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Molecular survey of atheromatous plaques for the presence of DNA from periodontal bacterial pathogens, archaea and fungi

A. R. L. Aquino[1], K. C. Lima[1], M. S. Paiva[2], I. N. Rôças[3], J. F. Siqueira Jr[3]

Departments of [1]Preventive Dentistry and [2]Medicine, Federal University of Rio Grande do Norte, Natal, RN, Brazil, [3]Department of Endodontics and Molecular Microbiology Laboratory, Estácio de Sá University, Rio de Janeiro, Brazil

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Background and Objective: Chronic infections, such as periodontitis, have been associated with the development and progression of atherosclerosis. The mechanisms through which this occurs have yet to be elucidated. This study was carried out to detect periodontopathic bacteria as well as archaea and fungi in atheromatous plaques and search for factors associated with their occurrence in atheromas.

Material and Methods: A cross-sectional study was carried out including 30 patients diagnosed with atherosclerosis in the carotid, coronary or femoral arteries. Plaques were collected during surgery and analysed using PCR to detect *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola* and members of the *Synergistetes* group. Samples were also surveyed with universal primers for bacterial, archaeal and fungal DNA. Patients responded to a questionnaire to determine factors associated with PCR results.

Results: All dentate individuals (66.7%) had periodontal disease, 95% of which was severe and 65% extensive. None of the targeted periodontopathic bacteria was found in the atheromas. No sample yielded positive results for fungal and archaeal DNA. Four samples (13%) were positive for the presence of bacterial DNA. Of these, three participants were dentate (two with severely chronic generalized periodontitis and one with severely chronic localized periodontitis).

Conclusion: This study did not confirm previous findings of periodontal pathogens in atheromas, making it impossible to establish factors associated with their presence in plaques. Presence of bacterial DNA in some samples indicates that periodontal or nonoral bacterial species other than the ones targeted in this study may be involved with some cases of atherosclerosis.

Ana Rafaela Luz de Aquino, DDs, MSc, Doctorate student, Oral Pathology Department, Federal University of Rio Grande do Norte. Rua Desembargador Túlio Bezerra De Melo, 3720, Candelária, 59064-585, Natal/RN, Brazil
Tel:/Fax: +84 3234 3833/9994 0684
e-mail: anarafaela.luz@terra.com.br

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Periodontal infections consist of a group of inflammatory conditions caused by microbial biofilms formed on tooth surfaces. The global prevalence of periodontitis is high, and chronic periodontitis affects about 10–15% of most populations (1). As these infections are mostly chronic in nature, recent studies have suggested

an association between periodontitis and the development and progression of atherosclerosis. The latter is a chronic progressive condition that affects the inner layer of large and medium-sized arteries (2–4). Traditional risk factors, such as hypertension, hypercholesterolemia, diabetes mellitus and smoking, may not suffice to explain the high number of atherosclerosis cases in the population (5,6).

The exact connection between periodontal diseases and atherosclerosis is not yet completely clear. Some authors (7,8) suggest two hypotheses for the association involving periodontitis, systemic inflammation and atherosclerosis. One theory centers on the chronic infection load that periodontitis represents to the host. Bacteria or bacterial endotoxins from periodontal pockets may repeatedly gain access to bloodstream and exert continuous noxious effects that cause vascular wall lesions and promote the formation of atheromatous plaques. The second theory is that periodontitis stimulates the release and spread of inflammatory mediators in the host in such a way that their elevated levels accelerate the progression of atherosclerosis.

Several studies (3–5,9–11) investigated the association between periodontitis and atheroslerosis by looking for periodontal pathogens in atheromata. Atheroma samples have been shown to be positive for *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Treponema denticola*, *Prevotella intermedia* and *Tannerella forsythia*. Consequently, a relationship between periodontitis and atheromatous disease has been suspected. However, data from the literature are conflicting, as other studies (12,13) failed to detect any of these classic periodontal pathogens in atheromatous plaques. Also, Padilla *et al*. (11) identified only *A. actinomycetemcomitans* in a few atheroma samples, but not *P. gingivalis*, *T. denticola*, *T. forsythia* or *Campylobacter rectus*.

To the best of our knowledge, there are no published studies in the literature that investigated other groups of potential oral bacterial pathogens, such as members of the *Synergistetes* group, as well as other microorganisms, such as archaea and fungi, for association with atheromas. Recent advances in molecular identification have included members of the *Synergistetes* group (14) and archaea (15) in the set of potential periodontal pathogens. Most representatives of these groups of microorganisms are difficult to culture or even as-yet-uncultivated species. Fungi have also been associated with periodontal diseases (16).

