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Correlation between Detection Rates of Periodontopathic Bacterial DNA in Carotid Coronary Stenotic Artery Plaque and in Dental Plaque Samples

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Utilizing PCR, the 16S rRNA detection rates for *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, *Treponema denticola*, and *Campylobacter rectus* in samples of stenotic coronary artery plaques were determined to be 21.6, 23.3, 5.9, 23.5, and 15.7%, respectively. The detection rates for *P. gingivalis* and *C. rectus* correlated with their presence in subgingival plaque.

It has been estimated that several hundred different species of bacteria inhabit the oral cavity. Among these, periodontal disease-associated bacteria adhere to and colonize the subgingival pocket, forming a biofilm (dental plaque). Once these bacteria are incorporated into biofilms, attenuation of the immune response against them may take place as a result of the reduction of phagocytosis, and the effectiveness of antibiotics is diminished (4, 15). These effects of biofilms may induce persistent infection in periodontal lesions. It has been suggested that periodontal disease-associated bacteria can penetrate gingival tissues and enter the bloodstream (8, 19). Microorganisms in the periodontal pocket may also induce a continuous benign bacteremia (5, 22, 32). Ross (30) reported that chronic infection can be one of the contributing factors involved in atherosclerosis. Several epidemiological studies have shown a positive correlation between periodontal disease and ischemic heart disease (2, 9, 27). However, Hujoel et al. (12) reported that their epidemiologic study did not find any evidence of an association between periodontal disease and heart disease. Nevertheless, members of our group and others have demonstrated that periodontal bacterial DNA can be detected in atherosclerotic lesions of aortic tissue (3, 11, 25, 34).

In this study, we sought to detect periodontal disease-associated bacterial DNA from stenotic coronary artery plaques recovered from 51 patients who were scheduled to receive surgical procedures to eliminate the plaque. We obtained informed consent from each subject in the present study. One week prior to surgery, we examined the periodontal status of each subject using a periodontal pocket probe. Thirty-four (30 males and 4 females; mean age, 64.3) of the subjects exhibited four or more periodontal lesions (probing depth, 4 mm and more). Seventeen (13 males and 4 females; average age, 63.5) demonstrated fewer than four periodontal lesions. Teeth were initially gently dried with sterile cotton swabs. After the removal of supragingival plaque with sterile cotton swabs, subgingival plaque samples were collected with sterilized scalers and transferred to 100 μl of sterilized phosphate-buffered saline (pH 7.4). The samples obtained from two periodontitis sites, which represented the deepest periodontal pockets, were pooled for analysis.

To eliminate blood contamination, the vascular endothelial samples were placed in sterilized phosphate-buffered saline and mixed gently, and tissue samples were transferred to fresh tubes. DNA was extracted by using a Puregene kit (Gentra Systems, Minneapolis, Minn.) according to the manufacturer's instructions. Briefly, samples (approximately 100 mg) were dissociated with a spatula, and 6 ml of cell lysis solution was added. Samples were then homogenized thoroughly with a tube pestle. Lysates were incubated at 65°C for 60 min, and further incubation was performed for 30 min after addition of RNase. After addition of protein precipitation solution, lysates were centrifuged for 10 min at 2,000 × *g*. DNA was concentrated by addition of 6 ml of 100% isopropanol to the supernatant and subsequent centrifugation. The DNA pellet obtained was then processed for PCRs. Samples from two young male patients (average age, 11.5) with Kawasaki disease who had healthy periodontal tissue were also examined in this study. One week prior to the cardiac surgical procedures, the periodontal disease status of the 53 patients was assessed and dental plaque samples from the periodontal sites were collected. Standard precautions were taken in handling reagents and samples as well as double blinding the analysts.

To detect *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Bacteroides forsythus*, *Treponema denticola*, and *Campylobacter rectus*, PCR was performed by the method described by Ashimoto et al. (1). Amplified fragments were confirmed following nucleotide sequencing by the dideoxy-chain termination method (31), using a 310A DNA sequencer (Applied Biosystems, Foster, Calif.).

