Title: Detection of Periodontal Microorganisms in Coronary Atheromatous Plaque Specimens of Myocardial Infarction Patients: A Systematic Review and Meta-Analysis

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Abstract

Background: Microbial translocation from inflamed periodontal pockets into coronary atheroma via systemic circulation is one of the proposed pathways that links periodontitis and myocardial infarction (MI). The purpose of this systematic review is to determine the reported prevalence of periodontal microorganisms in coronary atheroma and/or aspirated clot samples collected from MI patients with periodontal disease.

Methodology: The "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) guidelines were followed. Six databases were systematically searched using Medical Subject Headings/Index and Entree terms. After a thorough screening, fourteen publications spanning over ten years (2007-2017) were eligible for this systematic review and meta-analysis.

Results: Out of 14 included studies, 12 reported presence of periodontal bacterial DNA in coronary atherosclerotic plaque specimens. Overall, *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were the most frequently detected periodontal bacterial species. Meta-analysis revealed that the prevalence of *P. gingivalis* was significantly higher than *A. actinomycetemcomitans* in coronary atheromatous plaque samples. Apart from periodontal microbes, DNA from a variety of other microbes e.g. *Pseudomonas fluorescens, Streptococcus species, Chlamydia pneumoniae* were also recovered from the collected samples.

Conclusion: Consistent detection of periodontal bacterial DNA in coronary atheroma suggests their systemic dissemination from periodontal sites. It should further be investigated whether they are merely bystanders or induce any structural changes within coronary arterial walls.

Systematic Review Registration: PROSPERO Registration Number: CRD42017082259

Keywords: Myocardial Infarction, Periodontitis, Coronary Artery Disease, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Systematic Review

INTRODUCTION

Myocardial Infarction (MI) remains a major cause of morbidity and mortality worldwide.[1] Almost 10% to 15% of MI patients lack the presence of any classical risk-factors, indicating the contribution of alternative mechanisms.[2] It has now been accepted that the chronic inflammation and immune activation play a central role in atherosclerotic plaque instability, triggering a thromboembolic episode.[3] Several inflammatory conditions are believed to contribute to an increased coronary inflammatory burden.[4] One such prevalent disease is periodontitis, characterized by loss of tooth-supporting tissues, which is caused by host-mediated inflammatory and immune activities in response to complex microbial biofilms that colonise periodontal pockets.[5] In an untreated generalised periodontitis, the total epithelial surface area of deep periodontal pockets is up to 20 cm^2, which is approximately size of an average human hand [6]. The areas of discontinuity within inflamed periodontal pocket epithelium facilitate systemic dissemination of periodontal microbes and inflammatory biomolecules e.g. interleukins and matrix metalloproteinases (MMPs).[7] The consensus report of 'the world workshop on Periodontitis and Systemic Diseases' has concluded that the periodontal bacteria within atherosclerotic lesions produce endotoxins and express various virulence factors that interact with TLR2 receptors on the endothelial cells. This results in upregulation of host inflammatory and immune responses, endothelial adhesion molecules to create a prothrombotic environment.[8] (Fig. 1)

Growing number of literature has implicated periodontitis as an independent risk factor for coronary artery disease. Persistent translocation of microorganisms from inflamed periodontal sites into coronary arteries is believed to cause atheromatous plaque instability and ultimately increasing the risk of acute myocardial infarction.[9] This systematic review aims to assess the current knowledge on detection rates of specific periodontal microbes from atheromatous plaque and/or aspirated thrombus specimens recovered from MI patients with periodontal disease.

METHODS

Scope of review

The “Preferred Reporting Items for Systematic Reviews and Meta-Analyses” (PRISMA) guidelines were followed. An adapted “Population, Intervention/Exposure, Comparator, Outcome (PICO)” criterion was used to frame the review question- What is the prevalence of periodontal microbes in coronary atheromatous plaque and/or aspirated thrombus specimens (O) collected from adult MI patients (P) with periodontitis (I). From the ethical standpoint, it is not possible have atheromatous plaque samples collected from healthy participants (C). The systematic review is registered with PROSPERO (2017: CRD42017082259).