This study aimed to investigate the prevalence of well-established periodontal pathogens and members of the *Synergistetes* group, archaea and fungi in atheromatous plaques and identify factors associated with periodontal conditions and participants' general health in relation to the presence of these microorganisms in atheromata.

Material and methods

Subjects and sample taking

Samples for this observational cross-sectional study were taken from individuals diagnosed with atherosclerosis in the carotid, coronary or femoral arteries. Subjects were treated by stent implant angioplasty, endarterectomy or bypass surgery at the following hospitals: Natal Hospital Center, Hospital do Coração, Incor-Promater, Casa de Saúde São Lucas and the Hospital Universitário Onofre Lopes (HUOL), all of them located in the city of Natal, Rio Grande do Norte, Brazil. Patients were examined in the infirmary after elective stent implant angioplasty, endarterectomy or bypass surgery. Signed informed consent was obtained to authorize periodontal examinations. Extremely weak patients, in whom atheromata fragments could not be obtained or a periodontal examination performed, were excluded from the study. Cases where no atheromatous plaques were found in distal protection filters during angioplasty were also excluded. The study protocol was approved by the Research Ethics Committee of the Universidade Federal do Rio Grande do Norte.

Sample size consisted of 30 selected individuals and was calculated according to the major prevalence of peridontopathic bacteria in atheromata reported by Kozarov *et al*. (3), with a confidence level of 95% through the two-tailed hypothesis test with 85% power. Calculation resulted in the number of 21 individuals and to allow greater dispersion of data, the sample was closed with 30 participants.

Tools used for data collection included a structured interview, periodontal clinical examination and athermanous plaque collection. A questionnaire was applied to all participants to identify possible factors related to the presence or absence of periodontal bacteria in atheromatous plaques. These factors might help explain the occurrence of these bacteria in atheromata when associated with periodontal conditions and bacteremic episodes.

Patients were subjected to a clinical periodontal examination to determine health/periodontitis. Examination was carried out by a single operator using a Goldman/Fox Williams periodontal probe (Trinity, São Paulo, SP, Brazil) to determine values for probing depth, gingival recession and gingival bleeding rates. The procedure was calibrated and its reliability tested using the intraclass correlation coefficient.

Periodontal diagnosis was carried out in accordance with the American Academy of Periodontology. Sites with no bleeding and a probe depth up to 2 mm were considered healthy. Gingivitis sites were those with a probe depth between 2 and 3 mm, no recession and bleeding on probing. Cases of chronic periodontitis showed insertion loss. Sites with insertion loss of 1–2 mm were classified as mildly chronic periodontitis; insertion loss between 3 and 4 mm was considered moderately chronic; and sites with insertion loss of 5 mm and above were classified as severely chronic periodontitis. Disease was also classified according to its extent as localized (affecting up to 30% of the sites) or generalized (> 30% of the sites).

Diagnosis of atherosclerosis was in accordance with the guidelines of the Brazilian Cardiology Society and based on clinical examination, catheterization and angiography. Atheromatous plaques were obtained from endarterectomy, bypass surgery and

distal protection filters used in stent implant angioplasty. Samples were stored in sterile microcentrifuge tubes containing trypticase soy broth and dimethyl sulfoxide and immediately frozen at −20°C. The DNA was extracted from tissue samples using the QIAamp® DNA mini kit (Qiagen, Valencia, Spain) following the manufacturer's directions. Extracted DNA was stored at −20°C until further molecular analysis.

PCR

Aliquots of extracted DNA were used in 16S rRNA gene-based PCR protocols using universal primers for members of the domains Bacteria or Archaea, and in an 18S rRNA gene-based assay with universal primers for fungi (domain Eukarya). A group-specific PCR protocol was carried out to identify members of *Synergistetes* bacteria (most are uncultivated), and species-specific PCR assays were used to detect the periodontal pathogens *P. ginigivalis*, *T. denticola* and *A. actinomycetemcomitans*. These group-specific and species-specific PCR assays were based on the 16S rRNA gene. Sensitivity of the species-specific single PCR assays was approximately 100 bacterial cells per reaction. Primer sequences are displayed in Table 1.

