

Detection and Characterization of *Prevotella Intermedia* and Its In Vitro Susceptibility to Selected Antimicrobial Agents in Chronic Periodontitis and Acute Myocardial Infarction



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Abstract

Background: Periodontal disease has been reported to play a causative role in acute myocardial infarction (AMI), which may add to the various risk factors associated with coronary heart disease. The objective of the present study was to investigate the presence of *Prevotella intermedia* – an established periodontal pathogen – in subgingival plaque samples of chronic periodontitis and AMI patients in order to identify a possible association, and to evaluate the susceptibility of *Prevotella intermedia* to nine antimicrobial agents. **Methods:** After undergoing screening for eligibility, a total of 50 subjects were included in the present study. Twenty patients were diagnosed with AMI and generalized chronic periodontitis (Group I), 20 patients were diagnosed with only AMI (Group II), and 10 subjects were healthy controls (Group III). The isolated *Prevotella intermedia* strains were tested for susceptibility to bacitracin, chloramphenicol, penicillin G, polymyxin, gentamycin, neomycin, tetracycline, cefotaxime, and cefoxitin using an antibiotic zonescale to determine minimum inhibitory concentrations (MICs). **Results:** Periodontal pathogens were identified by phenotypic and enzymatic methods. The mean bacterial load of *Prevotella intermedia* species was higher in Group I compared to Group II and Group III. It was also found that penicillin G, gentamycin, neomycin, tetracycline, cefotaxime, and cefoxitin inhibited 90% of *Prevotella intermedia*, whereas bacitracin, chloramphenicol, and polymyxin inhibited 80% of *Prevotella intermedia*. Thus, only 10% of *Prevotella intermedia* were resistant to these antibiotics. **Conclusion:** The present study confirms that *Prevotella intermedia* is associated with chronic periodontitis and AMI.

Keywords: Acute myocardial infarction; periodontal disease; *Prevotella intermedia*; antimicrobial susceptibility.

Introduction

Coronary heart disease results from several environmental and genetic risk factors such as age, abnormal serum lipids, diabetes, smoking, hypertension, and viral and bacterial infections. These known risk factors, independently or combined, are involved in both myocardial infarction and atherosclerosis which are the leading cause of adult mortality and morbidity throughout the world.^{1,2,3} A relationship between periodontal disease and systemic diseases such as cardiovascular disease has been proposed,

as they share common risk factors including age, abnormal serum lipids, diabetes, smoking, and viral and bacterial infections. Haffajee and Socransky suggested that several subgingival species, including *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Aggregatibacter actinomycetemcomitans*, were associated with atheromatous lesions in the carotid arteries and found that over 40% of atheromas contain antigens for these periodontal pathogens. The endotoxins released from subgingival species may pass into systemic circulation and contribute to the formation of atheromas. The basis for the

biological mechanism of this relationship has been acknowledged and has driven an active field of investigation into possible association and causality.^{2,3}

Black pigmented, gram negative anaerobic pathogens such as *Prevotella intermedia* are involved in both periodontal infections and systemic conditions including myocardial infarction. A variety of microbes with different antimicrobial susceptibility profiles may cause periodontal disease, and as such, selection of antimicrobial agents should be based on proper microbial diagnosis and sensitivity testing while taking into consideration the patient's medical status. Patients who fail to stabilize following mechanical or surgical therapy are often administered antibiotics.⁴ Thus, the aim of the present study was to evaluate the presence of *Prevotella intermedia* as a possible bacterial species of significance in patients who present with acute myocardial infarction (AMI) and chronic periodontitis, and also to examine the susceptibility of *Prevotella intermedia* to nine antimicrobial agents.

