systemic infection with *Helicobacter pylori* impaired endothelium-dependent vasodilation in healthy male subjects.²⁸ Patients with periodontal disease are also ideal models for evaluating how endothelium-dependent vasodilation is affected by inflammation. Indeed, periodontal disease is associated with endothelial dysfunction.^{21–25} We have also shown that periodontal therapy improves endothelium-dependent vasodilation in patients with periodontitis.^{24,25} These findings suggest that endothelial dysfunction observed in the poor oral health group is due to increase in systemic inflammation and that oral health care, including tooth brushing, may have a beneficial effect on endothelial function.

Chronic inflammation induced by poor oral health may contribute to endothelial dysfunction through a decrease in NO bioavailability, a decrease in NO production and/or an increase in NO inactivation. Under the condition of chronic systemic inflammation, production of pro-inflammatory cytokines down-regulates the expression of endothelial NO synthase (eNOS) and decreases the half-life of eNOS mRNA.^{29,30} Interestingly, these cytokines attenuate endothelium-dependent vasodilation in vivo.³¹ CRP also directly decreased eNOS mRNA and protein levels and enzymatic activity in human aortic endothelial cells.³² It is thought that endothelial dysfunction causes inflammation of the vascular wall, leading to a vicious circle between

endothelial dysfunction and inflammation. These findings suggest that poor oral health activates several pro-inflammatory pathways, resulting in downregulation of the expression of eNOS and decrease in enzymatic activity and NO production.

Study Limitations

Although the method of self-reported assessment for dental status is well established, we used a questionnaire on the frequency of tooth brushing at the time of FMD measurement.

It is thought that lifestyle modifications, including physical activity and diet status, impair endothelial function, leading to cardiovascular outcomes.^{33–36} Unfortunately, in the present study, we did not obtain data on exercise status and diet. We cannot deny the possibility that differences in frequency of exercise and/or dietary fish intake affect the differences in FMD between patients with more and less frequent tooth brushing.

Not only the frequency of tooth brushing but also the quality and quantity of tooth brushing may influence FMD. Tooth brushing only once a day but done carefully and thoroughly at night immediately before bed might be more effective than poor tooth brushing twice a day for a short time. Evaluation of the effects of both quality and quantity of tooth brushing on vascular function would enable more specific conclusions to be drawn.

Conclusions

Poor oral health, that is, frequency of tooth brushing, is associated with endothelial dysfunction. Tooth brushing is a simple approach for improving poor oral condition, and it is expected that appropriate oral health care will improve endothelial function. Future large-scale clinical studies are needed to confirm whether long-term tooth brushing $\geq$ twice/day improves vascular function and reduces mortality and morbidity of cardiovascular disease in subjects with poor oral health.

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Disclosures

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