Periopathogens in atherosclerotic plaques of patients with both cardiovascular disease and chronic periodontitis

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Abstract

BACKGROUND: Atherosclerosis and periodontitis are both chronic inflammatory diseases. Although a strong relationship between the two has already been established, the underlying mechanism is unknown. The present study was conducted aiming to detect the deoxyribonucleic acid (DNA) of Aggregatibacter actinomycetemcomitans (A.a), Campylobacter rectus (C.r), and Porphyromonas gingivalis (P.g) in subgingival and atherosclerotic plaques of patients with both chronic periodontitis and cardiovascular disease (CVD).

METHODS: In this cross sectional study, patients with coronary artery disease (CAD) and moderate to severe periodontitis which were scheduled for coronary artery bypass grafting (CABG) were enrolled in the study. The subgingival plaques were collected before surgery. All samples were examined for the detection of selected periopathogens using polymerase chain reaction (PCR).

RESULTS: The subgingival and atherosclerotic plaque samples of 23 patients were examined. The DNA of P.g, A.a, and C.r were found to be positive in 43.47%, 43.47%, and 78.26% of subgingival plaques, and 13.04%, 17.39%, and 8.69% of atherosclerotic plaques, respectively. In all cases, the bacterial species found in atherosclerotic plaques were also found in the subgingival plaques of the same patient.

CONCLUSION: This study demonstrated the presence of periopathogens in atherosclerotic plaques of patients with chronic periodontitis. More studies are required to ascertain the exact role of these periopathogens in atherosclerotic plaque formation.

Keywords: Atherosclerosis, Coronary Artery Disease, Chronic Periodontitis, Porphyromonas Gingivalis, Aggregatibacter Actinomycetemcomitans, Campylobacter Rectus

Introduction

Gingiva embraces teeth in a collar-like fashion. There is a space between the tooth and gingival tissue termed gingival sulcus, which is lined by sulcular epithelium.1 Periodontitis is a chronic inflammatory disease in which microbial plaque is a causative factor. In periodontitis, the gingival sulcus is deepened and periodontal pocket is formed. The periodontal pocket is a preferable site for colonization of periopathogens. Bacterial challenge in this site aggravates the inflammatory process.2 Due to inflammation, the inner wall of the periodontal pocket will usually become ulcerated. In this situation, the impeding role of the inner epithelium against systemic circulation is disrupted and microorganisms and their products and inflammatory mediators can enter the well-vascularized periodontal tissues and subsequently into the circulation.3 Through the circulatory system, periodontal bacteria can reach distant organs like vascular cells.2

Atherosclerosis is a progressive chronic inflammatory process of the vessels, which may cause an increase in arterial wall thickness.4 To prevent this condition, understanding the underlying pathomechanisms is vital.5 The risk

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Periodontal pathogens are associated with atherosclerosis and prognosis of coronary artery disease (CAD). Some studies investigated the deoxyribonucleic acid (DNA) of various periodontal pathogens in arterial atheromatous plaques removed in carotid endarterectomy (CEA), however, there is a significant inconsistency of available data.

Apart from them, some studies mentioned that infectious pathogens are associated with atherosclerosis and prognosis of coronary artery disease (CAD). Some studies investigated the deoxyribonucleic acid (DNA) of various periodontal pathogens in arterial atheromatous plaques removed in carotid endarterectomy (CEA), however, there is a significant inconsistency of available data.

Considering the high incidence of periodontal and cardiovascular disease (CVD) worldwide, the present study was carried with the aim of assessing the presence of periopathogens [Porphyromonas gingivalis (P.g), Aggregatibacter actinomycetemcomitans (A.a) and Campylobacter rectus (C.r)] using polymerase chain reaction (PCR) in atherosclerotic plaques and subgingival plaques among patients with chronic periodontitis and CVD undergoing coronary artery bypass graft (CABG) in Isfahan, Iran.

Materials and Methods

This cross sectional study was performed in accordance with the World Medical Association (WMA) Declaration of Helsinki and subsequent revisions and was approved by the ethics committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran, with the code P/17/1/21952. Written informed consents were obtained from patients before entering the study and after fully explaining the protocol. The patients admitted to Isfahan Shahid Chamran hospital from January to May 2013 and scheduled for CABG due to CAD were selected. Periodontal conditions of dentate patients (at least having 10 teeth) were examined and patients with signs of periodontal inflammation and at least two teeth with periodontal pocket ≥ 5 mm were enrolled in the study. The exclusion criteria comprised of history of periodontal treatments in the previous 6 months, suffering from other major systemic diseases including malignancies and antibiotic consumption. At least, 23 patients were enrolled in the study.