All PCRs were performed in 50 μL of reaction mixture containing 1 μM concentration of each primer, 5 μL of 10 × PCR buffer (Fermentas, ON, Canada), 2 mM $MgCl_2$, 1.25 U of *Taq* DNA polymerase (Fermentas) and 0.2 mM of each deoxyribonucleoside triphosphate (Biotools, Madrid, Spain). Positive and negative controls were included in each batch of samples analysed. Positive controls consisted of DNA extracted from *A. actinomycetemcomitans* (ATCC 43718), *P. gingivalis* (ATCC 33277), *T. denticola* (B1 strain; Forsyth Institute, Boston, MA, USA), *Methanobrevibacter arboriphilicus* (DSMZ 744) and *Candida albicans* (ATCC 10231). Negative controls consisted of sterile ultrapure water instead of sample.

The PCR amplifications were performed in a DNA thermocycler (Mastercycler personal; Eppendorff, Hamburg, Germany). Cycling conditions were as follows. For *Synergistetes*: initial denaturation at 94°C for 2 min, 36 cycles at 94°C for 1 min, 50°C for 1 min and 72°C for 1 min, and final extension at 72°C for 5 min. For Archaea: initial denaturation at 94°C for 2 min, 36 cycles at 94°C for 30 s, 58°C for 30 s and 72°C for 1 min, and final extension at 72°C for 10 min. For Bacteria (universal) and *T. denticola*: initial denaturation step at 95°C for 2 min, 36 cycles at 95°C for 30 s, 60°C for 1 min and 72°C for 1 min, and a final extension at 72°C for 2 min. For *P. gingivalis*: initial denaturation step at 95°C for 2 min, 36 cycles at 94°C for 30 s, 60°C for 1 min and 72°C for 2 min, and a final step at 72°C for 10 min. For *A. actinomycetemcomitans* and fungi (universal): initial denaturation step at 95°C for 30 s, 36 cycles at 95°C for 30 s, 55°C for 1 min and 72°C for 2 min, and a final step at 72°C for 10 min.

The PCR products were subjected to electrophoresis in a 1.5% agarose gel–Tris-borate–EDTA buffer. The gel was stained with GelRed (Biotium, Hayward, CA, USA) and visualized under ultraviolet illumination. The presence of amplicons of the expected size in each primer was considered a positive result. A 100 bp DNA ladder (Biotools) was used as a parameter for amplicon size.

A descriptive statistical analysis was used to characterize the sample and analyse the specific periodontal pathogens identified in atheromata. Descriptive statistics was also used to characterize cases where bacteria were found in atheromatous plaques.

Results

Sample characterization

Table 2 shows sample distribution according to the sex, age and schooling of participants. Data relating to mean, standard deviation, median and quartiles 25 and 75 are also shown. A high percentage of male patients underwent atherosclerosis surgery. A high mean age was observed; most participants were elderly and with only a few years of education.

Table 3 shows data related to the periodontal analysis of the participants.

Table 1. Primer sequences used to detect different microorganisms in samples from atheromas

Microorganism	Gene	Sequence (5′–3′)	Amplicon size (bp)	Reference
Archaea (universal)	16S rRNA	TCC AGG CCC TAC GGG YCC GGC GTT GAM TCC AAT T	626	15
Bacteria (universal)	16S rRNA	GAT TAG ATA CCC TGG TAG TCC AC CCC GGG AAC GTA TTC ACC G	602	17
Fungi (universal)	18S rRNA	ACT TTC GAT GGT AGG ATA G TGA TCR TCT TCG ATC CCC TA	690	16
Aggregatibacter actinomycetemcomitans	16S rRNA	AAA CCC ATC TCT GAG TTC TTC TTC ATG CCA ACT TGA CGT TAA AT	557	17
Porphyromonas gingivalis	16S rRNA	AGG CAG CTT GCC ATA CTG CG ACT GTT AGC AAC TAC CGA TGT	404	17
Treponema denticola	16S rRNA	TAA TAC CGA ATG TGC TCA TTT ACA T TCA AAG AAG CAT TCC CTC TTC TTC TTA	316	17
Synergistetes group	16S rRNA	AGA GTT TGA TYM TGG CTC AG CAG GTA AGG TTC TTC GGT	980	18

Table 2. Absolute and percentile distribution in accordance with participants' sex; mean, standard deviation, median, Q_{25} and Q_{75} according to the age and schooling of the participants (Natal, Brazil 2010)

	Sex		*n* (%)	
	Male		19 (63.3)	
	Female		11 (36.7)	
	Mean ± SD	Median	95% CI	Q_{25}–Q_{75}
Age in years	70.60 ± 10.88	71	66.54–74.66	62.25–79.25
Schooling in years	6.43 ± 5.33	5.5	4.44–8.43	1.75–9.25

Q, quartile.

