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Detection of oral bacteria in cardiovascular specimens

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Background/aims: Oral bacteria, including cariogenic and periodontal pathogens, are thought to be etiological factors in the development of cardiovascular diseases. To define this relationship, we analyzed the distribution of oral bacterial species in cardiovascular specimens.
Method: Following acceptance into the study, 203 consecutive patients were analyzed, from whom 82 aortic valve specimens, 35 mitral valve specimens, and 86 aortic aneurysmal wall specimens, of which 16 contained aneurysmal thrombus tissues, were obtained. In addition, a total of 58 dental plaque specimens were collected from the same group of patients who underwent heart valve replacement or removal of aortic aneurysms. Bacterial DNA was extracted from both cardiovascular tissues and dental plaque in those cases and then species-specific polymerase chain reaction assays were used to analyze the occurrences of six oral streptococcal and six periodontal bacterial species.
Results: *Streptococcus mutans* was the most frequently detected species in the cardiovascular specimens, followed by *Aggregatibacter actinomycetemcomitans*. As for dental plaque specimens from patients who underwent cardiovascular operations, most of the tested periodontitis-related species as well as oral streptococci were detected at high frequencies. Furthermore, the positive rate of *S. mutans* in cardiovascular specimens from patients whose dental plaque specimens were also positive for *S. mutans* was 78%, which was significantly higher than any other tested species when the same analysis was performed.
Conclusion: Our results suggest that specific oral bacterial species, such as *S. mutans* and *A. actinomycetemcomitans*, are related to bacteremia and may be etiologic factors for the development of cardiovascular diseases.

Key words: aortic aneurysm; heart valve; molecular approach; polymerase chain reaction; *Streptococcus mutans*

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Dental caries and marginal periodontitis are two of the major conditions generally encountered in the field of dentistry, with both considered to be caused by pathogenic oral bacterial species (18, 19). It is becoming more apparent that oral microbes comprise a complex community, and that oral health or disease depends on the interface between the host and microbial community as a whole (6). Oral bacteria are also known to cause systemic diseases, such as infective endocarditis, which has been shown to be associated with oral streptococci (15). Furthermore, the association of periodontitis with cardiovascular diseases has recently become an issue of great concern.

In the field of cardiovascular surgery, heart valve disease and aortic aneurysms are major diseases. Recent population-based surveys in the USA found that the national prevalence of valve diseases was approximately 2.5% in adults (17). These valve diseases mostly develop in aortic or mitral valves, and are diagnosed as regurgitation, stenosis, or both (2). On the other hand, aortic aneurysms are defined as permanent localized dilation of the aorta with a diameter at least 1.5 times greater than the expected normal diameter of the given aortic segment, and are mainly classified into thoracic aortic aneurysms (TAAs), abdominal aortic aneurysms (AAAs) and thoracoabdominal aortic aneurysms (TAAAs), based on the location of the diseased aorta (5). The estimated prevalence of aortic aneurysms in the USA was recently reported to be 1–2% of the adult population (10).

In our previous report, we noted that oral streptococci and periodontitis-related bacteria were detected in cardiovascular lesions extirpated from 62 patients using polymerase chain reaction (PCR) assays (16). However, the number of these cases studied was not adequate to provide conclusive results, while no other systematic large-scale study demonstrating the presence of oral bacterial species in cardiovascular tissues has been conducted. In the present study, we collected heart valve and aneurysm clinical specimens from 203 patients with various clinical diagnoses, and analyzed the prevalence of six oral streptococcal and six periodontitis-related bacterial species in them, to define the relationship between oral bacteria and cardiovascular diseases. In addition, we determined if the occurrences of these bacteria were related to cardiovascular pathologic conditions and diseased sites. Dental plaque samples were also collected from some of the patients, and used to analyze the relationships between oral and cardiovascular bacterial profiles.

