



## Molecular analysis of oral bacteria in dental biofilm and atherosclerotic plaques of patients with vascular disease



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### ABSTRACT

**Background:** Oral bacteria have been detected in atherosclerotic plaques at a variable frequency; however, the connection between oral health and vascular and oral bacterial profiles of patients with vascular disease is not clearly established. The aim of this study was to evaluate the presence of oral bacterial DNA in the mouth and atherosclerotic plaques, in addition to assessing the patients' caries and periodontal disease history.

**Methods:** Thirty samples of supragingival and subgingival plaque, saliva and atherosclerotic plaques of 13 patients with carotid stenosis or aortic aneurysm were evaluated, through real-time polymerase chain reaction, for the presence of *Streptococcus mutans* (SM), *Prevotella intermedia* (PI), *Porphyromonas gingivalis* (PG) and *Treponema denticola* (TD). All patients were submitted to oral examination using the DMFT (decayed, missing and filled teeth) and PSR (Periodontal Screening and Recording) indexes. Histopathological analysis of the atherosclerotic plaques was performed.

**Results:** Most of the patients were edentulous (76.9%). SM, PI, PG and TD were detected in 100.0%, 92.0%, 15.3% and 30.7% of the oral samples, respectively. SM was the most prevalent targeted bacteria in atherosclerotic plaques, detected in 100% of the samples, followed by PI (7.1%). The vascular samples were negative for PG and TD. There was a statistically significant difference ( $p < 0.05$ ) between the presence of PG and TD in the oral cavity and vascular samples.

**Conclusion:** SM was found at a high frequency in oral and vascular samples, even in edentulous patients, and its presence in atherosclerotic plaques suggests the possible involvement of this bacterium in the disease progression.

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### To the Editor:

The connection between periodontal and vascular disease has been investigated, and periodontal microorganisms have been identified in atherosclerotic plaque samples at a variable frequency, which suggests that these bacteria can be related to the etiopathogenesis of atherosclerosis. Few studies have investigated the presence of cariogenic microorganisms, such as *Streptococcus mutans* (*S. mutans*), in vascular tissue,

although these bacteria are capable of invading endothelial cells and stimulating the production of inflammatory markers, in addition to being detected at a high frequency in these lesions [1–3].

Furthermore, detailed evaluation of oral health status of all patients enrolled in the study, that has been performed only by a few authors and in a small group of patients [4], could provide evidence of a connection between oral diseases and the frequency of oral microorganisms in vascular samples. Thus, to evaluate the presence of DNA of cariogenic and periodontopathogenic bacteria in the mouth and in atherosclerotic plaque samples of the same patients, in addition to evaluating their oral health status, 13 patients with carotid stenosis or aortic aneurysm were enrolled in this study.

All patients underwent bedside oral examination, which was performed by two previously calibrated examiners (Kappa values: 0.80 to

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0.97), up to one day before surgery. The indexes DMFT and PSR were used to evaluate caries history and periodontal condition respectively. All participants gave their informed consent, and the study was approved by the Ethics Committee of Hospital Universitário Walter Cantídio of the Federal University of Ceara.

Supragingival and subgingival plaque samples were collected for dentate patients, according to a previously reported protocol [4]. For edentulous patients, saliva samples were collected (Supplemental methods).

A total of 14 atherosclerotic plaques were collected, aseptically, during endarterectomy surgical procedures from the carotid artery or surgery of abdominal or thoracic aortic aneurysm. All oral and vascular samples were stored in a sterile vial containing phosphate-buffered saline (PBS) at  $-20^{\circ}\text{C}$  for further analysis using real-time PCR. Fragments of vascular samples were fixed in 10% formalin for histopathological analysis.

Total DNA was extracted from each sample with a protocol based on the cetyltrimethylammonium bromide method [5]. A total of 30 samples of DNA extracted from saliva, supragingival plaque, subgingival plaque and atherosclerotic plaque were subjected to real-time PCR (Supplemental methods) for the detection of DNA from 4 different bacterial species: *S. mutans*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Treponema denticola*. TaqMan probes to 16S bacterial ribosomal DNA (Supplemental Table 1) were specifically designed for this study (Life Technologies).

Data regarding demographic characteristics, vascular disease, oral health status and medical history of the patients are summarized in Table 1. The mean number of missing teeth was high, and most of the patients included in the present study were edentulous. Edentulous patients were included in the present study because in previous studies, even when these patients were considered to be

part of a control group, samples of atherosclerotic plaques were positive for oral bacteria [2,6]. Furthermore, of the studies that have included edentulous patients, none examined the oral bacterial profile of these patients [2,6].

In the present study, histopathologically, all specimens showed features of severe atherosclerotic lesions (Supplemental results). Although there was no morphologic evidence of bacterial colonization in the atherosclerotic plaques, all samples were positive for at least one-studied bacteria. In addition, at least two investigated bacteria were detected in all oral samples, although the majority of the patients were edentulous.

The presence of oral bacteria in atherosclerotic plaques of edentulous patients may result from recurrent bacteremia that occurred while the patient still had teeth, considering that the process of atherosclerotic plaque formation is a long-term process that can begin in childhood [6]. Another hypothesis is that these bacteria could enter the bloodstream through ulcers or epithelial lesions present in the oral cavity of edentulous patients, adhering to existing atherosclerotic lesions [6].

*S. mutans* was identified in all oral samples (100.0%) and atherosclerotic plaques (100.0%) (Supplemental Table 2), which suggests that this bacterium may have been originated from the oral cavity and most likely reached atherosclerotic plaques through bacteremia. The detection of *S. mutans* at a high frequency in atherosclerotic plaques may be correlated with a high average of lost teeth.