Search strategy

The search strategy for this systematic review was constructed primarily using the Medicine Medical Subject Headings (MeSH) terms and relevant free-text terms.(Table 1) A systematic electronic search was conducted using MEDLINE/PubMed, EMBASE, SCOPUS, Web of Science, Cochrane Controlled Trials Register and Google Scholar databases. Additionally, PROSPERO systematic review registry was searched for any existing registered protocol on this topic. Two reviewers (CJ, RB) carried out literature searches independently. Reference lists of the selected articles were searched for any additional relevant papers.

Inclusion and exclusion criteria

Studies considered eligible were: i) original studies published in English language, assessing the association between periodontitis and MI in adults (age>18 years); ii) studies investigating presence of microorganisms within atheromatous plaque samples collected from MI patients; iii) observational studies; iv) studies conducted between January-1989 and May-2018. This time period was chosen because in 1989, an interest

concerning impact of periodontal disease on cardiovascular health was revived.[10] Narrative-reviews, mini-reviews, dissertations, short commentaries, letter-to-editor, *in-vitro* and animal studies were excluded. A PRISMA flow diagram indicates the identified, screened, eligible and included articles.(Fig. 2) Following removal of duplicate articles, two reviewers (CJ and RB) assessed titles and abstracts of all the included research papers independently. The full texts of relevant articles were then critically reviewed according to the inclusion and exclusion criteria specified above. Although there were no disagreements, an arbitrator (WA) was available for mediation.

Data extraction and quality assessment

The following information was extracted from each included study by the two reviewers independently: the study ID (first author and year of publication), country, study design, characteristics of the subjects (including the number of patients, their age and gender distribution), adjusted or matched confounding factors, periodontal parameters i.e., clinical attachment loss (CAL), probing depth (PD), bleeding on probing (BoP), plaque index (PI), number of missing teeth, periodontal microorganisms identified in subgingival plaque and/or atheromatous plaque specimens, presence of any other microbial species and brief conclusions. For qualitative assessment of included articles, both reviewers used the Newcastle-Ottawa Scale (NOS)[11] independently.(Table 2) WA reviewed the extracted data against the selected articles for any missing information.

RESULTS

Search results

A systematic search resulted in 4054 original published articles, narrowed to 3606 after removal of duplicates. With careful screening of titles and abstracts, 43 studies were found to be within the scope review. Later, 29 studies were excluded for the various reasons: 1} inadequate information on prevalence of periodontitis in recruited patients-13 2} no mention of case-definition used to diagnose periodontitis and/or MI-8 3} narrative reviews-3, 4} collection of samples from arteries other than coronaries e.g. aorta, carotid arteries etc.-5. This yielded a total of 14 research papers. No additional publications were identified by hand searching bibliographical lists of these 14 full-text publications.(Fig. 2)

Characteristics of studies

The review comprises of 14 cross-sectional studies, published between 2007-2017. The shortlisted studies were carried out in 6 different countries: Poland[12,23], France[13], India[19, 24, 25], Brazil[14-17,20,21], Finland[18], Denmark[22]. Subjects in the included studies were

adults with an age range of 32-80 years. MI was defined according to contemporaneous European Society of Cardiology criteria. However, a great variation was observed with periodontitis definitions.(Table 4)

Assessment of periodontitis

Case definition used to assess the severity of periodontitis

The case definition of periodontitis to categorise patients into moderate and severe periodontitis differed within the selected publications. Two studies[16,17] utilised the “1999-Classification of Periodontal diseases” by American Academy of Periodontology, while one study[14] used the “2005-case definition of periodontitis by Tonetti & Claffey”.

