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ORIGINAL ARTICLE

Detection of Porphyromonas gingivalis (fimA) In Coronary Plaque

MAHENDRA J* , MAHENDRA L , KURIAN V M***, JAISHANKAR K******

ABSTRACT

Background: Recent studies indicate that chronic bacterial infections are an important risk factor for coronary artery disease. The inflammation initiated by bacteria and their components might be the common causal factor in the progression of atherosclerosis. The ability of oral pathogens to colonize in the coronary atherosclerotic plaque is well known.

Aims: The aim of this study was to detect the presence of the periodontal *oral pathogens;* namely, *Porphyromonas gingivalis (fimA) gene, Porphyromonas gingivalis, Tannerella forsythia and Prevotella nigrescens* in the atherosclerotic plaque samples of the patients undergoing Coronary Artery Bypass Grafting.

Settings and Design : The study population consisted of 51 patients with chronic periodontal infection in the age group of 40 to 80 years and were recruited consecutively from the institute of Cardiovascular disease, Madras Medical Mission, Chennai. These patients were suffering from Coronary Artery Disease and were undergoing Coronary Artery Bypass Grafting.

Methods and Materials: DNA was extracted from the subgingival plaque and from the coronary atherosclerotic plaque samples from the same patients. Polymerase chain reaction was used to amplify the part of the 16S rRNA gene to detect the presence of the above microorganisms.

Statistical Analysis: Kappa Measures of Agreement, Percentage Prevalence

Results: Bacterial DNA was detected in all the samples of subgingival and atherosclerotic plaques. *Tannerella forsythia, Porphyromonas gingivalis, Porphyromonas gingivalis (fimA) gene and Prevotella nigrescens* were detected in 31.4%, 45.1%, 39.2% and 21.6% of atherosclerotic plaque samples. In both subgingival plaque and coronary atherosclerotic plaque samples, *T. forsythia was detected in* 19.6%, *P.gingivalis in* 39.2%, *P.gingivalis (fimA) gene in* 33.3% *and P. nigrescens in 15.7%* of the samples. 11.8 % of *T.forsythia, 5.9% of P.gingivalis, 5.9% of the P.gingivalis (fimA) gene and 5.9 % of P.nigrescens* were found only in coronary plaques without the presence of these microorganisms in subgingival plaques.

Conclusion: Our study confirmed the detection of the DNA of the above microorganisms in coronary plaque samples. This may explain that the inflammation in the oral cavity triggers and influences the atherosclerotic process.

Key Words: 1. Atherosclerosis, 2. Periodontitis, 3. Polymerase Chain Reaction, 4. Inflammation and Coronary Heart Disease

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Introduction

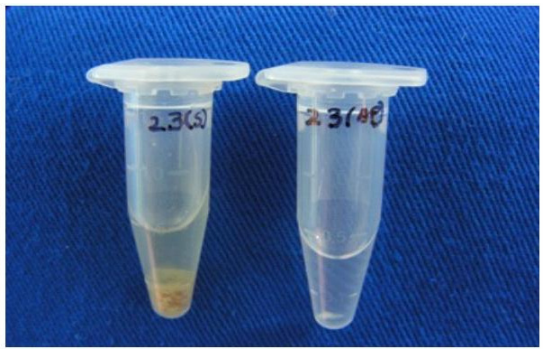
The recent data suggest that oral bacteria are implicated in the aetiology of atherosclerosis [1],[2] Associations have since been reported between atherosclerosis and chronic infections with Helicobacter pylori, herpes viruses and chronic dental infection [3]. Porphyromonas gingivalis fimbriae (fimA) is one of the major cell surface component of the Porphyromonas gingivalis which plays a major role in the colonization and progression of periodontal disease [4],[5]. The aim of the study was to identify the detection of *Porphyromonas gingivalis (fimA), Porphyromonas gingivalis, Tanerella forsythia and Prevotella nigrescens* in the atherosclerotic plaque of the patients with Coronary Artery Disease (CAD) by using Polymerase Chain Reaction (PCR).

