IJM Iranian Journal of Microbiology
Volume 5 Number 4 (December 2013) 391-395

Microbiological tests to identify a link between periodontitis and acute myocardial infarction-an original research

Mohan Kumar Pasupuleti[1], Ravindra Reddy Nagireddy[2], Roopa Dinahalli[2], Deepa Anumala[2], Avula Kishore Kumar[2], Vinay Chavan[3]

[1]St. Joseph Dental College, Duggirala, Eluru, Andhra Pradesh, India. [2]CKS Teja Institute of Dental Sciences, Chadalawada Nagar, Renigunta Road, Tirupathi (AP), India. [3]Meghana Institute of Dental Sciences, Nizamabad, Andhrapradesh, India.

Received: December 2012, **Accepted:** August 2013.

ABSTRACT

Background and Objectives: Gingival and periodontal diseases are associated with specific bacterial infections. The main aim of the study was to know whether the periodontitis is associated with an increased risk for acute myocardial infarction (AMI) and to know the distribution of *Porphyromonas gingivalis* in patients with acute myocardial infarction associated with chronic periodontitis and acute myocardial infarction Groups.

Materials and Methods: Out of 50 patients, 20 were diagnosed as acute myocardial infarction associated with chronic periodontitis (Group I), twenty patients were suffering from AMI (Group II) and 10 patients were healthy (control Group III).

Results and Conclusion: Periodontal pathogens were identified by phenotypic, enzymatic and hybridization methods. The total bacterial load and the number of *Porphyromonas gingivalis* pathogens were more in Group I when compared to Group II and Group III. Thus, the present study confirmed an association between periodontitis and AMI.

Keywords: Acute myocardial infarction, periodontal disease, *Porphyromonas gingivalis*

INTRODUCTION

Acute myocardial infarction is the leading cause of mortality and morbidity throughout the world. Risk factors like age, gender, familial history, environmental factors like smoking, alcohol, pollution, stress, diabetes, hypertension, genetic factors, bacterial and viral infections result in the development of acute myocardial infarction (1-3).

Periodontal diseases are a Group of inflammatory diseases in which bacteria and their by-products are the principal etiologic agents. It is the result of uncontrolled development of facultative anaerobic bacteria. When the equilibrium between the hygiene-bacteria-immune system is destabilized, the specific periodontal pathogens destroy the soft tissue and hard tissue components of the periodontium and results in periodontal disease (4, 5).

When the periodontium gets infected with specific periodontal pathogens like *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia*, the periodontal tissues become permeable and thus these bacteria enter the blood circulation (6, 7).

As mouth is the principal entry door for these pathogens in the human body. It represents multiple connections between oral and general health. Epidemiologic surveys confirmed the relation between cardiovascular diseases, preterm birth, diabetes, HIV and osteoporosis with periodontal diseases (8, 9).

As oral health is the principal entry door of pathogens, it is obvious that oral health must be

* Corresponding author: Dr. Mohan Kumar. P
Address: Flat no. 303, R.K Gold Apartments, Sriram nagar, 5 th Road, Eluru, West Godavar District, Andhrapradesh, India.
E-mail: suppimohan@gmail.com

followed in a regular way with the means of tests going beyond clinical observation.

The aim of the present study was to investigate the possible association between the periodontal disease and acute myocardial infarction and to know whether the *Porphyromonas gingivalis* infection increase the risk for acute myocardial infarction.

MATERIALS AND METHODS

A total of 50 patients, 20 with acute myocardial infarction and generalized chronic periodontitis (Group I), 20 with AMI only (Group II) and 10 without AMI and generalized chronic periodontitis (Group III) were included in the study. The Group I and Group II patients were admitted to the Department of Cardiology, Sri Venkateswara Institute of Medical Sciences (SVIMS). Ethical board from SVIMS has given approval to conduct the cross sectional study. AMI was verified by typical changes in the electrocardiogram and alteration of serum enzymes [high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG) and cholesterol.