Subgingival plaque samples were collected one day prior to the CABG surgery. To perform this, selected teeth were carefully dried using sterile cotton swaps. Following supragingival plaque removal, subgingival plaques were obtained using a curette from two sites with the greatest pocket depth. The samples were placed in Stuart transport medium and sent to the laboratory for microbiologic analysis.

The surgeon provided a biopsy from the coronary atherosclerotic plaque during the CABG procedure. A 0.5-1 mm tissue from the periphery of the coronary plaque was resected during arteriotomy. The samples were then soaked in saline with sulfate buffer in order to eliminate blood contamination and were then placed in Stuart transport medium. The atherosclerotic plaques were homogenized before the PCR procedure. In all cases, the atherosclerotic plaque and the subgingival plaque were obtained from the same patient.

Following DNA isolation, PCR was performed on all atherosclerotic and subgingival plaque samples and the products were then sequenced for further analysis as described in the study by Mahendra et al. The primers utilized were described in table 1. The PCR protocol for all microorganisms included the following: 100 ng of DNA template of sample was added to 50 µl of working stock reaction mixture (containing 10 mM of PCR buffer, 1.25 unit of Taq DNA polymerase, 0.2 mM of each deoxyribonucleotides (dNTPs), primers, 0.3 mM and Mgcl₂ 1.5 mM). Biometra Thermocycler was used to perform PCR which comprised of initial denaturation (95 °C for 3 min) stage followed by 35 cycles of denaturation (94 °C for 30 s), annealing (60 °C for 1 min), extension (72 °C for 1 min) with a final extension of 72 °C for 5 min. After amplification, 10 µl of PCR product was subjected to electrophoresis in a 1% agarose gel containing 0.5 mg/ml ethidium bromide in 1x Tris-Borate electrophoresis buffer (TBE).

Table 1. Bacterial target and primer sequences used in the polymerase chain reaction (PCR) detection

| Bacteria                          | Primers                                           |
|-----------------------------------|---------------------------------------------------|
| Aggregatibacter actinomycetemcomitans | Forward primer: CTT ACC TAC TCT TGA CAT CCG AA  |
|                                   | Reverse primer: ATG CAG CAC CTG TCT CAA AGC      |
| Porphyromonas gingivalis          | Forward primer: AGG CAG CTT GCC ATA CTG C         |
|                                   | Reverse primer: ACT CTT AGC AAC TAC CGA TGT      |
| Campylobacter rectus              | Forward primer: TTT CCG AGC GTA AAC TCC TTT TC   |
|                                   | Reverse primer: TTT CTG CAA GCA GAC ACT CTT      |

Periodontal pathogens are associated with atherosclerosis and prognosis of coronary artery disease (CAD). Some studies investigated the deoxyribonucleic acid (DNA) of various periodontal pathogens in arterial atheromatous plaques removed in carotid endarterectomy (CEA), however, there is a significant inconsistency of available data.
Table 2. Data on number, age, and gender of participants and the presence of periopathogens in subgingival and atherosclerotic plaques