The protocol used for a dental examination

Out of 14 studies, 12 opted for clinical periodontal examination employed a standard protocol of full-mouth periodontal examination around 6 sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual) per tooth. The parameters recorded were CAL, PD, and recession in millimeters. Periodontal probes used to examine periodontal parameters were PCP-UNC15[14-16] and Goldman Fox probe [17,20]. In remaining two studies, periodontitis was defined either by assessment of bone loss pattern and presence of intra-bony pockets using Dental Panoramic Tomogram (DPT)[18] or by retrospectively telephonic evaluation to assess whether they had periodontitis diagnosed by their dentists at the time of percutaneous coronary intervention (PCI) using a standardised questionnaire.[22](Table 4)

Examiner calibration and statistical power calculation

Out of 14 selected studies, 5 studies mentioned that the periodontal examination was carried out by a single examiner.[13,14,17,21,23] Out of these 5 studies, 4 reported that intra-examiner calibration was carried out prior to the periodontal examination.[12,17,21,23] Pessi T *et al*.[18] assessed the radiographic alveolar bone level but it is unclear whether a single calibrated examiner carried out all the radiographic analyses. Only one out of 14 studies [17] mentioned about the power calculation to determine sample size prior to recruitment.(Table 3)

Collection and storage of specimens

Source/sites of procurement of atheromatous plaque

As part of their medical management, the subjects in the selected studies underwent one of three treatment alternatives: i) percutaneous coronary intervention (PCI)[18,21,22] ii) endarterectomy[14,15,20,25] or iii) coronary artery bypass surgery (CABG)[12,13,16,17,19,23,24]. Across all the included studies, a great variation was observed with the techniques used to collect atheromatous plaque samples. In one study, during CABG, sterile paper-points were inserted into atherosclerotic plaque present within surgically-exposed coronary vessels.[12] In another study, the atheromatous plaque was collected during angioplasty by capturing it in distal protection filters.[17] Pessi *et al.*, collected aspirated coronary thrombi along with arterial blood during primary PCI procedure.[18] Two studies[21, 22] collected the first PCI balloon that came in contact with atheromatous plaques.

Segments of angioplasty catheter 5-7cm proximal to balloon[22], blood sample from the arterial sheet[18,21], specimens from internal mammary and femoral artery[17] and saphenous vein graft[13] served as a comparator group in the respective studies. In ten articles, the collected atheromatous plaque specimens were stored under dry conditions without addition of any storage medium and flash frozen at either

-20˚C or -80˚C until further analysis. While in remaining four studies, the storage medium of choice was either a phosphate buffer saline solution[19,24] or a Tris buffer solution[20,21].

The technique employed for detection of microorganisms in atheromatous plaque specimens

Most of the included studies(8) utilised a conventional polymerase chain reaction (PCR) technique and 2 studies used real-time PCR) technique to target a 16S rRNA gene segment of selected periodontal bacteria. Additionally, in two studies, bacterial 16S rRNA gene-PCR amplicon was obtained using universal primers and then sequenced by Sanger method to identify bacterial phylogenic diversity.[20,21] Remaining two studies employed DNA-DNA hybridization technique.[12,13] Few other advanced techniques such as immunohistochemistry and transmission electron microscopy were used to detect the presence of either fragments or whole cells of bacteria, respectively.[18]

Qualitative analysis of microbiological findings

Presence of periodontal microorganisms' DNA in coronary artery atheromatous plaque or aspirated thrombus samples

Periodontal bacterial DNA was consistently detected in atheromatous plaque specimens collected from MI patients. In 12 of 14 selected studies, DNA of at least one known periodontal bacteria was found in atheromatous plaque samples.[12-16,18-21,23-25] In the remaining two studies, all the collected atheromatous plaque samples were negative for periodontal bacterial DNA.[17, 22] As a common theme across the included studies, 10 reported detection of *Porphyromonas* gingivalis[12,14-16,18,19,21,23-25] in atheromatous plaque samples while *Aggregatibacter actinomycetemcomitans* was detected 6 studies[12, 14-16,18,20]. The role of these two periodontal microbes in initiation as well as progression of periodontal disease is well-established.[26] Many of the studies[14-16,19-21,24,25] reported the presence of more than two periodontal bacterial species implying the presence of a complex microbial ecology within collected atheromatous plaque samples. The

other known periodontal bacteria in these samples, though less frequently, were *Tanerella forsythia*[14,16,19,25]; *Treponema denticola*[17,24,25]; *Prevotella nigrescens*[14]; *Eikenella corrodens*[13]; *Campylobacter rectus*[13].