The patients should have at least 14 teeth and they should neither received periodontal surgery nor antibiotics 6 months prior to this study. The probing pocket depths should be greater than 5 mm and clinical attachment loss ≥ 1 in Group I and less than 3 mm probing pocket depth and CAL = 0 mm in Group II and Group III.

Oral examination. AMI patients were clinically examined 3 to 4 days after the admission to the cardiology department. Clinical examination was carried out by assessing the periodontal status by plaque index (PI), gingival index (GI), Russel's periodontal index.

Laboratory analysis. Blood samples were taken on admission from all patients. Serum total cholesterol, high density lipoprotein, low density lipoprotein and triglycerides were determined by autoanalyzer in the clinical laboratory.

Microbiological tests. Several methods have been employed for the detection of putative periodontal pathogens in subgingival samples and also to identify the link between periodontal disease and acute myocardial infarction. As oral health reflects general health, it is important to advice more specific, advanced, molecular biologic techniques which allows the risk detection prior to clinical observations. In this study, the association between periodontal disease and acute myocardial infarction was confirmed by the *Porphyromonas gingivalis* infection. Preliminary identification of *Porphyromonas gingivalis* was by Grams staining and by its pleomorphic morphology.

After plaque samples were collected, they immediately transferred into thyoglycolate agar medium. Pure culture was done in order to identify *Porphyromonas gingivalis* in blood agar anaerobic medium. As *P. gingivalis* is an aggressive periodontal pathogen, the specific pathogenic characteristics were identified by its proteolytic, hemolytic, coagulase, catalase, fibrinolysin activity and the presence of capsule.

DNA hybridization. There is only a genetic identification based on DNA or RNA to know the type and quantity of bacteria present in the periodontal pockets. The IAI Pado Test 4.5 (IAI Eschhenweg6. CH-4528 Zuchwil/ Switzerland) is a biologic molecular test which allows the identification and quantification (Total Bacterial Load, TBL) of best indicators of periodontitis. The specific periodontal pathogens like *Aggregatibacter actinomycetemcomitans* (Aa), Porphyromonas gingivalis (Pg), *Tannerella forsythia* (Tf) and *Treponema denticola* (Td) were identified by this test. As *P. gingivalis* is the most common periodontal pathogen associated with severe diseased states along with Aa, we studied on the occurrence of *P. gingivalis* in periodontal disease associated with acute myocardial infarction (Fig. 1).

Statistical analysis. Means and proportions for major risk factors and clinical parameters were calculated for the three Groups (Table 1).

RESULTS

Table 1 shows the means (± SD) and clinical parameters and major risk factors for Group I and Group II. Mean Plaque index, Gingival index , Pocket depth, Russel's index were more in Group I when compared to Group II and Group III (Table 1). These results are in agreement with stein et al. (8).

The major risk factors which include serum enzymes showed more elevated levels of low density lipoprotein (LDL), triglycerides (TG) and total cholesterol (CHO)

Table 1. Periodontal parameters and lipid profile.

Groups	PI	GI	Mean PPD	Russels index	HDL Mean	LDL Mean	TG Mean	CHO Mean
Group I	2.73	2.58	6.10	3.22	41.45	162.30	341.70	260.50
Group II	2.35	1.67	2.20	0.66	42.05	154.90	313.35	225.25
Group III	1.16	1.08	1.90	0.70	45.60	121.20	164.00	184.00
Significance	F = 49.42	F = 58.17	F = 113.43	F = 108.9	F = 2.322	F = 57.832	F = 51.530	F = 62.499

The above table demonstrates high periodontal parameters (PI = plaque index, GI = gingival index, PPD = mean probing pocket depth, Russel's periodontal index) in Group I when compared to Group II and Group III. F – value is significant (it is the value of the test/ mean of the within Group variances done by ANOVA test).
It also demonstrates low HDL (High Density Lipoprotein) and high LDL (Low Density Lipoprotein), TG (Triglycerides) and CHO (Cholesterol) levels in Group I when compared to Group II and Group III.

in Group I when compared to Group II and Group III. Whereas high density lipoproteins were decreased in Group I when compared to Group II and Group III (Table. 1).