| No | Age (year) | Gender (M/W) | Subgingival plaque P.g | A.a | C.r | Atherosclerotic plaque P.g | A.a | C.r |
|----|------------|--------------|------------------------|-----|-----|----------------------------|-----|-----|------------------------|-----|-----|
| 1  | 60         | M            | -                      | -   | +   | -                          | -   | -   | -                      | -   | -   |
| 2  | 71         | W            | -                      | -   | +   | -                          | -   | -   | -                      | -   | -   |
| 3  | 59         | M            | -                      | -   | +   | -                          | -   | -   | -                      | -   | -   |
| 4  | 64         | M            | -                      | -   | +   | -                          | -   | -   | -                      | -   | -   |
| 5  | 60         | M            | +                      | +   | +   | +                          | +   | +   | +                      | -   | -   |
| 6  | 60         | M            | -                      | +   | +   | -                          | -   | -   | -                      | -   | -   |
| 7  | 67         | M            | -                      | +   | -   | -                          | -   | -   | -                      | -   | -   |
| 8  | 60         | M            | +                      | -   | +   | -                          | -   | -   | -                      | -   | -   |
| 9  | 58         | M            | -                      | -   | +   | -                          | -   | -   | -                      | -   | -   |
| 10 | 57         | W            | +                      | -   | +   | -                          | -   | -   | -                      | -   | -   |
| 11 | 63         | M            | -                      | -   | +   | -                          | -   | -   | -                      | -   | -   |
| 12 | 61         | M            | +                      | -   | -   | -                          | -   | -   | -                      | -   | -   |
| 13 | 58         | M            | -                      | -   | +   | -                          | -   | -   | -                      | -   | -   |
| 14 | 63         | M            | +                      | -   | -   | -                          | -   | -   | -                      | -   | -   |
| 15 | 64         | M            | -                      | +   | +   | -                          | -   | -   | -                      | -   | -   |
| 16 | 59         | M            | -                      | -   | +   | -                          | -   | -   | -                      | -   | -   |
| 17 | 53         | W            | +                      | -   | -   | -                          | -   | -   | -                      | -   | -   |
| 18 | 65         | M            | +                      | -   | +   | -                          | -   | -   | -                      | -   | -   |
| 19 | 65         | M            | -                      | +   | +   | -                          | -   | -   | -                      | -   | -   |
| 20 | 61         | M            | +                      | +   | -   | +                          | +   | -   | -                      | -   | -   |
| 21 | 58         | M            | +                      | +   | +   | -                          | -   | -   | -                      | -   | -   |
| 22 | 60         | M            | +                      | +   | +   | -                          | -   | -   | -                      | -   | -   |
| 23 | 59         | M            | +                      | +   | +   | +                          | +   | +   | +                      | -   | -   |

M: Man; W: Woman; P.g: Porphyromonas gingivalis; A.a: Aggregatibacter actinomycetemcomitans; C.r: Campylobacter rectus

Results

A total of 23 patients including 20 men and 3 women with mean age of 61.00 ± 4.01 years participated in this study. Totally, 23 atherosclerotic plaque samples and 23 subgingival plaque samples were examined and compared for the incidence rate of three periopathogens (P.g, A.a, C.r). All 23 (100%) subgingival plaque samples and only 4 (17.39%) atherosclerotic plaque samples tested were positive for at least one periopathogen. Table 2 shows the rate of periopathogens in subgingival and atherosclerotic plaques of each patients. The DNA of P.g was found positive in 10 subgingival samples (43.47%), from which 3 (13.04%) atherosclerotic samples were positive as well. The DNA of A.a was found in 10 (43.47%) and 4 (17.39%) subgingival and atherosclerotic plaque samples, respectively. In addition, the DNA of C.r was positive in 18 (78.26%) and 2 (8.69%) subgingival and atherosclerotic samples, respectively. McNemar analysis showed the significant differences between subgingival plaque and atherosclerotic samples for all the three microorganisms (P = 0.016, P = 0.031, and P < 0.001 for P.g, A.a, and C.r, respectively).

In overall, 2 patients tested were positive for all the three bacteria and one patient had P.g and A.a in both atherosclerotic and subgingival samples. All patients who tested positive for a bacterium in the atherosclerotic plaque sample also had the same bacterium in the subgingival plaque sample, although, the presence of a bacterium in subgingival plaque was not necessarily associated with its presence in the atherosclerotic plaque sample.

Discussion

Several epidemiologic studies have reported a statistical correlation between periodontitis and CAD. Microorganisms have been proposed to contribute to CVD by direct and indirect mechanisms. Direct invasion of microorganisms to arterial wall or atherosclerotic plaques was assessed with evaluation of bacterial DNA. Microorganisms can produce infectious agents and also exacerbate and maintain inflammation and hence affect CVDs indirectly. Studies aiming at detecting DNA of periopathogens in atherosclerotic and subgingival plaques have yielded different results. Totally, in the present study, 23 subgingival and 23 atherosclerotic plaques have yielded different results. Totally, in the present study, 23 subgingival and 23 atherosclerotic plaques have yielded different results.
plaque species, host immune responses, study populations, and sampling volume techniques. Szulc et al. concluded that the method of sample collection may affect the results and isolation of microorganisms, and sampling the atherosclerotic plaque is more predictable compared to using paper points which contact with atherosclerotic plaque.

Limitation to this study was the rather small sample size which could be addressed in future studies.

**Conclusion**

In conclusion, this study demonstrates the presence of periodontopathogens in atherosclerotic plaques of patients with chronic periodontitis. These periodontopathogens may contribute to the pathogenesis of atherosclerosis directly or indirectly. Further studies are required to ascertain the underlying mechanism of these periodontopathogens in the atherosclerotic process.

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**Conflict of Interests**

Authors have no conflict of interests.

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