Virulence factors of periodontal bacteria

One of the studies analysed the genes encoding fimbriae of *P. gingivalis* in twenty-one *P. gingivalis*-positive atheromatous samples and *fimA type-II* was detected in 11 samples. 10 of these atheromatous samples belonged to periodontitis patients while only 1 subject was periodontally-healthy.[14] Other encountered genotypes were *fimA type-IV*, *fimA type-V* in the decreasing order of detection rates. In another study, out of 51 patients, 33.3% patients had *P. gingivalis-fimA gene* in subgingival as well as coronary plaque specimens.[19] In a different study, a statistical significance was reported for presence of *P. gingivalis-fimA type-II* and *T. forsythia-bspA genes* in atheromatous and subgingival plaque samples collected from ischemic heart disease patients.[25]

Detection of other microorganisms

Three studies that targetted other microorgnisms reported that DNA of *Chlamydia pneumoniae*[16], *Enterococcus faecalis*[15], *Porphyromonas endodontalis*[15] and *Streptococcus* species mainly *S. mitis* group[18] were detected in the atheromatous samples. Furthermore, two studies that employed Sanger method to sequence universal bacterial 16S rRNA gene-PCR amplicon, identified a taxonomic diversity within collected atherosclerotic plaque.[20,21] In one study, alpha diversity of 12 atherosclerotic plaque samples in terms of species richness, revealed prevalence of *Pseudomonadaceae* phylum followed by *Betaproteobacteria* and *Alphaproteobacteria*. Interestingly, fifteen phylotypes i.e. 60.9% of entire detected bacterial DNA, belonged to “yet to be cultivable or characterized species”. *Aggregatibacter actinomycetemcomitans* was the only known periodontal bacteria that was detected (20%) in the tested samples.[20] While in the other study,[21] alpha diversity of

coronary balloon specimens collected from 40 MI patients identified 68 diverse species. *Acinetobacter, Pseudomonas, Alloprevotella, Enterobacter, Sphingomonas* and *Moraxella* were prevalent genera detected around coronary balloon samples ($p<0.05$). Although not statistically significant, authors also recovered DNA from other genera such as GN02 [G-1], *Burkholderia*, *Stenotrophomonas*, *Parvimonas* and *Propionibacterium*.[21] Among known periodontal microbes, prevalence of *Porphyromonas gingivalis* was highest (67%).The beta diversity analysis comparing results from these two studies[20,21] demonstrated the highest prevalence of *Pseudomonas fluorescens.*

Quantitative analysis of microbiological findings

The objective of this review is to evaluate the prevalence of periodontal bacteria within coronary atheroma. The results of included studies, which combined targeted and community based analyses of microbial diversity, suggested the highest prevalence of *P. gingivalis* followed by *A. actinomycetemcomitans* in coronary atheromatous plaque samples. A heterogeneity analysis of the results revealed that a wide variation in detection rates of both periodontal bacteria within coronary atheromatous plaque samples exists across the included studies ($Q =114$; $P <0.001$, $I^2= 76.3\%$). For this reason, a random-effect model was selected to combine the reported detection rates of *P. gingivalis* and *A. actinomycetemcomitans*. Forest plot for prevalence of *P. gingivalis* illustrates that the higher levels of bacteria were consistently detected from the collected atheromatous plaque samples (average prevalence of 0.4, 95% CI: 0.237 to 0.556; P=0.00003).(Fig. 3a) On the other hand, meta-analysis of *A. actinomycetemcomitans* detection rates indicated that the overall prevalence of bacteria in atheromatous plaque samples was significantly low (average prevalence of 0.042, 95% CI: -0.398 to 0.282; P=0.311).(Fig. 3b)

Difference in population and sample size may also have impacted the detection rates significantly as evident by wider confidence intervals and differences in the effect size means in the forest plot. (Fig. 3). Further exploration of the potential sources of heterogeneity revealed method of

sample collection has no effect on the microbial detection rates (I^2=0) but microbial detection techniques certainly have a significant impact with I^2 of 71.(Table 5)

Covariates

The most commonly recorded are hypertension, diabetes mellitus, smoking, body mass index, lipid profile. Additionally, few studies also included white blood cell count (WBC), % neutrophils, the previous incidence of MI, the severity of coronary artery disease (single-vessel versus multi-vessels disease).(Table 4)

DISCUSSION:

Our meta-analysis indicates that levels of *P. gingivalis* were significantly higher than *A. actinomycetemcomitans* in the atheromatous plaque samples. Consistent detection of the key periodontal bacteria-*P. gingivalis* in atheromatous plaque specimens collected from MI patients bolsters the concept of bacterial translocation from inflamed periodontal sites into coronary arteries. These bacteria produce multiple virulence factors that play a cardinal role not only in destruction of periodontal apparatus but also facilitate their systemic dissemination. Among the arsenal of various virulence factors, fimbriae (fimA) of *P. gingivalis* is a critical factor that aids its colonisation within periodontal pockets. It also helps *P. gingivalis* to adhere and invade endothelial cells of blood vessels.[27,28] In the present review, 3 studies showed a high prevalence of fimA type-II and type-IV, in atheromatous samples.[14,19,25] It is noteworthy to mention that most of these atheroma specimens were obtained from MI patients having either moderate or severe periodontitis. Both type-II and IV fimA genotypes are known to be associated with severe periodontitis[29,30] and demonstrated to directly impact the process of atherosclerosis thus affecting the progression of coronary artery disease.[31] Additionally, in another case-control study, a statistical significance was observed for *P. gingivalis prtC* gene *and T. forsythia* bspA gene in atheromatous as well as subgingival plaque samples.[25] prtC gene regulates collagenase activity of *P. gingivalis* that hydrolyses type-I collagen and facilitate tissue invasion.[32] While *BspA* gene governs the expression of cell surface protein (bacteroides surface protein A), a known virulence factor of *T. forsythia*. This protein has been shown to mediate bacterial adherence and invasion of epithelial cells.[33]

Apart from known periodontal bacteria, coronary atheromatous plaque samples were also found to be positive for an array of different bacterial and viral species.(Fig. 4) Detection of multiple phylotypes within atheroma[20,21] underscores the importance of taxonomic analysis through high-throughput sequencing over targeting specific bacterial species. This claim is further bolstered by the results of a study where 15 phylotypes i.e. 60.9% of bacterial DNA, belonged to uncultivable or uncharacterized species.[20] Even though both the studies were performed in Brazil, Calandrini et al.[20] reported highest prevalence of *A. actinomycetemcomitans* while it was *P. gingivalis* in the study by Filho et al.[21] The difference in type of atherosclerotic plaque sample used i.e. coronary endarterectomy specimen[20] versus PCI balloon[21], might explain the disparity in the results.

Nonetheless, courtesy to the phylogenic diversity studies, it is evident that a variety of microbes coexist within atheroma specimens. We would like to draw readers' attention to the fact that not all the species detected within atheromatous plaque samples were commensals. For example, *Pseudomonas fluorescens* and *Enterobacter* species are the multidrug-resistant opportunistic pathogens that are known to cause bacteremia and have been associated with soft tissue and systemic infections, including endocarditis and meningitis.[34-39] The origin of these species and their role in atherosclerosis should further be investigated. Future studies need to broaden their horizon beyond the confines of the oral cavity and look for other potential sources that lead to translocation of microorganisms into the atheromatous plaques. One needs to delve deeper into analysing the role of bacteria in atherosclerosis considering most of them are known to cause opportunistic systemic infections and possess multidrug resistance genes. To elucidate the interspecies metabolic as well as ecological interactions within phylogenetic diversity of atheroma, metagenomic and metatranscriptome analyses need to be employed. Also, it is important to investigate if the presence of certain systemic risk factors create a conducive environment for microorganisms to form complex biofilms within coronary arteries.

With limited data, it is hard to conclude with certainty, if detected bacterial DNA belong to live bacteria residing within coronary atheroma or they are merely the fragments of bacterial DNA phagocytosed by host immune cells without any pathological significance. Interestingly, Pessi *et al.*[18] demonstrated the presence of viable bacteria in nine randomly selected thrombus samples (n=101) using electron microscopy. Three of the nine specimens were found to contain whole bacteria, whereas numerous bacterial components were found in the remaining six specimens. All the thrombus specimens were already termed positive for bacterial DNA using qPCR assay.