The role of *S. mutans* in atherogenesis has been investigated. Several in vitro studies have shown that *S. mutans* has the ability to adhere to collagen type 1 [7], induce platelet aggregation [8], invade human endothelial cells [9] and induce increased production of interleukin (IL) 1, IL-6, monocyte chemoattractant protein 1 (MCP-1) and foamy macrophages, which are strongly associated with the pathogenesis of atherosclerosis [9]. Studies using animal models observed that an infection with the invasive strain of *S. mutans* OMZ175 accelerates the development of atherosclerotic plaques and increases the inflammatory response in an ApoE-null mouse when compared to the control without *S. mutans* infection [10]. These results suggest that invasive strains of *S. mutans* may be related to vascular disease in humans, possibly contributing to the progression of atherosclerotic lesions.

In the present study, *P. intermedia* was detected in the oral samples of 12 patients (92.0%). Only one patient (7.1%) presented positivity for this bacterium in the vascular sample, but not in the oral sample. This fact supports the theory that this microorganism may have colonized the atherosclerotic plaque during previous bacteremia while the patient still had teeth.

Two patients (15.3%) presented positive oral samples for *P. gingivalis*, and 4 patients (30.7%) for *T. denticola*. However, these bacteria were not identified in vascular samples. Regarding the frequency of bacteria in atherosclerotic plaques, *S. mutans* presented a higher prevalence ( $p < 0.05$ ) compared to the other bacteria (Fisher/ $X^2$  exact test with Bonferroni correction) (Supplemental Fig. 1). There was a statistically significant difference regarding the presence of *P. gingivalis* and *T. denticola* in the oral cavity compared to the vascular sample ( $p < 0.05$ ) (Fisher/ $X^2$  exact test with Bonferroni correction) (Fig. 1), suggesting that these bacteria have greater difficulty in entering the bloodstream when compared to *S. mutans*.

The oral and vascular bacterial profiles of the patients in the present study were distinct because only *S. mutans* and *P. intermedia* were detected in atherosclerotic plaques. *S. mutans* was found at a high frequency in vascular and oral samples, even in edentulous patients, and its presence in atherosclerotic plaques suggests a possible involvement of this pathogen in the disease progression. Therefore, routine dental appointments are important for dentate and edentulous patients, whether they are cardiac patients or patients at risk for atherosclerotic disease, to maintain oral health and to reduce recurrent episodes of bacteremia.

During the execution of the present study, the authors found difficulty in obtaining the samples, considering that there are currently

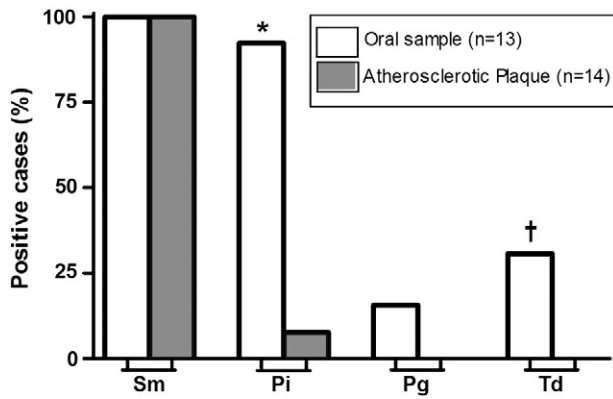
**Table 1**

Demographic, clinic and dental characteristics of patients with carotid stenosis and aortic aneurysm. Fortaleza — CE, Brazil, 2013.

Variables	Samples
Demographic	13 patients
Age	68.5 ± 10.1 (45–82)
Sex	
Male	6 (46.1)
Female	7 (53.9)
Clinical diagnosis	14 atherosclerotic plaques
Carotid stenosis	8 (57.1)
Aortic aneurysm	6 (42.9)
Medical comorbidities	13 patients <sup>a</sup>
Hypertension	9 (69.2)
Dyslipidemia	8 (61.5)
Diabetes	2 (15.4)
Other	9 (69.2)
None	3 (23.1)
Smoking history	13 patients
Smoker	1 (7.7)
Ex-smoker	6 (46.2)
Never smoked	6 (46.2)
Dental profile	13 patients/78 sextants
DMFT	31.5 ± 1.4 (27–32)
Healthy teeth	7 (1.7)
Decayed teeth	7 (1.7)
Filled teeth	2 (0.5)
Missing teeth	400 (96.1)
PSR	
Health sextants	0 (0)
Bleeding sextants	0 (0)
Sextants with dental calculus	1 (1.3)
Sextants with periodontal pockets 4–5 mm	1 (1.3)
Sextants with periodontal pockets >6 mm	0 (0)
Excluded sextants (≤1 dente)	76 (97.4)

- DMFT, decayed, missing, filled teeth; PSR, Periodontal Screening and Recording. Quantitative data expressed by "Mean ± SD (minimum–maximum)". Qualitative data expressed by "n (%)".

<sup>a</sup> Comorbidities may be associated (n<sub>comorbidities</sub> = 31).



**Fig. 1.** Percentage distribution of periodontopathic and cariogenic bacteria in oral samples (saliva, supragingival plaque and subgingival plaque) and atherosclerotic plaques. \*Statistically significant difference ( $p < 0.05$ ) between the frequency of Pi in oral samples and in atherosclerotic plaque samples. †Statistically significant difference ( $p < 0.05$ ) between the frequency of Td in oral samples and in atherosclerotic plaque samples (Fisher exact test or  $\chi^2$  test with Bonferroni correction). Sm, *Streptococcus mutans*; Pi, *Prevotella intermedia*; Pg, *Porphyromonas gingivalis*; Td, *Treponema denticola*.

less invasive and more widely used methods for treating carotid stenosis than endarterectomy, which was a study limitation.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ijcard.2014.04.201>.

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