Materials and methods

Cardiovascular and dental plaque specimens

The study procedures were approved by the Ethics Committee of Osaka Rosai Hospital and informed consent was obtained from each subject. Two hundred and three consecutive cases were analyzed, from which 82 aortic valve specimens, 35 mitral valve specimens, and 86 aortic aneurysmal wall specimens, 16 of which contained aneurysmal thrombus tissue, were obtained at the Department of Cardiovascular Surgery, Osaka Rosai Hospital, from December 2004 to November 2007. Table 1 summarizes the characteristics of the cases, such as number of patients, diagnosis, gender, and age. In addition, supragingival and subgingival plaque samples from some of these patients, referred for oral care before their cardiovascular operations, were taken from the mesial and buccal subgingival sites of all teeth with sterile Gracey curettes at the Department of Dentistry and Oral Surgery of the same hospital. A total of 58 dental plaque specimens were collected from 29 patients who underwent heart valve replacement and 29 patients who underwent removal of aortic aneurysms. The cardiovascular and dental plaque specimens were stored in sterile saline after collection with sterile instruments, then preserved at −20°C until analysis, as described previously (16).

Detection of 12 oral bacterial species

Six oral streptococcal species (*Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus salivarius*, *Streptococcus sanguinis*, *Streptococcus oralis*, and *Streptococcus gordonii*), as well as six periodontitis-related species (*Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans*, *Campylobacter rectus*) were detected with PCR using species-specific sets of primers, as described previously (1, 4, 9, 22, 23). The primers used and other details are shown in Table 2.

Statistical analysis

Statistical analyses were carried out using the computational software package StatView 5.0 (SAS Institue Inc., Cary, NC). Fisher's protected least-significant difference test was utilized to compare the detection frequency of each species.

Results

Distribution of oral bacterial species in cardiovascular specimens

The detection rates of all tested species were classified according to clinical diagnosis based on pathological and anatomical conditions, as shown in Fig. 1. A majority of the specimens (80% of heart valves, 90% of aortic aneurysms) were found to contain more than one species. There were no significant relationships between bacterial occurrences and clinical classification of the specimens. Regardless of diagnosis, *S. mutans* was the most frequently detected species, with detection rates in the heart valve and aneurysm wall specimens of 42.7% and 62.8%, respectively, while *A. actinomycetemcomitans* was the second most often detected, in 35.0% and 30.2%, respectively. As for the other species, *S. sanguinis*, *P. gingivalis*, and *T. denticola* were detected at frequencies ranging from 15 to 20%, each of which was significantly lower than *S. mutans* ($P < 0.05$), while the detection rates of *S. sobrinus*, *S. salivarius*, *S. oralis*, *S. gordonii*, *P. intermedia*, *T. forsythia*, and *C. rectus* were extremely low.

Detection of oral bacterial species in dental plaque and cardiovascular specimens from the same patients

The bacterial profiles of the dental plaque and cardiovascular specimens collected from the same patients were compared (Fig. 2). Periodontitis-related species were often detected in dental plaque, at rates of more than 70% for *C. rectus*, *T. forsythia*, and *T. denticola*, while the occurrences of *P. gingivalis* and *A. actinomycetemcomitans* were 50–60%. In contrast, *P. intermedia*, the least often occurring periodontitis-related species, was detected

Table 1. Summary of cases analyzed in the present study

Clinical diagnosis	Number of cases			Age[1] [mean ± SD (years)]
	Male	Female	Total	
Aortic regurgitation (AR)	27	4	31	66.8 ± 9.1 (36–84)
Aortic stenosis (AS)	15	17	32	69.1 ± 8.1 (49–81)
Aortic stenosis and regurgitation (ASR)	9	10	19	67.8 ± 11.3 (46–87)
Total aortic valves	51	31	82	67.9 ± 9.2 (36–87)
Mitral regurgitation (MR)	6	17	23	64.0 ± 8.4 (49–78)
Mitral stenosis (MS)	3	2	5	66.8 ± 2.9 (63–71)
Mitral stenosis and regurgitation (MSR)	3	4	7	69.6 ± 9.7 (58–84)
Total mitral valves	12	23	35	65.5 ± 8.3 (49–84)
Abdominal aortic aneurysm (AAA)	39	8	47	70.6 ± 7.1 (56–84)
Thoracic aortic aneurysm (TAA)	18	10	28	69.4 ± 8.0 (53–78)
Thoracoabdominal aortic aneurysm (TAAA)	8	3	11	73.8 ± 5.7 (64–82)
Total aneurysms	65	21	86	69.9 ± 9.8 (53–84)

[1]Parentheses indicate the range of age distribution.