Apart from coronary artery samples, few studies also collected specimens from an internal mammary artery (IMA), saphenous veins (SVG) and peripheral arterial blood from the same patients. Higher quantity of periodontal bacterial DNA was detected in the saphenous vein of patients diagnosed with generalised severe periodontitis than in patients with generalised moderate periodontitis.[13] It has been shown that the IMA endothelium has fewer fenestrations and lower intercellular junction permeability as compared to SVG which possibly could prevent not only lipoproteins from entering the subendothelial space but also microbial attachment and colonization.[40] With the recovery of bacterial DNA from the donor vessels that are thought to be resistant to the process of atherosclerosis, one must wonder if these blood-borne microorganisms are: i) merely bystanders or, ii) if they actually induce any structural changes. None of the included studies commented on the health and patency of donor vessels. The focus of future research should be broadened to evaluate the atherosclerotic status of donor vessels and the role of above-detected microbes affecting donor vessels.

HETEROGENEITY OF STUDIES

As indicated in the quantitative analyses, a high level of heterogeneity in terms of study design was noted. A variety factors such as storage conditions, reaction reagents and primer designs, might have cumulatively contributed to the heterogeneity of the results. Their individual effect

could not be assessed either due to lack of information or great variation within them. Also, the periodontitis case definition differed greatly across all the studies. A disparity in the detection rates of periodontal bacterial species can be explained by differences in sample collection and storage, experimental techniques and conditions, host immune response, ethnic, and socio-economic status of the population examined. Unfortunately, information about these factors and thresholds of species detection were not indicated in any of the included studies. None of the included studies analysed and reported the difference in the detection rate of microorganisms obtained from MI patients with and without periodontitis. In our view, this would be a crucial aspect of future study designs in order to understand the impact of a longstanding periodontal disease on the severity of coronary artery disease.

RECOMMENDATIONS FOR FUTURE RESEARCH

Several recommendations for future research on this topic are enlisted in table 6.

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Figure 1: Possible pathway illustrating microbial dissemination from distant sources into the coronary atheromatous plaques via systemic circulation

Figure 2: Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram of literature search and paper selection process

Figure 3: a) Forest plot of studies describing prevalence of *P. gingivalis* DNA in collected coronary atheromatous plaque samples. Random-effect models estimated a significant average mean prevalence of *P. gingivalis* of 0.4 (95% CI: 0.237 to 0.556; P=0.00003), b) Forest plot of studies describing prevalence of *A. actinomycetemcomitans* DNA in collected coronary atheromatous plaque samples. Random-effect models estimated average mean prevalence of *A. actinomycetemcomitans* of 0.042 (95% CI: -0.398 to 0.282), which is statistically insignificant (P=0.311).

Figure 4: Prevalent microorganisms found within atheromatous plaque and their association with a variety of systemic illnesses

Table1: MeSH terms used to search “Population intervention/exposure comparator outcome” question

Table 2: Quality assessment of included studies using Newcastle-Ottawa Scale (N = 14)

Table 3: Characteristics of included studies (n=14)

Table 4: Periodontitis case definition, Protocol for periodontal examination, Periodontal outcomes, Source of collected atheroma, Targeted microorganisms/DNA and method detection, Covariates, Outcome of studies, Statistical significance and Reference

Table5: Heterogeneity analyses summary

Table 6: Research recommendations based on the format provided by Brown *et al.,* 2006

PICO	Search terms
Population	Cardiovascular diseases, Myocardial infarction, Coronary Artery disease, Atherosclerosis, Coronary angiography, Coronary Thrombosis, Venous Thrombosis, Thrombosis, Thromboembolism
Intervention/exposure	Periodontal diseases, Periodontitis, Chronic periodontitis, Periodontal pocket, Alveolar Bone loss
Outcome	Microbiota, microbiome, human microbiome, microbiology, bacteria, biofilm, dental biofilm, oral biofilm, dental deposits, dental plaque, *Porphyromonas gingivalis,* Bacteroides gingivalis, *Fusobacterium*, *Prevotella intermedia*, *Bacteroides intermedius, Aggregatibacter actinomycetemcomitans*, *Actinobacillus actinomycetemcomitans*, *Tannerella forsythia*, *Treponema denticola*, *Campylobacter rectus*