Porphyromonas gingivalis was higher in Group I [D.F = 12] compared to Group II [D.F = 6], and in Group III shows no growth.

DNA hybridization test (IAI Pado Test 4.5) shown that the Total bacterial load was higher in Group I when compared with Group II (Table 2).

Gram staining and bacterial enzymatic reactions shown that the presence of *Porphyromonas gingivalis* were more in Group I when compared to Group II and Group III in the following order (Group I > Group II > Group III).

Finally, our results revealed that the total bacterial load and specific periodontal pathogens like *P. gingivalis* were significantly higher in Group I than GroupII.

DISCUSSION

Periodontal diseases are characterized by inflammatory processes of the tissues surrounding the teeth in response to bacterial accumulation. The transmission of periodontal pathogens into main blood stream is because of the frequent bleeding events of the gingival tissues loaded with periodontal bacteria (10).

Approximately three people out of four suffer at one time from periodontal disease during their lives. In adults, 70% of teeth losses are due to periodontitis (11, 12). Wide lesions thus become often apparent only in the second half of the life, after a long time of evolution. If the disease is severe and the environmental and genetic risk factors co-exist, the periodontal pathogens can penetrate the periodontal tissues and enter the blood circulation to become the source of problems in other parts or organs of the body (13).

Porphyromonas gingivalis (Pg), a Gram-negative anaerobic black-pigmented rod, has been implicated as a crucial etiologic agent in the initiation and progression of chronic marginal periodontitis. It is one of the most frequently associated specific periodontal pathogen that has a plausible role in the development of cardiovascular disease (13, 14).

A study by Kuramitus *et al.* (2003) stated that *Porphyromonas gingivalis* has specific role in stimulating monocyte migration to endothelial sites in blood vessels as well as the transformation of these cells into foam cells which is important for the development of cardiovascular disease. However, additional approaches are necessary to establish an exact role in the progression of cardiovascular disease.

In the present study an association between chronic periodontitis and acute myocardial infarction was found (15, 16). After assessing the periodontal status and lipid profile in all the three Groups included in

Table. 2. *P. gingivalis* detection after DNA hybridization.

Group		No of subjects	Detection frequency	Mean	Standard deviation
TBL	I	4	0.62 millions	100.9050	26.7313
	II	4	1.13 millions	46.4600	35.9401

p Value = 0.043; D.F = Detection Frequency; T.B.L= Total Bacterial Load.
This table demonstrates P.gingivalis detection frequency and Total Bacterial Count which is more in Group I (AMI with chronic periodontitis) than Group II (AMI only).

Fig. 1. Plaque sample collected by paper points.

the study, we collected the plaque sample and tested for identification of *P. gingivalis* by phenotypic characteristics which include bacterial Grams staining and culture methods and shown the presence of *P. gingivalis*.

At the same time, the collected samples were also tested for bacterial enzymatic reactions, which were specific for *P. gingivalis* and resulted in positive reactions for coagulase, catalase, fibrinolysin, haemolysin, proteolysis, DNase and the presence of capsule. These biochemical reactions confirmed the presence of *P. gingivalis* (Fig. 2).

A precise microbiologic test which can help in quantifying very weak number of pathogens, which also shows the extension of bacterial infection is needed to diagnose the periodontitis and its associated systemic link at an early stage prior to the clinical observation (17). In this regard, we have chosen IAI Pado Test 4.5, which is the only genetic test helps in quantifying and identifying four targeted bacteria which are pathogenic to periodontitis and acute myocardial infarction.

This biomolecular test is essential to: 1) detect periodontitis as earliest as possible, 2) measure the seriousness and extension of disease, 3) determine which antibiotic must be prescribed if necessary and 4) to helps in targeting a second and different treatment if necessary.

It is important to stabilize the periodontal condition in patients with cardiovascular diseases both by non-surgical therapy that includes scaling and root planing (SRP) and adjunctive therapy which includes administration of non-steroidal anti-inflammatory drugs (NSAIDs), aminobisphosphonates, inhibitors against MMPs, and proinflammatory cytokine inhibitors.