Table 2. List of polymerase chain reaction primers used in the present study

Purpose	Sequence (5′ to 3′)	Size (bp)	Reference
Detection of oral streptococci			
S. mutans	GGC ACC ACA ACA TTG GGA AGC TCA GTT GGA ATG GCC GCT AAG TCA ACA GGA T	433	(4)
S. sobrinus	GAT GAT TTG GCT CAG GAT CAA TCC TC ACT GAG CCA GTA GTA GAC TTG GCA ACT	328	(4)
S. salivarius	GTG TTG CCA CAT CTT CAC TCG CTT CGG CGT TGA TGT GCT TGA AAG GGC ACC ATT	544	(4)
S. sanguinis	GGA TAG TGG CTC GGG CAG CCA GTT GAA CAG TTG CTG GAC TTG CTT GTC	313	(4)
S. oralis	TCC CGG TCA GCA AAC TCC AGC C GCA ACC TTT GGA TTT GCA AC	374	(4)
S. gordonii	CTA TGC GGA TGA TGC TAA TCA AGT G GGA GTC GCT ATA ATC TTG TCA GAA A	440	(4)
Detection of periodontitis-related species			
Porphyromonas gingivalis	TGT AGA TGA CTG ATG GTG AAA ACC ACG TCA TCC CCA CCT TCC TC	197	(22)
Prevotella intermedia	TTT GTT GGG GAG TAA AGC GGG TCA ACA TCT CTG TAT CCT GCG T	575	(1)
Treponema denticola	TAA TAC CGA ATG TGC TCA TTT ACA T TCA AAG AAG CAT TCC CTC TTC TTC TTA	311	(23)
Tannerella forsythia	GCG TAT GTA ACC TGC CCG CA TGC TTC AGT GTC CAG TTA TAC CT	641	(1)
Aggregatibacter actinomycetemcomitans	CTA GGT ATT GCG AAA CAA TTT G CCT GAA ATT AAG CTG GTA ATC	262	(9)
Campylobacter rectus	TTT CGG AGC GTA AAC TCC TTT TC TTT CTG CAA GCA GAC ACT CTT	598	(1)