Materials and Methods

A total of 50 subjects were included in the present study: 20 were diagnosed with AMI and chronic generalized periodontitis (Group I), 20 were diagnosed with only AMI (Group II), and 10 healthy controls (Group III).⁵ Group I and Group II patients were admitted to the Department of Cardiology, Sri Venkateswara Institute of Medical Sciences (SVIMS). Approval was obtained from the ethical board of SVIMS to conduct the present cross-sectional study. AMI was verified by typical changes on an electrocardiogram and alteration of serum levels of total cholesterol (CHO), high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), creatine kinase (CK), and creatine kinase-muscle/brain (CK-MB). All subjects had at least 14 teeth and had not received periodontal therapy or antibiotics six months prior to their recruitment in the present study. Group I patients had probing pocket depths (PPD) greater than 5 mm and a clinical attachment loss (CAL) greater than 1 mm in >30% of sites. Group II and Group III subjects had probing pocket depths of less than 3 mm and no CAL.

I. Oral Examination

AMI patients were clinically examined three to four days after admission to the cardiology department. A clinical examination was

administered which assessed the periodontal status of each patient and included the following parameters: plaque index (PI), gingival index (GI), PPD, Russell's periodontal index, and CAL.⁶

II. Laboratory Analysis

Blood samples were withdrawn from all patients upon admission. Serum CHO, HDL, LDL, TG, CK, and CK-MB were all determined by means of an AutoAnalyzer in the clinical laboratory.

III. Microbiological Tests

Culture methods for recovery of bacteria are still the gold standard in microbiology. After subgingival plaque samples were collected, they were immediately transferred to a thyoglycolate agar medium (Figure 1). *Prevotella intermedia* was identified in a pure culture grown in a blood agar anaerobic medium. Gram staining was employed for preliminary identification of *Prevotella intermedia* and its pleomorphic morphology. As *Prevotella intermedia* is an aggressive periodontal pathogen, specific pathogenic characteristics were identified including its proteolytic and fibrinolytic activity and the presence of a capsule. Its catalase negative characteristic was also identified.

Figure 1. Plaque sample collected with paper point



IV. Antimicrobial Susceptibility

An inoculum of 10⁵ CFU was delivered with a multipoint inoculator to supplemented Mueller-Hinton agar. The plates were incubated at 37°C for 48 hours in jars filled with a gas mixture (85% N₂, 5% CO₂, 10% H₂). The isolated *Prevotella intermedia* strains were tested for susceptibility to bacitracin, chloramphenicol, penicillin G, polymyxin, gentamycin, neomycin, tetracycline, cefotaxime, and cefoxitin using a antibiotic zonescale^a to determine minimum

^a HiMedia® Laboratories Pvt. Ltd.

inhibitory concentrations (MICs). The antibiotic zonescale was provided in two hexa disks: Hexa Anaerobic-1 and Hexa Universal-1 with antibiotics; Hexa Anaerobic-1 contained tetracycline, cefotaxime, and ceftiofur, while Hexa Universal-1 contained bacitracin, chloramphenicol, penicillin G, polymyxin, gentamicin and neomycin. The lowest concentration of antimicrobial agents that showed no growth was recorded as the MIC. The susceptibility of *Prevotella intermedia* was determined by referring to the MICs zone size interpretive standards chart of the National Committee for Clinical Laboratory Standards (NCCLS), which was provided by HiMedia®.

Statistical Analysis

The means and proportions of the major risk factors and diagnostic markers for AML, as well as the clinical periodontal parameters were

calculated for the three groups and the value of the F-test and mean within group variances was determined by analysis of variance (ANOVA) (Tables 1 and 2). The F-test was used to specify if a group of variables was jointly significant.

Results

Table 1 demonstrates the mean and standard deviation (SD) of the clinical periodontal parameters for all groups. Mean PI, GI, PPD, Russell’s index, and CAL were higher in Group I compared to Group II and Group III. The F-value was clinically significant. Table 2 demonstrates the mean and SD of the major risk factors and diagnostic serum enzymes for AML for all groups. Group I showed higher levels of CHO, LDL, TG, CK, and CK-MB compared to Group II and Group III, whereas HDL were lower in Group I compared to Group II and Group III . The F-value was clinically significant.