Recently, food-derived polyphenols have received considerable attention as therapeutic reagents because of their safety and scientifically proven positive effects, such as anticarcinogenic, anti-inflammatory, and antimicrobial activities, as well as alleviation of cardiovascular disease (18).

Very few studies determined that, with definitive elimination of potential periodontal infections from

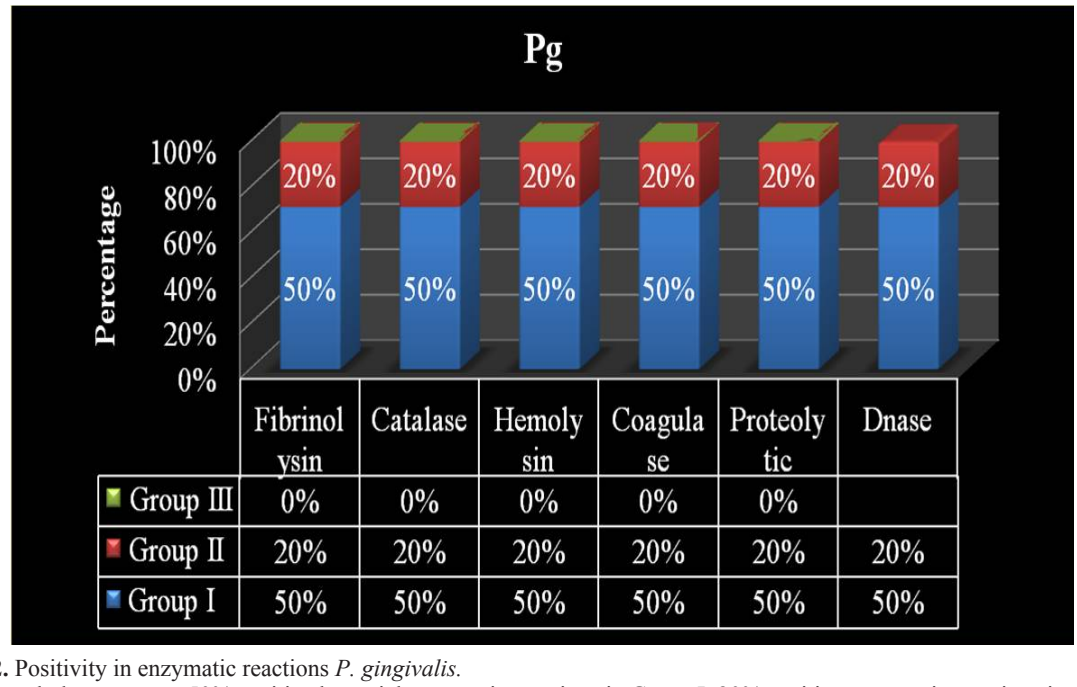

Fig. 2. Positivity in enzymatic reactions *P. gingivalis*.
This graph demonstrates 50% positive bacterial enzymatic reactions in Group I, 20% positive enzymatic reactions in Group II and 0% in Group III.

any cardiovascular disease patients resulted in lowering the cardiovascular disease risk overtime when compared with patients who had not received any treatment for specific periodontal disease (19, 20).

CONCLUSION

The results of our study confirm the association between periodontal disease and acute myocardial infarction. It also helps in identifying each specific periodontal pathogen (*Porphyromonas gingivalis* in this study) and the connecting link between periodontal disease and systemic disease at an early stage prior to the clinical observation of the disease. In particular, in this study Pg might be considered as risk indicator for AMI.