In 51 adult patients, detection rates for 16S rRNA from *P. gingivalis*, *A. actinomycetemcomitans*, *B. forsythus*, *T. denticola*, and *C. rectus* in the coronary artery plaque samples were 21.6, 23.3, 5.9, 23.5, and 15.7%, respectively. The detection frequencies for *P. gingivalis*, *A. actinomycetemcomitans*, *B. forsythus*, *T. denticola*, and *C. rectus* in subgingival plaque were 54.9, 33.3,

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TABLE 1. Comparison of detection rates of 16S rRNA of *P. gingivalis*, *A. actinomycetemcomitans*, *B. forsythus*, *T. denticola*, and *C. rectus* in samples of stenotic coronary artery plaque and subgingival dental plaque from patients possessing fewer than four periodontal lesions and those from patients with four or more periodontal lesions

Species	Detection rate (%)			
	Patients possessing <4 periodontal lesions ($n = 17$)		Patients possessing 4 or more periodontal lesions ($n = 34$)	
	Subgingival	Coronary artery	Subgingival	Coronary artery
P. gingivalis	47.1	5.8	58.8	29.4
A. actinomycetemcomitans	41.2	17.6	29.4	26.5
B. forsythus	41.2	5.8	41.2	5.9
T. denticola	58.8	11.8	67.7	29.4
C. rectus	29.4	17.6	41.2	14.7

41.2, 64.7, and 37.3%, respectively. *A. actinomycetemcomitans* and *C. rectus* were detected in a coronary artery sample from one of the two young patients with Kawasaki disease. No defined gingival inflammation, dental plaque accumulation, or dental calculus was found in these patients. Kawasaki disease often accompanies coronary aneurysms; however, this disease is not normally associated with periodontitis. In our previous studies, no periodontopathic bacterial DNA was detected in healthy arterial walls. Recently, Lalla et al. (18) reported that oral infection by *P. gingivalis* accelerates early atherosclerosis and that two of nine infected mice tested demonstrated the presence of DNA for this organism. These reports suggested that there is a relationship between the detection of periodontopathic bacterial DNA in atherosclerotic lesions and periodontal disease.

Table 1 shows a comparison of the detection rates for periodontal pathogens from subgingival plaque and stenotic coronary artery plaque in patients possessing four or more periodontal lesions with those for patients with fewer than four lesions. The detection rates for *P. gingivalis*, *A. actinomycetemcomitans*, and *T. denticola* in patients possessing four or more periodontal lesions were higher than those for patients with fewer than four lesions. The *P. gingivalis* 16S rRNA locus was detected from the coronary artery in 10 of 34 patients possessing four or more periodontal lesions and in 1 of 17 patients possessing fewer than four lesions. We detected *P. gingivalis* in both coronary artery and subgingival plaque samples from 10 of 11 patients. *A. actinomycetemcomitans* in coronary artery samples was detected for 9 of 34 patients possessing four or more periodontal lesions and 3 of 17 patients with fewer than four lesions. Unexpectedly, we detected *A. actinomycetemcomitans* in both coronary artery and subgingival plaque samples for only 2 of 12 patients. The sampling sites for subgingival plaque in the present study were the deepest periodontal pockets. Normally anaerobic conditions might be expected to produce these results, because *A. actinomycetemcomitans* is a facultative anaerobic bacterial species. *B. forsythus* in coronary artery samples was the least frequent of the periodontal pathogens examined. We detected this organism in only 1 of 17 patients possessing fewer than four periodontal lesions and 2 of 34 patients possessing four or more periodontal lesions. We detected the microorganisms in samples of both coronary artery plaque and subgingival plaque from two of the three patients. *T. denticola* was detected in coronary artery samples from 2 of 17 patients possessing fewer than four periodontal lesions and 10 of 34 patients possessing four or more lesions. We detected *T. denticola* in subgingival plaques from 7 of 10 patients whose coronary artery samples were positive for these microorganisms. Detection of *C. rectus* in coronary artery samples was positive for 3 of 17 patients possessing fewer than four periodontal lesions and 5 of 34 patients possessing four or more lesions. We detected the microorganisms in samples of both coronary artery plaque and subgingival plaque from four of these five patients. Statistical analysis using a chi-square test showed that detection of 16S rRNA of *P. gingivalis* and *C. rectus* in coronary artery plaque samples significantly correlated with colonization by these organisms in subgingival sites ($P < 0.01$).

