



Research Brief

Molecular analysis shows the presence of periodontal bacterial DNA in atherosclerotic plaques from patients with coronary artery disease



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ABSTRACT

Links between periodontitis and atherosclerosis can be predicted based on inflammatory mechanisms initiated by bacteria associated with periodontal lesions, which then influence the initiation or propagation of the atherosclerotic lesion. This study aimed to detect the presence of three periodontal pathogens, in atheromatous plaques of patients with coronary artery disease. Subgingival and atherosclerotic plaque samples were obtained from 80 patients scheduled for CABG or angioplasty. A nested PCR was done for the detection of the pathogens in the plaque samples. *Porphyromonas gingivalis*, *Tanarella forsythia*, and *Treponema denticola* were detected in 10%, 12.5%, and 1.3% of the atherosclerotic plaque samples respectively. It was also observed that patients whose atherosclerotic plaques tested positive for one or more of the pathogens had chronic periodontitis.

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1. Introduction

The surfaces lining the oral cavity are coated with a plethora of bacteria, some of which have been implicated in oral diseases like chronic periodontitis (CP) and caries.¹ CP is a bio-film induced chronic inflammatory disease affecting the tooth-supporting tissues and resulting in progressive loss of attachment and bone loss.² Healthy gingiva, limit the colonization by periodontal pathogens and trigger a well-defined immune response.³ This delicately balanced host-microbe interaction changes upon colonization with keystone pathogens like *Porphyromonas gingivalis* which have been reported to dysregulate the host immune response leading to an inflammatory condition by remodeling a normal, benign oral microbiota into a dysbiotic one.⁴ Periodontal pathogens like *P. gingivalis*, *Treponema denticola*, and *Tanarella forsythia* together constitute the red complex and has been frequently isolated in

patients with CP.⁵ They evade the human immune system by expressing proteases that help them in avoiding complement-dependent phagocytosis.⁶ It has been hypothesized that CP could lead to persistent low-level bacteremia, and bacterial invasion of endothelial cells could further cause endothelial dysfunction triggering a pro-inflammatory and a pro-atherogenic response. Increased levels of pro-inflammatory cytokines and chemokines induced by periodontal pathogens could in turn activate vascular endothelial cells and trigger foam cell formation which is an early stage in atherogenesis.⁷

Atherosclerosis is a progressive cardiovascular disease characterized by the thickening of the arteries. It is believed to be mediated by inflammatory events, which include infiltration of the blood vessel wall by activated monocytes and mature macrophages.⁸ Patients with CP have been found to have elevated levels of systemic inflammatory markers like C-reactive protein and Platelet-activating factor which are known to be associated with increased cardiovascular risk.⁹

We surmise that the invasion of endothelial cells by periodontal pathogens has a definitive role in the development of atherosclerosis. Their identification in the atherosclerotic plaques in our study

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would lead to a better understanding of the underlying mechanisms involved in the development of infectious atherosclerosis and add to the growing evidence¹⁰ implicating periodontitis as an independent risk factor for coronary artery disease (CAD).

2. Methods

A total of 80 patients between the age of 35–76 who were scheduled to undergo either Coronary artery bypass grafting (CABG) or angioplasty for coronary artery disease and consented to the study were included. Patients with less than 12 teeth or those having undergone periodontal treatment during the last six months were excluded. The research protocol was according to the ethical guidelines and approved by the Central Ethics Committee of the University (NU/CEC/2017-2018/0121, NU/CEC/2017-2019/0213).

A full mouth oral examination was done by a Periodontist and data on the number of teeth present, gingival index (GI), probing depth (PD), and clinical attachment loss (CAL) was recorded. PD and CAL were recorded at six sites per tooth excluding the last molars. Based on the number of sites involved CP was further classified into localized and generalized CP. Subgingival plaque samples were then collected from the site with the deepest PD in each quadrant using sterile paper points. Atherosclerotic plaque samples were taken from the ascending aortic artery (0.5–1 mm) during the CABG procedure. In patients undergoing angioplasty, the distal part of the balloon catheter was cut and atherosclerotic samples were retrieved from these catheters. Only angioplasty balloons used for pre-dilatation were sampled. All plaque samples were transferred to sterile micro-centrifuge tubes with Tris-EDTA buffer and stored at - 20 °C.

2.1. Microbiological analysis

Genomic DNA was extracted from the plaque samples using the QIAamp DNA Mini Kit (Qiagen, Germany). The atherosclerotic samples obtained through surgical excision were homogenized before DNA extraction. PCR amplification of the eubacterial 16S rDNA was carried out in a 30 µl master mix using universal primers as per the protocol described by Ashimoto et al.¹¹ Amplification was done using 4 µl of the extracted DNA as a template in a thermocycler. The second step Nested PCR was carried out using specific primers. PCR products were electrophoresed in a 2% agarose gel, stained with ethidium bromide (0.5 µg/mL), and analyzed using a gel documentation system (Gel Doc XR+, BioRad, USA).

The PCR products of representative samples were sequenced (Eurofins Genomics India Pvt. Ltd. India) and bioinformatics analysis was carried out using the BLAST tool. The generated sequences were submitted to NCBI GenBank (MG457721, MG457718, MN847610, MT509413, MT509412, MN847612).