Materials and Methods

Study Population

Out of the 150 patients recruited, 51 patients (11 females and 40 males) with chronic periodontitis in the age group of 40 to 80 years, who met the inclusion criteria, were selected for the study. These patients had Coronary Artery Disease (CAD), with no other systemic complication. The subjects were scheduled to undergo Coronary Artery Bypass Grafting (CABG). The exclusion criteria included major systemic illness, advanced malignancy, antibiotic intake and periodontal treatment in the previous 6 months.

The medical and dental history of each subject was obtained by an interview. Patients fulfilling the inclusion criteria were informed about the study and consent was obtained from them. The study was approved by the ethics committee of Madras Medical Mission.



(Table/Fig 1)Samples from the subgingival and atheromatous plaques

Method of Collection

Subgingival plaque sample

The samples were taken the day before the patients underwent the CABG. The deepest periodontal sites with probing pocket depth ≥ 5 mm were selected for the microbial sampling. The teeth were gently dried with sterile cotton swabs. After the removal of supragingival plaque, the subgingival plaque samples were obtained with the help of curette from the two deepest periodontitis sites and were pooled for analysis.

Atherosclerotic plaque sample

A biopsy was obtained from the coronary atherosclerotic plaque during the CABG. The surgeon excised one or two small bits of plaque (0.5 to 1 mm) from the edge of the coronary arteriotomy which was performed for anastomising the graft. The DNA extraction of both the subgingival plaque and coronary atherosclerotic plaque samples was undertaken as per Saiki et al, 1988 [6].

PCR Amplification

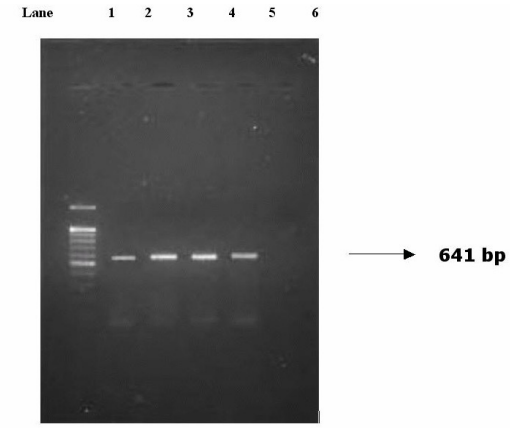
16S rRNA PCR amplification was carried out to detect the presence of the microorganisms. PCR primers were designed as per Larsen *et al*, (1993)[7] [Table/Fig 2].

Microorganisms	Product Size
Tannerella forsythia GCG TAT GTA ACC TGC CCG CA TGC TTC AGT GTC AGT TAT ACC	120-760 (641)bp
Porphyromonas gingivalis AGG CAG CTT GCC ATA CTG C ACT GTT AGC AAC TAC CGA TGT	729-1,132 (404)bp
Porphyromonas gingivalis(fimA gene) 5'-ATA ATG GAG AAC AGC AGG AA-3' (sense), 5'-TCT TGC CAA CCA GTT CCA TTG C-3' (antisense)	131bp
Prevotella nigrescens ATG AAA CAA AGG TTT TCC GGT AAG CCC ACG TCT CTG TGG GCT GCG	219-1,022 (804)bp
Ubiquitous primer (universal primer for 16s RNA of bacterial species) GAT TAG ATA CCC TGG TAG TCC AC CCC GGG AAC GTA TTC ACC G	602 bp