Table 3. Absolute and percentile distribution in accordance with the presence of dental elements, periodontitis and its severity and extent (Natal, Brazil, 2010)

	n	%
Dentate		
Yes	20	66.7
No	10	33.3
Presence of periodontitis		
Yes	20	100
No	0	0
Severity of periodontitis		
Mild	0	0
Moderate	1	5.0
Severe	19	95.0
Extent of periodontitis		
Localized	7	35.0
Generalized	13	65.0

Most participants had at least one tooth in their inferior and superior arches. All 20 patients had periodontitis and almost all of these were classified as severely chronic cases with clinical attachment loss ≥5 mm. Most patients had generalized chronic periodontitis. More than half of the periodontal sites showed clinical attachment loss of 5 mm or more (mean, 54.85 periodontal sites). Gingival bleeding during periodontal probing was also observed in a high percentage of sites (mean, 32.85 periodontal sites). In the edentate patients, a mean time of 10 years had elapsed since loss of the last tooth.

Most patients reported no endodontic (63.3%), periodontal (86.7%) or exodontic treatment (76.7%) in the last 6 mo. In theory, this may indicate a low risk of recent bacteremic episodes arising from dental procedures.

The majority of participants were smokers and ex-smokers (56.7%). Smokers were individuals who smoked or quit smoking over the last year. Individuals who had not smoked for >1 year were considered ex-smokers. Subjects who had never smoked or had not smoked for >5 years were considered nonsmokers. Both smokers and ex-smokers used tobacco for an extended period (mean, 39.39 years).

In relation to arterial hypertension risk, the results showed that more than two-thirds of the individuals had arterial hypertension (76.7%) and a smaller portion of these were diabetic (46.7%). Hypertensive medication was used by most of the patients (80%) together with antibiotics in the last 6 mo (80%). This is in accordance with the HUOL protocol of prescribing antibiotics before bypass surgery, which was the most common surgery among the subjects.

Table 4 shows the association between the origin of atheromatous plaques and the surgery performed. Origin of the plaques refers to the location of the arteries where the atherosclerotic samples were removed. The femoral artery was predominant in relation to the carotid and coronary arteries. As a result, the surgery most commonly performed was in femoral arteries.

Data were collected on the use of antiseptic mouthwash, time and use of dental prostheses, number of times teeth were brushed per day and the last dental visit. The majority of participants did not use mouthwash (86.7%), and more than two-thirds reported using a dental prosthesis for a number of years. Oral hygiene was poor among participants, with 33.3% brushing their teeth only once a day and only 20% brushing three times a day. The mean time since the last dental visit was 6.27 years, meaning an extensive period without dental care.

PCR results

No atheromatous plaque sample yielded positive results for DNA from the periodontopathic bacterial species *P. gingivalis*, *T. denticola* and *A. actinomycetemcomitans*. Likewise, negative results were observed for PCR specific for *Synergistetes* group, archaea and fungi. Thus, no association could be established with the factors and oral conditions studied. Four positive samples were positive for bacterial DNA when universal 16S rRNA gene primers were used, representing 13% of the cases. These bacteria were, however, not identified. All positive controls exhibited only the amplicons of predicted size. No PCR products were observed in the negative samples.

Three of the four participants with bacteria present in their atheromata were males (75%) over 60 years old, and only one was female, aged 55 years. Two patients were diabetic and all four were hypertensive. Only one patient reported never having smoked and the others were smokers or long-term ex-smokers. In two bacteria-positive samples, atheromata fragments were removed from the carotid artery; one was obtained through

Table 4. Absolute distribution and percentage of the sample according to the origin of atheromatous plaque and surgery performed (Natal, Brazil 2010)

	Origin of atheromatous plaques							
	Carotid		Coronary		Femoral		Total	
Surgery	*n*	%	*n*	%	*n*	%	*n*	%
Endarterectomy	2	100	0	0	0	0	2	6.7
Bypass	0	0	0	0	17	100	17	56.7
Angioplasty with filter	6	54.5	5	45.5	0	0	11	36.7
Total	8	26.7	5	16.7	17	56.7	30	100

angioplasty and the other during endarterectomy. The other two fragments were removed from the femoral artery during bypass surgery.