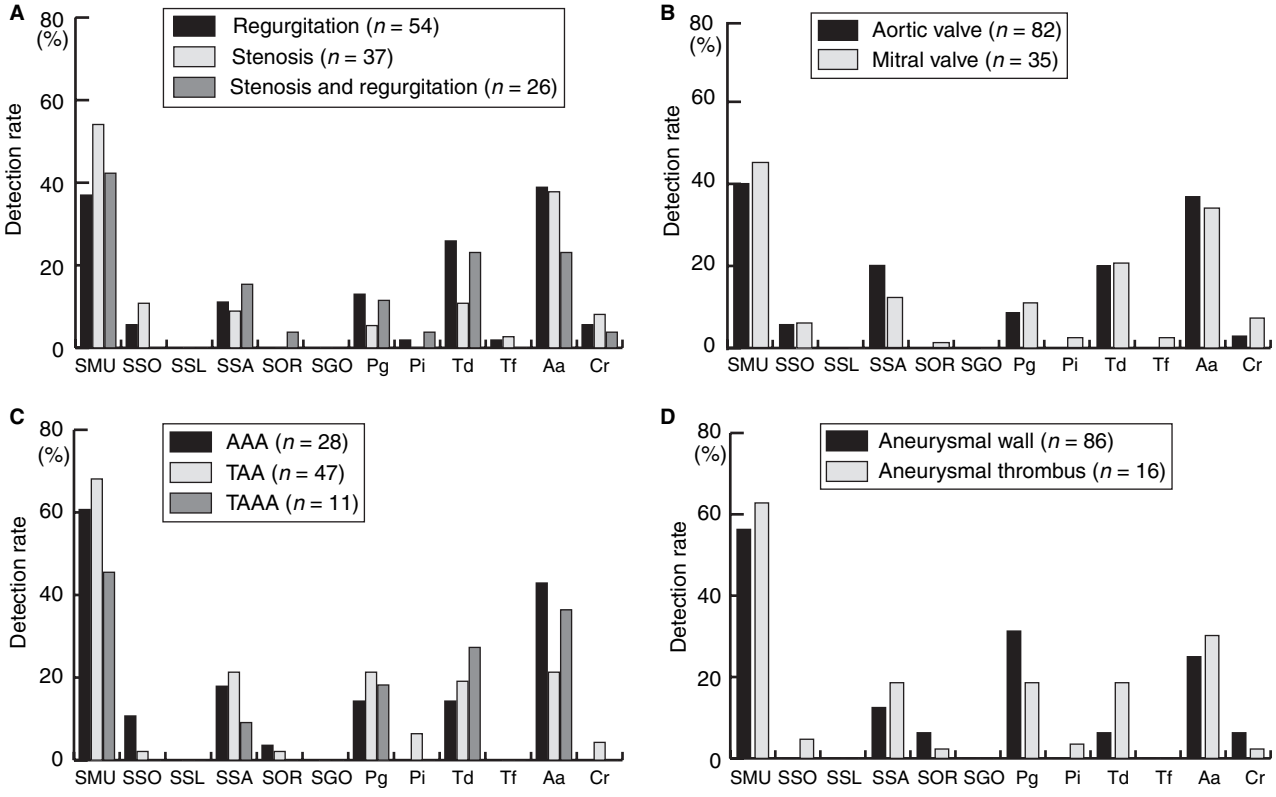

Fig. 1. Detection of oral bacterial species in cardiovascular specimens. The detection rates of the tested bacterial species in 117 heart valve specimens (A and B), 86 aortic aneurysmal wall specimens (C), and 102 aortic aneurysm specimens (D) were determined by polymerase chain reaction using species-specific sets of primers. Abbreviations: SMU, *Streptococcus mutans*; SSO, *Streptococcus sobrinus*; SSL, *Streptococcus salivarius*; SSA, *Streptococcus sanguinis*; SOR, *Streptococcus oralis*; SGO, *Streptococcus gordonii*; Pg, *Porphyromonas gingivalis*; Pi, *Prevotella intermedia*; Td, *Treponema denticola*; Tf, *Tannerella forsythia*; Aa, *Aggregatibacter actinomycetemcomitans*; Cr, *Campylobacter rectus*.

at a frequency of approximately 20%. Oral streptococcal species, including *S. mutans*, *S. sanguinis*, and *S. oralis*, were also often detected in the dental plaque samples at rates of more than 60%, while the occurrences of *S. sobrinus*, *S. salivarius*, and *S. gordonii* were limited to approximately 10–15%. As shown in Fig. 2, there was a slight tendency for species prevalent in

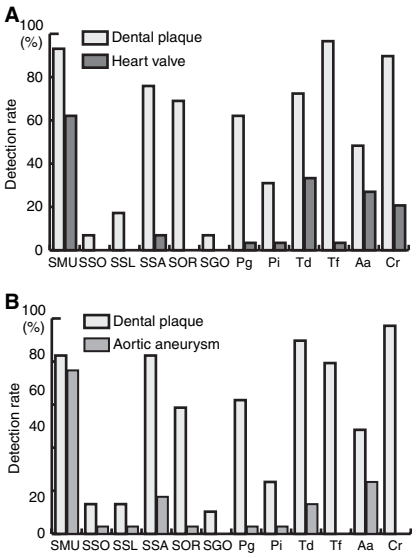

Fig. 2. Detection of oral bacterial species in patients from whom both dental plaque and cardiovascular specimens were collected ($n = 58$). The detection rates of the tested bacterial species in dental plaque and cardiovascular specimens from patients who underwent heart valve replacement (A; $n = 29$) and aortic aneurysm removal (B; $n = 29$) procedures were determined by polymerase chain reaction using species-specific sets of primers. Abbreviations as in Fig. 1.

dental plaque to be detected in cardiovascular tissues, though this was not statistically significant.