Sr No	Author, year	Selection	Comparability	Outcome	Score	Reference
1	Zambera M *et al.,* 2007	***	**	*	6	12
2	Elkaïm R *et al.,* 2008	****	**	*	7	13
3	Gaetti-Jardim E Jr *et al.,* 2009	****	-	*	5	14
4	Marcelino SL *et al.,* 2010	****	-	*	5	15
5	Oliveira FJ *et al.,* 2010	***	-	**	5	16
6	Aquino AR *et al.,* 2011	***	-	*	4	17
7	Pessi T *et al.,* 2013	****	**	**	8	18
8	Mahendra J *et al.,* 2013	***	-	*	4	19
9	CA Calandrini *et al.,* 2014	***	**	*	6	20
10	Serra e Silva Filho W *et al.,* 2014	***	**	**	7	21

11	Hansen GM *et al.,* 2015	***	**	**	7	22
12	Szulc M *et al.,* 2015	****	*	**	7	23
13	Mahendra J *et al.,* 2015	***	**	**	7	24
14	Mahalakshmi K *et al.,* 2017	****	**	**	8	25

Sr No	Author, year	Selection	Comparability	Outcome	Score	Reference
1	Zambera M *et al.,* 2007	***	**	*	6	12
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4	Marcelino SL *et al.,* 2010	****	-	*	5	15
5	Oliveira FJ *et al.,* 2010	***	-	**	5	16
6	Aquino AR *et al.,* 2011	***	-	*	4	17
7	Pessi T *et al.,* 2013	****	**	**	8	18
8	Mahendra J *et al.,* 2013	***	-	*	4	19
9	CA Calandrini *et al.,* 2014	***	**	*	6	20
10	Serra e Silva Filho W *et al.,* 2014	***	**	**	7	21

11	Hansen GM *et al.,* 2015	***	**	**	7	22
12	Szulc M *et al.,* 2015	****	*	**	7	23
13	Mahendra J *et al.,* 2015	***	**	**	7	24
14	Mahalakshmi K *et al.,* 2017	****	**	**	8	25

Table 4: Author and year, Periodontitis case definition, Protocol for periodontal examination, Periodontal examination outcome, Source of collected atheroma, Targeted microorganisms/DNA and method detection, Covariates, Results of studies, Statistical significance and Reference

Author	Periodontitis case definition	Protocol for periodontal examination	Periodontal outcomes	Source of collected atheroma	Targeted microorganisms/ DNA and method of detection	Covariates	Outcome of studies	Statistical significance	Reference
1. Zaremba M *et al.,* 2007	Severe generalized chronic periodontitis: >30% of dental pockets having CAL>5 mm and at least two pockets with PD>5 mm)	Full mouth periodontal examination with clinical parameters recorded: simplified plaque, and bleeding indices, percentage of (%) PD>4 mm, CAL>5 mm	1] Prevalence of periodontitis within recruited MI patients is not mentioned.	Atherosclerotic plaque samples were collected by sterile paper points from coronary arteries during CABG.	1] Detection of Aa, Pg, Pi, Ec, Tf, Cr, Td and Fn. 2] Method of detection- DNA hybridization using a nitrocellulose membrane (‘‘slot blot’’ procedure)	1] White blood cell count (WBC), 2] % neutrophils	1] In atheromatous plaques, Pg was the most frequently detected (in 10 individuals) followed by Tf (in 6 individuals)	No	12
2. Elkaïm R *et al.,* 2008	1] Moderate generalized chronic periodontitis: PD<3 mm, CAL<4 mm, *2*] Severe generalized	Full mouth periodontal examination with clinical parameters recorded: GI, SBI, PI, PD, CAL	All the patients had periodontitis and were divided into two groups 1] moderate generalized chronic periodontitis	Atheromatous plaque along with specimens of internal mammary artery and saphenous vein grafts were harvested from the	1] Detection of Aa, Pg, Pi, Cr, Ec, Tf, Fn, Td along with *Actinomyces naeslundii* (An), *Prevotella nigrescens* (Pn), *Streptococcus*	Three patients had Diabetes Mellitus.	In collected atheromatous plaque samples, Er was more prevalent in mGCP group while Cr was found to be	No	13