Concerning oral health, three of the four participants were dentate. Two had severely chronic generalized periodontitis and one severely chronic localized periodontitis. None of them reported having been subjected to procedures related to possible bacteremic episodes, such as endodontic treatment, exodontia in the last 6 mo or any dental surgery. Only one individual reported daily use of antiseptic mouthwash for approximately 7 years and two wore dental prostheses.

Discussion

Recent studies have suggested that periodontitis may have an important role in the development of atherosclerosis (1,19–21). However, complete evidence of this has not been definitely established, and studies have questioned such an association (13,22). This study was undertaken to provide additional information as to the involvement of periodontal bacteria and other microorganisms in atherosclerosis using a set of patients from a specific geographical location in Brazil.

Individuals participating in this study were classified as dentate (66.7%) or edentate (33.3%). The latter patients were included to serve as a possible 'control' group in case periodontal bacteria were detected in the atheromata. Periodontal disease was present in all dentate patients and classified as severely chronic in 95% of cases and generalized in 65%. Edentulous patients had been without teeth for a substantial time and therefore had no periodontal sites capable of housing periodontopathic bacteria. However, it should be considered that periodontal pathogens might have occurred in other oral habitats, including the dental prostheses used for a significant period by 76.7% of the patients. Also, the possibility that periodontopathic bacteria could have been present in periodontal sites when the edentulous patients had teeth and thus be found in atheromatous plaques cannot be discarded.

The possible bias related to the use of a questionnaire in search of possible factors associated with the presence of periodontal pathogens in atheromatous plaques may have been minimized in the present study. In adddition to the fact that the data set produced represents only an estimate, one may conceive that loss of a tooth is a significant event in an individual's lifetime. Furthermore, all patients had good cognition and whenever they were in doubt, the response was not considered. Finally, because no target microorganism was detected at all, possible memory loss affecting the questionnaire results was not expected to influence interpretation of the results.

In relation to the epidemiology of atherosclerosis, our results are apparently in line with various epidemiological studies that suggested a positive association between periodontitis and cardiovascular disease, with the main cause being atherosclerosis (10). However, some studies (23) could not establish a statistically significant association between cardiovascular disease and periodontitis. According to Armitage (24), although most studies show an association, the results of epidemiological studies are rather inconclusive.

Given the connection between periodontitis and atherosclerosis as suggested by epidemiological research, many studies have been carried out to determine the nature of this connection. Among these are studies looking for the presence of periodontal pathogens in atheromata. The rationale is that the infection load to which the individual is exposed in the oral cavity, especially due to periodontitis, may contribute to the development and progression of atherosclerosis (25). These studies have shown variations in the detection of putative periodontal pathogens in atheromatous plaques using molecular assays (12).

In the present study, we targeted three classic periodontal pathogens that have also been previously detected in atherosclerotic lesions (4,5,9). Ishihara *et al.* (4) used PCR and suggested that *P. gingivalis*, *A. actinomycetemcomitans*, *T. denticola*, *T. forsythia* and *C. rectus* may enter the bloodstream and be involved in the progression of atheromata. These findings were somewhat corroborated by many other studies (2,3,5,9,26).

However, detection of periodontal pathogens in atheroma samples has not been confirmed by other studies. A cross-sectional study (11) used PCR to detect periodontal bacteria in subgingival biofilm and atheromatous plaques from 12 patients with chronic periodontitis and atherosclerosis. Although *A. actinomycetemcomitans* was identified in the periodontal pockets and atheroma in two cases, *P. gingivalis*, *T. denticola*, *T. forsythia* and *C. rectus* were not found. In a case–control study (13) of dentate and edentate patients using the PCR technique to detect *F. nucleatum*, *P. gingivalis*, *P. intermedia* and *T. forsythia*, these periodontal bacteria were found in the subgingival biofilm but none of them was present in carotid atheromatous plaques. Likewise, Aimetti *et al.* (12) used PCR and found periodontal bacteria in the subgingival biofilm of patients with chronic periodontitis but not in carotid atheromas.

The present study is in line with these previous reports in that none of the targeted periodontal pathogens, *A. actinomycetemcomitans*, *P. gingivalis* and *T. denticola*, was found in the atheromatous plaques examined. Other suspected pathogens, such as *Synergistetes*, archaea and fungi, were not detected either. Therefore, no association could be established between periodontal disease and atherosclerosis through the presence of periodontal species and other microbial pathogens.