Table 1. Clinical Periodontal Parameters

	Mean PI	Mean GI	Mean PPD (mm)	Russell’s Periodontal Index	Mean CAL (mm)
Group I	2.73	2.58	6.10	3.22	2.23
Group II	2.35	1.67	2.20	0.66	0
Group III	1.16	1.08	1.90	0.70	0
Significance	F = 49.249	F = 58.175	F = 113.436	F = 108.915	F = 52.253

High clinical periodontal parameters in Group I compared to Group II and Group III. F-value is significant. **PI** = plaque index; **GI** = gingival index; **PPD** = probing pocket depth; **CAL** = clinical attachment loss.

Table 2. Lipid Profiles and Diagnostic Serum Enzymes for Acute Myocardial Infarction

	Mean CHO (mg/dL)	Mean HDL (mg/dL)	Mean LDL (mg/dL)	Mean TG (mg/dL)	Mean CK (U/L)	Mean CK-MB (U/L)
Group I	260.50	41.45	162.30	341.70	343.85	71.25
Group II	225.25	42.05	154.90	313.35	223.55	43.80
Group III	184.00	45.60	121.20	164.00	191.40	37.70
Significance	F = 62.499	F = 2.322	F = 57.832	F = 51.530	F = 6.981	F = 57.832

Low HDL and high LDL, TG, and CHO levels in Group I compared to Group II and Group III. F –value is significant. **CHO** = total cholesterol; **HDL** = high density lipoprotein; **LDL** = low density lipoprotein; **TG** = triglycerides; **CK** = creatine kinase; **CK-MB** = creatine kinase-muscle/brain.

Gram staining and bacterial enzymatic reactions demonstrated a higher quantity of *Prevotella intermedia* in Group I compared to Group II and Group III. Figure 2 demonstrates 50% positive bacterial enzymatic reactions in Group I, 30% positive enzymatic reactions in Group II, and 0% in Group III. Moreover, our results revealed that the total bacterial load and *Prevotella intermedia* in specific, was significantly higher in Group I compared to Group II and Group III in the following order: Group I > Group II > Group III.

Table 3 shows the susceptibility of *Prevotella intermedia* to various antibacterials by comparing the recorded MICs to the MICs zone size interpretive standards chart of the National Committee for Clinical Laboratory Standards (NCCLS). It was found that pencillin G, gentamycin, neomycin, tetracycline, cefotaxime, and cefoxitin inhibited 90% of *Prevotella intermedia* whereas bacitracin, chloramphenicol, and polymyxin inhibited 80% of *Prevotella intermedia*. Thus, only 10% of *Prevotella intermedia* were resistant to these antibacterial drugs.