Next, cardiovascular specimens from patients whose dental plaque specimens were positive for each species were further analyzed for bacterial occurrence (Table 3). We suspected that some oral bacteria were able to efficiently enter and spread in the bloodstream, which may be an etiologic event for the development of cardiovascular diseases. *S. mutans* had a detection rate of 78.0% in the cardiovascular specimens, which was significantly greater than any of the other tested species ($P < 0.01$). In addition, the detection rates of *A. actinomycetemcomitans* and *T. denticola* were 46.4% and 27.7%, respectively. In contrast, *P. gingivalis* was detected in only 5.6% of the cases and the occurrences of *S. oralis*, *S. gordonii*, and *T. forsythia* were also low (<3%).

Discussion

Dental treatment is known to be the major causative agent for bacteremia and procedures that generally cause bleeding, such as tooth extraction, periodontal surgery, scaling and root planing, are considered to frequently lead to its development (21). Furthermore, bacteremia has been reported after daily routine oral hygiene procedures including tooth brushing (0–26%) and dental flossing (20–58%), and even from chewing (17–51%) (20). In fact, a majority of the present specimens (approximately 80% of the heart valves, 90% of the aortic aneurysms) were positive for at least one of the tested oral bacterial species. In addition, there were no significant differences in detection rates for each species between aortic and mitral valves, as well as between valves associated with stenosis and regurgitation in the present study. These findings indicate that oral bacteria frequently enter the bloodstream and may invade cardiovascular tissues following dental treatments and after the performance of routines of daily life.

Recently, an association between periodontitis and cardiovascular diseases has been suggested, based on reports that bacterial DNA corresponding to periodontitis-related species was detected in cardiovascular tissues using PCR. Although several studies have described the detection of oral bacteria in cardiovascular specimens, few have performed simultaneous detection of gram-positive (oral streptococcal) and gram-negative (periodontitis-related) species. To our knowledge, this is the first report of both gram-positive and gram-negative bacterial profiles analyzed using cardiovascular and dental plaque specimens collected from a large number of subjects. In the present study, we focused on the detection of six oral streptococcal and six periodontal bacterial species, of which three periodontitis-related species and two oral streptococcal species were detected in more than 10% of cardiovascular specimens, with the detection rate of *S. mutans* being high (42.7% of the heart valve and 62.8% of aortic aneurysm specimens). In addition, *S. mutans* was detected in cardiovascular specimens with a significantly higher frequency in those subjects whose dental plaque specimens also showed a positive reaction for this species, as compared to the other species. A previous study that analyzed bacterial species in blood from child subjects using a blood culture technique also showed that the incidence of *S. mutans* tended to be higher than that of other species (12). Therefore, *S. mutans* likely enters the bloodstream more readily than the others, which may indicate it as a possible etiological factor for cardiovascular disease.

Table 3. Detection rates of oral bacteria in cardiovascular specimens from patients whose dental plaque specimens possessed the same species

	Patients with heart valve replacement				Patients with aortic valve removal				Total patients with cardiovascular operations			
Species[1]	Detection rate[2] (%)	*P*-values[3] vs. SMU	vs. Aa	vs. Td	Detection rate[2] (%)	*P*-values[3] vs. SMU	vs. Aa	vs. Td	Detection rate[2] (%)	*P*-values[3] vs. SMU	vs. Aa	vs. Td
SMU	17/26 (65.4)	–	NS	NS	22/24 (91.7)	–	<0.001	<0.001	39/50 (78.0)	–	0.006	<0.001
SSO	0/2 (0)	NS	NS	NS	1/4 (25.0)	0.012	NS	NS	1/6 (16.7)	0.006	NS	NS
SSL	0/5 (0)	0.012	0.045	NS	1/4 (25.0)	0.012	NS	NS	1/9 (11.1)	<0.001	NS	NS
SSA	2/22 (9.1)	<0.001	0.006	0.016	5/24 (20.8)	<0.001	NS	NS	7/46 (15.2)	<0.001	0.006	NS
SOR	0/20 (0)	<0.001	<0.001	0.001	1/17 (5.9)	<0.001	NS	NS	1/37 (2.7)	<0.001	0.001	0.002
SGO	0/2 (0)	NS	NS	NS	0/3 (0)	0.003	NS	NS	0/5 (0)	0.001	NS	NS
Pg	1/18 (5.6)	<0.001	0.004	0.011	1/18 (5.6)	<0.001	NS	NS	2/36 (5.6)	<0.001	<0.001	0.010
Pi	1/9 (11.1)	0.007	0.040	NS	1/7 (14.3)	<0.001	NS	NS	2/16 (12.5)	<0.001	0.045	NS
Td	9/21 (42.9)	NS	NS	–	4/26 (15.4)	<0.001	NS	–	13/47 (27.7)	<0.001	NS	–
Tf	1/28 (3.6)	<0.001	<0.001	0.001	0/23 (0)	<0.001	0.005	NS	1/51 (2.0)	<0.001	<0.001	<0.001
Aa	8/14 (57.1)	NS	–	NS	5/14 (35.7)	0.001	–	NS	13/28 (46.4)	0.006	–	NS
Cr	6/26 (23.1)	0.005	0.043	NS	0/28 (0)	<0.001	0.002	0.047	6/54 (11.1)	<0.001	<0.001	0.043