In this study, sampling from periodontal pockets was performed 1 week in advance of sampling from coronary arteries. It is possible to cause bacteremia by sampling the periodontal pockets. Roberts et al. (28) reported that the highest yield of microorganisms from blood samples occurs at approximately 30 s after the onset of dentally induced bacteremia. The peak of bacteremia after injection of human oral microorganisms into the bloodstream was within a minute in animal experiments (33). The reduction of bacteremia by host defense systems occurs over several minutes after dental instrumentation (26). In addition, daily tooth brushing and mastication were also reported to induce bacteremia (10, 28). Moreover, the detection rate for *T. denticola* in our previous study is similar to that of the present study. Taken together, these results suggest that probing and sampling of subgingival plaque 1 week in advance of sampling from the heart should not affect the detection rates in arterial plaque.

A relationship between chronic inflammation and atherosclerosis has been reported (21, 29). Previously, we detected the 316-bp 16S rRNA of *T. denticola* in plaque samples from aneurysmal sites in 6 of 26 patients (23.1%), along with antigens of *T. denticola* in and around foam cells in the lesions (25). However, no other periodontal disease-associated bacterial DNA was detected. Presumably, *T. denticola* reaches aneurysmal sites by means of its high motility. Recently periodontal pathogens were detected from samples of carotid endarterectomy (3, 11). It has also been demonstrated that subgingival bacteria, such as *P. gingivalis* and *A. actinomycetemcomitans*, are able to invade both epithelial and endothelial cells (6, 7, 19, 24, 36). In addition, Madianos et al. (23) showed that *P. gingivalis* can persist and multiply within epithelial cells. In the present study, the detection rates for *P. gingivalis* and *C. rectus* in coronary artery plaques correlated with detection from subgingival plaque. The increased detection rates for *P. gingivalis* and *T. denticola* in coronary artery samples are also paralleled by their detection in periodontal pockets. These results suggest that these microorganisms in periodontal pockets may penetrate subgingival epithelial cells and invade the bloodstream.

Outer membrane vesicles of *P. gingivalis* in macrophages appear capable of inducing foam cell formation in these cells (16, 17). Likewise, it has been reported that when $ApoE^{+/-}$ atherosclerosis-prone mice were fed a high-fat diet and in-

fected with *P. gingivalis*, atherosclerosis was further accelerated (20). In addition, induction of atherosclerosis by oral infection with *P. gingivalis* in ApoE$^{-/-}$ mice was reported (18). Furthermore Jain et al. (13) reported that rabbits with experimentally induced periodontitis from *P. gingivalis* had more extensive accumulations of lipids in the aorta than did control animals, and there was a positive correlation between the severity of periodontal disease and the extent of lipid deposition. Poor oral hygiene was also found to be a high risk factor for infective endocarditis (35). Kiechl et al. (14) demonstrated that polymorphism of the toll-like receptor 4, which attenuates receptor signaling and diminishes the inflammatory response to gram-negative bacteria, is associated with a decreased risk of atherosclerosis. Thus, asymptomatic bacteremia due to periodontopathic gram-negative bacteria may accelerate stenotic coronary artery plaque progression. Taken together, the present study supports the hypothesis that periodontal disease-associated bacteria could enter the bloodstream and play a direct or indirect role in the progression of stenotic coronary artery plaque lesions.

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