(Table/Fig 2) PCR Primers

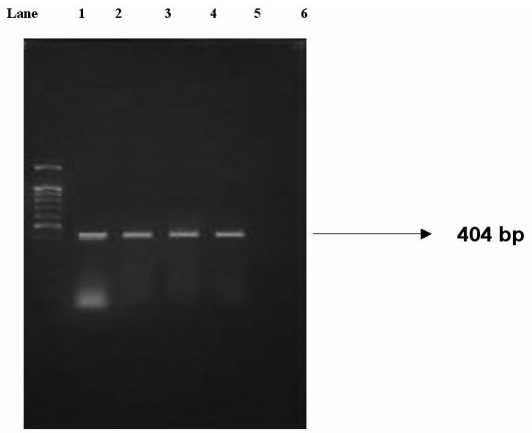
PCR was performed as described by *Saiki et al (1988)* [6]. 10 µl of the DNA template of the sample was added to 40 µl of the working stock reaction mixture containing 5 µl of 10 x PCR buffer, 1.25 unit of Taq DNA Polymerase (0.4 µl), 0.2 mM (1µl) of each deoxyribonucleotides (dNTP's), Primers (1 µl) forward and (1 µl) reverse of the specific microorganisms and 31.6 µl of milli Q water. The PCR reaction was carried out using a PCR thermocycler from Applied Bio-systems, (USA). The PCR temperature profile for *Tannerella forsythia and Porphyromonas gingivalis* included an initial denaturation of 95 ° C for 2 minutes, followed by 36 cycles of the denaturation step at 95 ° C for 30 seconds, the annealing step at 60 ° C for 1 minute, extension at 72 ° C for 1 minute and the final step at 72 ° C for 2 minutes.[7] The temperature profile for *Porphyromonas gingivalis(fimA gene) (*WUYan-min *et al 2000)*[8] included initial denaturation at 94° C for 5 minutes, followed by 35 cycles of denaturation step at 94° C for 1 minute, annealing at 50° C for 1 minute, extension at 72° C for 1.5 minutes and the final step at 72° C for 7 minutes. The temperature profile for *Prevotella nigrescens* included initial denaturation at 95 ° C for 2 minutes, followed by 36 cycles, denaturation at 94 ° C for 30 seconds, annealing at 55 ° C for 1 minute, extension at 72 ° C for 2 minutes and the final step at 72 ° C for 10 minutes [9].

After amplification, 10µl of the aliquot of the amplified PCR product was subjected to electrophoresis in a 0.75 % agarose gel containing 0.5 µg/mL^{-1} Ethidium bromide in 1XTAE buffer. The gel was photographed under 300 nm by using an ultraviolet light trans-illuminator. The PCR amplified products were sequenced in an automated sequencer (Genetic analyzer 3130, *Applied Bio Systems, U.S.A.*). The sequencer data was blasted with available data in the Gen Bank and were compared for possible homologies.



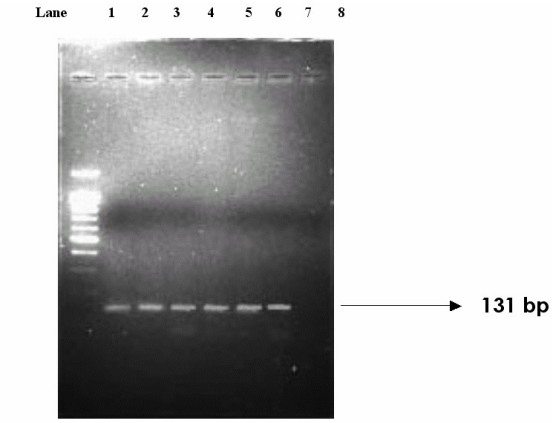
Lane 1 : DNA ladder – 100 bp
Lane 2 and 4 : Subgingival plaque samples
Lane 3 and 5 : Coronary artery plaque samples
Lane 6 : Negative control