3. Results

Subgingival and atherosclerotic plaques of 80 patients (56 males and 24 females) with a mean age of 58.29 ± 9.7 years were analyzed. The mean number of teeth present was 25.46 ± 5.8 with a mean PD of 1.65 ± 0.2. The mean GI score was 1.218 ± 0.325 based on which 31.2% had mild, 67.5% had moderate and 1.3% patients had a clinically severe degree of gingivitis respectively. The mean CAL was 0.611 ± 0.52 and 23 patients had a CAL of more than 5. Overall 10 patients were current smokers and the remaining were either non-smokers (58) or former smokers (12).

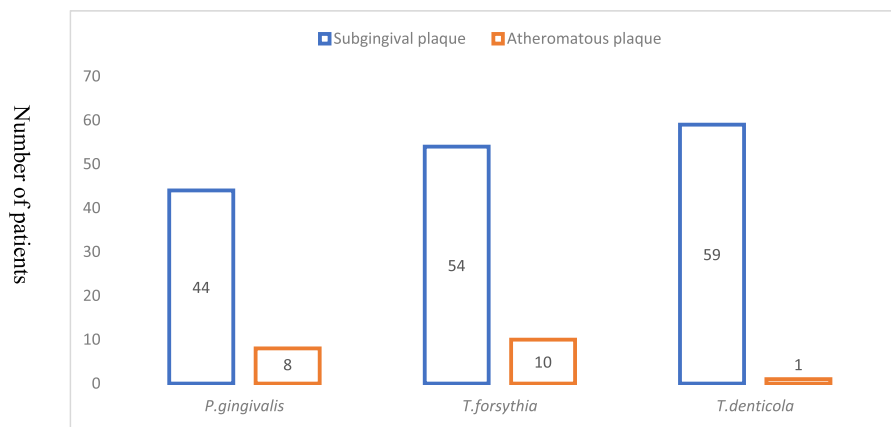
The prevalence for *P. gingivalis*, *T.forsythia*, and *T.denticola* in the subgingival plaques and atherosclerotic plaques has been shown in Fig. 1. Chi-square test showed a significant association for the presence of *P.gingivalis* (p = 0.007) and *T.forsythia* (0.001) and found no association for the presence of *T.denticola* (p = 1.00) in the subgingival and atherosclerotic plaques.

We found a statistically significant difference (p = 0.027) between the two procedures (CABG and balloon catheters) that were used for the collection of atherosclerotic plaques. The detection rate for the periodontal pathogens was higher when atheromatous samples were collected from balloon catheters.

4. Discussion

We attempted to examine the role of the red-complex bacteria in the development of atheromas. *P.gingivalis* in particular has been the focus of several studies that have pointed to its etiopathogenic role in the development of atheromatous lesions. Its fimbriae are reported to be involved in the attachment and invasion of endothelial cells; however, it has also been observed that mutant strains lacking major fimbriae fail to invade endothelial cells.¹² This differential virulence attribute was not ascertained in our study and could account for its lowered detection in the atherosclerotic plaque samples of our study.

The detection rates for the three pathogens based on the periodontal status have been shown in Table 1. We observed that patients whose atheromatous plaques tested positive for one or more



Frequency of pathogens detected by PCR in the subgingival and atherosclerotic plaques

Fig. 1. Frequency of pathogens detected by PCR in the subgingival and atherosclerotic plaques.

Table 1
Frequency of detection of pathogens based on the periodontal disease severity.

Periodontal status	n = 80	<i>Pg</i>	<i>Tf</i>	<i>Td</i>
Generalized CP	13			
Subgingival		9	11	13
Atheromatous		4	4	0
Localized CP	61			
Subgingival		35	41	42
Atheromatous		4	6	1
Gingivitis	5			
Subgingival		0	2	3
Atheromatous		0	0	0
Healthy Periodontium	1			
Subgingival		0	0	1
Atheromatous		0	0	0

Pg- *P.gingivalis*, *Tf*-*T.forsythia*, *Td*-*Treponema denticola*.
“n” denotes number of patients, CP-chronic Periodontitis.

periodontal pathogens had either localized or generalized CP. Only 21.3% of the patients had deep pockets which could have contributed to a lowered detection rate of the pathogens in the atherosclerotic plaques as it has been reported that patients suffering from severe forms of periodontitis have a higher concentration of *P.gingivalis* in periodontal pockets than patients presenting with moderate forms of the disease.¹³ Deep periodontal pockets provide a suitable environment for the growth of anaerobes.¹⁴

Studies to date have shown variations in the detection levels of putative periodontal pathogens in atherosclerotic plaques which could be attributed to different methodologies used in the collection of the plaque samples, lack of uniformity in defining CP, ethnic differences in the populations, and the type of PCR used as the sensitivity of the test varies between the different generations of the PCR.^{15,10} In this study the detection rate for the periodontal pathogens was higher when atheromatous samples were collected from balloon catheters and hence this could be a preferred method in the future.

The limitations of this study were that we examined only three known periodontal pathogens it may be possible that there may be other known and unknown bacteria that may play a role in atherogenesis. A complete characterization of all oral and atherosclerotic plaque samples utilizing the next-generation sequencing would have given us better insights into the role of oral pathogens in the development and progression of atherosclerotic plaques.

5. Conclusion

We observed that patients whose atherosclerotic plaques tested positive for the pathogens had chronic periodontitis. These findings further provide the much-needed local evidence that patients with CVD should be advised that effective periodontal therapy may have a positive impact on cardiovascular health.

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Declaration of competing interest

Nothing to declare.

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