One reason for discrepant results related to detection of periodontal bacteria in atheromatous plaques may be the different methodology used. The PCR procedures may vary significantly, from DNA extraction methods to the primers used or the conditions in which reactions are carried out (13), and may be responsible for differences (3). The PCR protocol used for detection of the three periodontally pathogenic species was based on the description by Ashimoto *et al.* (17). This is a single PCR assay, and the

possibility exists that more sensitive PCR assays, such as the nested PCR, could have been more effective by detecting a lower number of bacteria. However, other studies (9,11,22) that identified periodontal bacteria in atheromatous plaques also used the method described by Ashimoto *et al.* (17). Thus, it is possible that other factors may also have influenced the results, including epidemiological factors, such as nutrition, ethnicity, geographical factors and disease stage (12,13). Further studies comparing different technologies and populations may help elucidate these questions.

In relation to collection of the atheromatous plaques, three surgical procedures were used due to the difficulty of obtaining this material. Endarterectomy, in which atheromatous plaques causing arterial obstruction are removed, was used in several previous studies (11–13,22). Other authors (9,26,27) used bypass surgery, in which part of the obstructed artery is removed, followed by ligation of the mesial and distal end of the removed piece. All these studies found periodontal pathogens in the atheromata. No studies were carried out using distal protection filters in stent implant angioplasty. This method is based on the displacement of fragments of atheromatous plaques that may occur during stent implants. These fragments are then captured by the filter. The small amount of material collected in the filters may have contributed to the lack of detection of periodontal pathogens in some samples.

Many authors (4,5,11) believe that the migration of periodontal bacteria occurs through bacteremic episodes. This allows microorganisms to enter the bloodstream and then be involved with the development or progression of atherosclerosis. Most periodontal bacteria are strictly anaerobic, and among the pathogens targeted herein, only *A. actinomycetemcomitans* is regarded as a facultative bacteria. *Porphyromonas gingivalis*, *T. denticola*, *Synergistetes* bacteria and oral archaea are strictly anaerobic (14), and the presence of viable bacteria in such a very oxygenated arterial environment seems improbable. In one study (2), it was not possible to cultivate viable oral bacteria from atheromatous plaques of carotid or femoral arteries, in spite of detecting DNA of *P. gingivalis* and *P. intermedia*. Even bacteria that can be more tolerant to oxygen (e.g. *A. actinomycetemcomitans* and *C. rectus*) were not observed in plaques either by cultivation or by PCR.

Although no periodontal pathogens were detected in atheromatous plaques in the present study, four samples (13%) were positive for bacterial DNA. This prevalence is lower when compared with other studies. In one study (12), DNA of periodontopathic bacteria was not detected in atheromatous plaques, although 94% of the atheroma samples were found to be positive for the presence of bacterial DNA. Occurrence of bacterial DNA in atheroma samples has been shown in other studies to range from 72 to 100% (2,5). Differences may be related to DNA extraction methods, PCR sensitivity, sample contamination and epidemiological issues. Open-ended molecular methods, such as broad-range PCR followed by cloning and sequencing, are required to identify these nontargeted bacterial species and should be used in future studies.

In the cases positive for bacteria, the species present were not identified; therefore, the possibility exists that other periodontopathic bacteria not targeted here might be related to atherosclerosis. Furthermore, oral and nonoral bacteria, periodontopathic or not, have been associated with atheroma formation. Studies (28,29) have reported the presence of other microorganisms, such as *Chlamydia pneumoniae*, *Helicobacter pylori* and cytomegalovirus, in atheromatous plaques.

Patients who tested positive for bacterial DNA in their atheromatous plaques may have been affected by other chronic or acute infections of the atheromata. According to Rothenbacher *et al.* (30), the cumulative effect of different infectious agents (infection load), and not only persistent infection, may be crucial to the development of atherosclerosis and its complications.

Longitudinal, cohort or case–control studies should be used to determine more precisely the relationship between periodontitis and atherosclerosis. Other cultivable or as-yet-uncultivated periodontal pathogens and nonoral bacteria should be investigated to determine the extent to which treatment of these infections can reduce the deterioration or incidence of atherosclerotic complications.

Conclusions

The periodontal bacteria targeted here were not found in the atheromatous plaques. DNA from other microorganisms, such as archaea and fungi, which can also be involved in oral diseases, including periodontitis, was not detected either. Thus, an association of these pathogens with atheromatous plaques could not be determined. However, the occurrence of bacterial DNA in some atheroma samples confirms the suspected bacterial involvement in some cases of atherosclerosis. Although the focus in this study was placed on selected pathogens, it is possible that other oral and nonoral bacteria may play a role in the development of atheroma.

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