(Table/Fig 3) 16S rRNA-based PCR detection of T.forsythia,



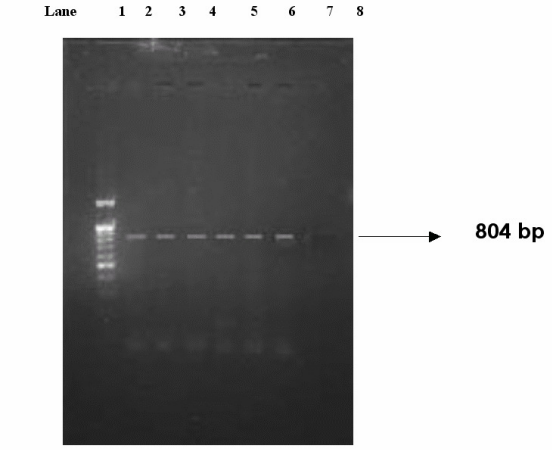
Lane 1 : DNA ladder – 100 bp
Lane 2 and 4 : Subgingival plaque samples
Lane 3 and 5 : Coronary artery plaque samples
Lane 6 : Negative control

(Table/Fig 4) 16S rRNA-based PCR detection of *P.gingivalis*



Lane 1 : DNA ladder – 100 bp
Lane 2, 4 and 6 : Subgingival plaque samples
Lane 3, 5 and 7 : Coronary artery plaque samples
Lane 8 : Negative control

(Table/Fig 5) 16S rRNA-based PCR detection of *P.gingivalis (fimA)*



Lane 1 : DNA ladder – 100 bp
Lane 2, 4 and 6 : Subgingival plaque samples
Lane 3, 5 and 7 : Coronary artery plaque samples
Lane 8 : Negative control

(Table/Fig 6) 16S rRNA-based PCR detection of *P.nigrescens*

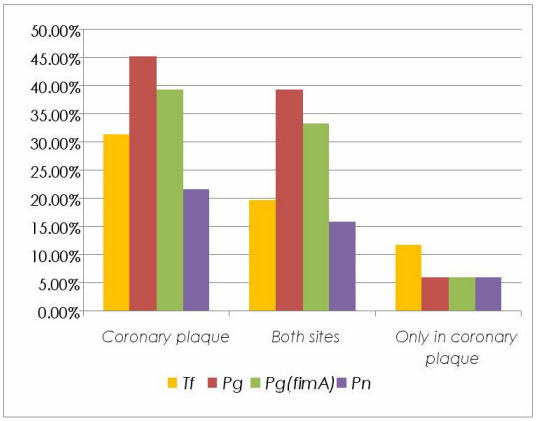
Statistical Analysis

The Kappa measures of agreement were applied to find out the microorganisms which were present in the subgingival as well as atherosclerotic plaques and the P-value and the percentage prevalence were calculated.

Results

Microorganisms	Detected in Coronary Atherosclerotic plaque	Detected in Both plaques	Detected only in coronary plaque samples	Kappa Statistics	P value
Tannerella forsythia	31.40%	19.60%	11.80%	0.256	0.059*
Porphyromonas gingivalis	45.10%	39.20%	5.90%	0.39	0.003*
Porphyromonas gingivalis(fimA) gene	39.20%	33.30%	5.90%	0.331	0.008*
Prevotella nigrescens	21.60%	15.70%	5.90%	0.419	0.002*

(Table/Fig 7)Prevalence of Periodontal Pathogens



(Table/Fig 8) The prevalence of periodontal pathogens in atherosclerotic plaque, both sites and only in coronary plaque samples

The Kappa measures of agreement indicated that a good agreement of the probability of the presence of oral bacteria existed between the subgingival plaque and the atherosclerotic plaque of the patients, which means that the patients who were harbouring microorganisms in the oral subgingval plaque will have chances of its presence in the atherosclerotic plaque also (and vice versa), thus contributing to the progression of atherosclerosis.

Discussion

The present study aimed to investigate and compare the presence of oral bacterial DNA in the subgingival and atherosclerotic plaques of the same patients undergoing CABG. Both the subgingival plaque and the coronary atherosclerotic plaque samples revealed at least one target bacterial DNA.