REFERENCES

1. Lockhart PB, Bolger AF, Papapanau PN, Osinbowale O, Trevisan M, Levison ME, et al. periodontal disease and atherosclerotic vascular disease: does the evidence support an independent association? A scientific statement from the American heart association. *Circulation* 2012; 125: 2520-44.
2. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. *Periodontol 2000*, Vol. 5, 1994, 78-111.
3. Emingil G, Buduneli E, Aliyev A, Akilli A, Atilla G. Association between periodontal disease and Acute Myocardial Infarction. *J Periodontol* 2000: 71: 1882 -1886.
4. Kuramitsu HK, Kang IC, Qi M. Interactions of *Porphyromonas gingivalis* with host cells: Implications for Cardiovascular Diseases. *J Periodontol* 2003: 74: 85-89
5. Golebiewska M, Taraszkiewicz-Sulik K, Kuklinska A, Musial WJ. Periodontal condition in patients with Cardiovascular diseases. *Adv Med Sc*i 2006; 51, suppl 1: 69-72.
6. Davey ME. Techniques for the growth of *Porphyromonas gingivalis* biofilms. *Periodontology 2000*; 42, 2006, 27-35.
7. Holt SC, Kesavalu L, Walker S, Genco CA. Virulence factors of *Porphyromonas gingivalis.* Periodontol 2000 1999; 20:168-238.
8. Stein JM, Kuch B, Conrads G, Fickl S, Chrobot J, Schulz S, et al. Clinical Periodontal and microbiologic parameters in patients with acute myocardial infarction. *J Periodontol* 2009; 80: 1581-1589.
9. Elkaim R, Dahan M, Kocgozlu L, Werner S, Kanter D, Kretz JG, et al. Prevalence of periodontal pathogens in subgingival lesions, atherosclerotic plaques and healthy blood vessels: A preliminary study. *J Periodontal Res* 2008; 43: 224-231.
10. F Ismail, W Heuer, N Stumpp, J Eberhard, A Winkel, I Ismail, et al. 16S rDNA- based metagenic analysis of human oral plaque microbiota in patients with atherosclerosis and healthy controls. *Indian J Med Microbiol* 2012; 30: 462-466.
11. Aimetti M, Romano F, Nessi F. Microbiologic analysis of periodontal pockets and carotid atheromatous plaques in advanced chronic periodontitis patients. *J Periodontol* 2007; 78: 1718-1723.
12. Ridker PM, Silvertown JD. Inflammation, C –Reactive protein, and Atherothrombosis. *J Periodontol* 2008; 79: 1544-1551.
13. Goteiner D, Ashmen R, Lehrman N, Janal MN, Eskin B. Presence and significance of interleukin-1 polymorphism in patients who present with acute coronary syndrome, angina and chronic periodontitis: An epidemiologic pilot study. *J Periodontol* 2008; 79: 138-143.
14. Kou Y, Inaba H, Kato T, Tagashira M, Honma D, Kanda T, Ohtake Y, Amano A. Inflammatory responses of gingival epithelial cells stimulated with *Porphyromonas gingivalis* vesicles are inhibited by Hop- associated polyphenols. *J Periodontol* 2008; 79: 174-180.
15. Friedewald VE, Kornman KS, Beck JD, Genco R, Goldfine A, Libby P, et.al. Periodontitis and Atherosclerotic cardiovascular disease. *J Periodontol* 2009; 80: 1021-1032.
16. Renvert S, Ohlsson O, Pettersson T, Persson GR. Periodontitis: A future risk of Acute coronary syndrome? A follow-up study over 3 years. *J Periodontol* 2010; 81: 992-1000.
17. Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. *J Periodontol* 2000: 71: 1554-1561.
18. Sakanaka S, Okada Y. Inhibitory effects of green tea polyphenols on the production of a virulence factor of the periodontal disease causing anaerobic bacterium Porphyromonas gingivalis. *J Agric Food Chem* 2004; 52: 1688-1692.
19. Hujoel PP, Drangsholt M, Spiekerman C, DeRouen TA. Examining the link between coronary heart disease and the elimination of chronic dental infections. *J Am Dent Assoc* 2001; 132: 883-889.
20. Scannapieco FA, Dasanayake AP, Chhun N. «Does periodontal therapy reduce the risk for systemic diseases?». *Dent Clin North Am.* 2010; 54: 163-181.