[1]Bacterial species are abbreviated as follows: SMU, *Streptococcus mutans*; SSO, *Streptococcus sobrinus*; SSL, *Streptococcus salivarius*; SSA, *Streptococcus sanguinis*; SOR, *Streptococcus oralis*; SGO, *Streptococcus gordonii*; Pg, *Porphyromonas gingivalis*; Pi, *Prevotella intermedia*; Td, *Treponema denticola*; Tf, *Tannerella forsythia*; Aa, *Aggregatibacter actinomycetemcomitans*; Cr, *Campylobacter rectus*.
[2]The detection rate is expressed as the ratio of cardiovascular specimens positive for the species in all individuals whose dental plaque specimens were positive for the same species.
[3]NS indicates not statistically significant (i.e. $P > 0.05$).

In a previous study, oral cariogenic and periodontal conditions were epidemiologically evaluated using clinical and radiographic examinations of patients with cardiac valve disease, and the results were used to analyze the relationships of oral conditions with cardiovascular diagnostic variations (8). It appeared that aortic valve disorders developed significantly more often than mitral site disorders in patients with severely infected oral cavities. Although no clinical records with regard to the oral cavity were available for the present subjects, there were no significant differences found for the detection rates of the tested oral streptococcal and periodontal bacterial species between the aortic and mitral valve specimens. In addition, the rates of detection for oral bacterial species between valves extracted under the diagnosis of stenosis and regurgitation were not statistically different. Therefore, bacterial colonization in blood is not likely affected by the anatomical and pathological characteristics of the disease.

The association of oral pathogens with cardiovascular diseases has recently received attention, with atherosclerotic lesions caused by *P. gingivalis* infection being widely investigated. Among those results, systemic challenge by *P. gingivalis* was reported to accelerate atherogenic plaque formation in an apolipoprotein E-deficient murine model (11). On the other hand, several previous reports identified multiple oral bacterial species in human atheromatous plaque specimens (3, 7, 13, 14). In the present study, multiple oral bacterial species were detected in aortic aneurysm specimens and the detection rate for each species was similar to that of those species found in the heart valve specimens. Therefore, it is possible to speculate that the species detected in the aortic aneurysm specimens were related to bacteremia. However, additional investigations are required, because the species identified in these specimens might also be relevant for the pathogenesis of aortic aneurysm formation.

We collected dental plaque specimens from only about 30% of the present subjects with cardiovascular disease because the patients were originally examined at the Department of Cardiovascular Surgery and did not have an opportunity to also visit the Department of Dentistry and Oral Surgery, unless there was a specific problem in the oral cavity. Nevertheless, we frequently detected oral bacterial species in the cardiovascular specimens, which might be correlated with the development of cardiovascular diseases. Therefore, it is important for cardiovascular physicians and surgeons to understand the importance of oral health care and of routine dental examinations before surgery, while additional analyses focused on the direct involvement of oral bacteria in cardiovascular diseases may provide important findings.

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