Tannerella forsythia is a gram-negative obligate anaerobe which produces several

proteolytic enzymes that are able to destroy immunoglobins and factors of the complement system and thus induce apoptotic cell death [10]. Our study revealed the presence of *T.forsythia* in 31.4% of the atherosclerotic plaque samples. This bacteria was also detected in 19.6% of both the sub-gingival and the coronary plaques, which is in accordance with the findings of Haraszthy et al [11], Zaremba et al [12], Ford et al [13], Gotsman et al [14], Zhong et al [15], Yuan-ming et al[16] and Ishihara et al [17], thus indicating the role of the microorganism in contributing towards the progression of atherosclerosis.

Porphyromonas gingivalis belongs to the Bacteroides genus and is a non-motile, gram-negative, rod-shaped, anaerobic pathogenic bacterium. It forms black colonies on blood agar [18] P.gingivalis was detected in 45.1% of the atherosclerotic plaque samples. In both the subgingival and the coronary plaque samples, the bacteria was detected in 39.2% of the patients, thus showing a significant correlation between both the plaques. This is an important finding which is in deviation from the findings of Nakano et al (2006) [19] who detected only 7.4% of the microorganisms of the coronary plaque.

It can be suggested that the presence of *P. gingivalis* has a potential role in the initiation of atherosclerosis by the activation of the phagocyte cells, the elevation of serum lipid levels, the impaired function of polymorphonuclear leukocytes and the induction of the monocyte attractant protein-1. *P. gingivalis* affects cytokine production (prostaglandin E_2, interleukin-1β, and tumour necrosis factor-alpha), C-reactive protein levels, lipid accumulation, and antibody production against bacterial heat shock proteins. Also, *P.gingivalis* induces platelet aggregation and matrix metalloproteinase production [20].

The study also detected the presence of ***P. gingivalis (fimA)*** in 39.2% of the atherosclerotic plaque samples and 33.3% of both the subgingival and the coronary plaque samples. Our findings supported those of the animal study conducted by Chou *et al,*[21] who demonstrated that *P. gingivalis* fimbria-mediated invasion upregulates inflammatory gene expression in HAEC (Human Aortic Endothelial Cells) and in aortic tissues. *Porphyromonas gingivalis* is one of the major causative factors of periodontal disease and its fimbriae (fimA). The filamentous components located on the cell surface play a vital role in the colonisation of the bacteria and in the invasion of the periodontal tissues [22].

Similarly, ***P.nigrescens*** was detected in 21.6% of the atherosclerotic plaques and in 15.7% of both the subgingival and the atherosclerotic plaque samples. Though the percentage prevalence of *P. nigrescens* was lower as compared to the other microorganisms, still this microorganism showed a significant association between the two samples. The detection of *P.nigrescens* in the coronary plaque is an important finding of this study, since this study is one of the few recent studies in the past which detected the presence of this microorganism.

Several possible mechanisms may operate independently or in concert to explain the association between oral infections and atherosclerosis. However, evidence suggests the direct effect of oral infectious agents in atheroma formation. The studies done by Haraszthy [11] and Chiu B [3] *at al* suggest the presence of *P. gingivalis* in Coronary atheromas. The findings of Deshpande and colleagues [23] have shown the invasion and proliferation of *P. gingivalis* in endothelial cells. The major evidence comes from the studies by Hersberg and Meyer [24] which showed that *P. gingivalis* is able to induce the aggregation of the platelets, which is thought to be associated with thrombus formation. The protease production by *P. gingivalis* and other periodontal pathogens may contribute the remodelling of the extracellular matrix in coronary plaques. The above findings could relate that the oral bacteria that infect the coronary plaques could contribute to their formation or to the thrombotic events associated with myocardial infarction [24].

Our study confirms the presence of ***P. gingivalis (fimA), P. Gingivalis, P.nigrescens*** *and* ***T.forsythia*** in the coronary atherosclerotic plaque samples. It is possible that multiple oral bacteria may interact with each other and with host vascular cells either directly or

indirectly and play a role in the initiation, development and progression of atherosclerosis. This observation can provide a base for further research regarding oral infections and atherosclerosis. This may also help to devise preventive treatment strategies such as primary oral prophylaxis to reduce the risk of coronary heart disease.

Acknowledgement

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