Figure 2. Positivity in enzymatic reactions of *Prevotella intermedia*

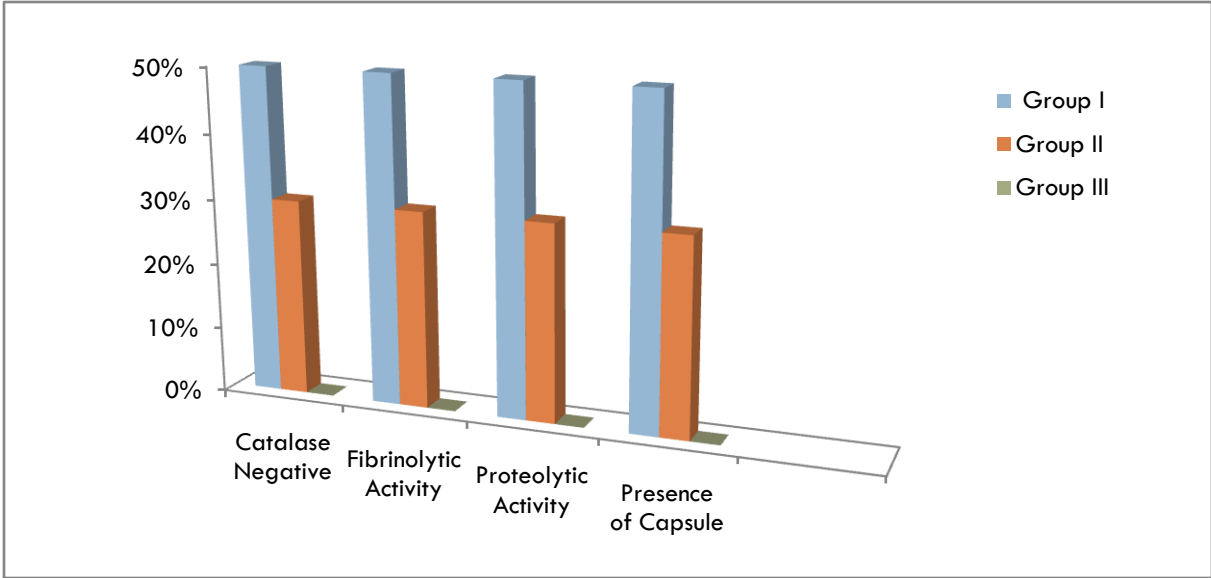


Table 3. Antimicrobial Susceptibility of *Prevotella Intermedia*

Antibiotic	Zone Size Interpretative Chart Provided by HiMedia®			MICs Of <i>Prevotella intermedia</i> to Antimicrobial Agents in the Present Study (%)		
	Resistant	Intermediate Sensitive	Sensitive	Resistant	Intermediate Sensitive	Sensitive
Bacitracin	8 mm	9-12 mm	13 mm	20%	30%	50%
Chloramphenicol	12 mm	13-17 mm	18 mm	20%	20%	60%
Pencillin G	13 mm	13-18 mm	18 mm	10%	0%	90%
Polymyxin	11 mm	10-12 mm	12 mm	20%	10%	70%
Gentamycin	12 mm	13-14 mm	15 mm	0%	20%	80%
Neomycin	14 mm	13-16 mm	17 mm	10%	10%	80%
Tetracycline	14 mm	15-18 mm	19 mm	0%	20%	80%
Cefotaxime	14 mm	15-22 mm	23 mm	0%	20%	80%
Cefoxitin	14 mm	15-17 mm	18 mm	0%	10%	90%

Pencillin G, gentamycin, neomycin, tetracycline, cefotaxime, and cefoxitin inhibited 90% of *Prevotella intermedia*, whereas bacitracin, chloramphenicol, and polymyxin inhibited 80% of *Prevotella intermedia*.

Discussion

Periodontal diseases are a group of inflammatory conditions of the supporting tissue of the teeth caused by bacteria. It is believed that bacteria associated with periodontal disease enter the bloodstream and travel to major organs such as the heart, thereby contributing to the formation of artery-clogging plaques.⁵ Black-pigmented, gram-negative oral anaerobes such as *Prevotella intermedia* are regarded as pathogens in chronic periodontitis and are involved in both oral and non-oral infections.

After assessing the periodontal status and lipid profiles in all three groups included in the present study, periodontal parameters and risk factors were found to be higher in Group I compared to Group II and Group III. These results are in agreement with Stein et al.¹⁰

Nonnenmacher et al. and Mane et al. found that *Prevotella intermedia* was a frequently detected microorganism in periodontal disease and was identified by its fibrinolytic and proteolytic activity and by being catalase negative and encapsulated.^{11,12} After assessing the periodontal status and lipid profiles of all three groups included in the present study, we collected subgingival plaque samples in order to test for *Prevotella intermedia* which was identified by phenotypic characteristics demonstrated through bacterial Gram staining and culture methods. These methods confirmed the presence of *Prevotella intermedia* in subgingival plaque samples of both chronic periodontitis and AMI patients.

Patients who do not respond to common mechanical or surgical periodontal therapy are often administered antibiotics as an adjunct to conventional treatment. According to Andrés et al. 14% of *Prevotella intermedia* were resistant to β -lactam antibiotics (pencillins and/or cephalosporins) whereas our study shows that only 10% were resistant to these antibiotics.⁴

It may be warranted to stabilize the periodontal condition of patients with cardiovascular disease through non-surgical therapy that includes both scaling and root planing (SRP) and adjunctive antimicrobial therapy. The fact that the load of *Prevotella intermedia* was higher in the chronic periodontitis and AMI group (Group I) compared to the other groups, and that the load was higher in the AMI group (Group II) compared to the healthy control group (Group III), hints to the possibility that this

particular pathogen may be linked to the onset of cardiovascular disease.

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