	chronic periodontitis: Mean PD>3 mm, CAL>4 mm		(mGCP) group (n=11) and severe generalized chronic periodontitis (sGCP) group (n=11)	patients during CABG	*mutans* (Sm), *Streptococcus sanguinis (*Ss), *Streptococcus intermedius* (Si), *Selenomonas noxia* (Sn), *Veillonella parvula* (Vp), *Streptococcus oralis* (So), *Capnocytophaga ochracea* (Co), *Porphyromonas endodontalis* (Pe), *Prevotella melaninogenica* (Pm), *Eubacterium nodatum* (En) 2] Method of detection-		prevalent in sGCP group. Although the inter-group differences were statistically insignificant.		

					modified checkerboard DNA–DNA hybridization				
3. Gaetti-Jardim E Jr *et al.,* 2009	Generalized chronic periodontitis: CAL more than 5 mm at ≥ 30% of sites	1] Full mouth periodontal examination with clinical parameters recorded: PD, CAL, % bleeding score, % plaque score	1] Based the periodontal examination participants were divided into periodontitis patients (n=39) and periodontally-healthy subjects (n=55).	Atheromatous plaques from the culprit coronary arteries were collected during endarterectomy procedure	1] Detection of Universal 16s rRNA bacterial gene, Aa, Fn, Pg, Pi, Tf and *Prevotella nigrescens* by real-time PCR 2] In twenty-one Pg-positive atheromatous samples genotyping of the gene *fim*A (Type I, Ib, II, III, IV and V) was performed	Not mentioned	1] In atherosclerotic samples of individuals with periodontitis, total bacterial DNA and periodontopathic bacteria DNA was detected in 94.9 and 92.3%, respectively. 2] Pi (59.0%), Pg (53.8%) and Aa (46.2%) were the most prevalent bacteria, followed by Tf (25.6%) and *P. nigrescens*	Yes	14

							(17.9 %), in the atheroma from individuals with periodontitis.		
4. Marcelino SL *et al.,* 2010	Chronic periodontitis patients (CAL≥5mm in 30% of teeth)	Full mouth periodontal assessment with parameters recorded: PD, PI, BoP, CAL.	Out of 30 recruited MI patients 28 individuals had periodontitis and 2 were periodontally-healthy subjects.	Atheromatous plaques samples obtained during endarterectomy procedure	Universal 16S rRNA bacterial gene, Aa, Pg, Pi, Tf, Td, Fn, Cr, and *P. endodontalis*, *P. nigrescens*, *E. faecalis* using conventional PCR	Not mentioned	1] Among the patients with periodontitis, 67.9% atherosclerotic plaque samples were positive for periodontal bacterial DNA. 2] In individuals with periodontitis, Pg was the most prevalent bacteria detected in 14 out of 28 atheromatous plaque samples	Yes	15

5. Oliveira FJ *et al*., 2010	1] Mild periodontitis- CAL<2mm and PD<3mm 2]Severe chronic periodontitis- > 4mm CAL for more than 30% of teeth and more than five periodontal pockets with PD>5 mm	1] Full mouth periodontal examination with clinical parameters recorded: CAL and PD	Out of 118 recruited MI patients, 20 individuals had severe periodontitis and remaining 68 had mild periodontitis	During CABG procedure, coronary atheromatous plaque samples were obtained from 17 individuals along with equal number of specimens of internal mammary graft artery as a controls	1] Detection of Universal 16S rRNA bacterial gene, Aa, Pg, Pi, Tf and *C. pneumonia* using conventional PCR	1] BMI, 2] lipid profile 3] hematological and glycaemic profiles.	1] The prevalence of periodontal bacteria within 17 coronary atheromatous plaques samples- *Pg* (52.9%), *Aa* (35.3%), *Pi* (23.5%), and *Tf* (11.7%). 2] Seven (41.1%) specimens were positive for two or more periodontal bacteria. 3] No periodontal bacterial DNA was found in any of internal mammary specimens.	Yes	16

6. Aquino AR *et al.,*2011	1] Mild periodontitis-CAL<2mm and PD<3mm 2]Severe chronic periodontitis-> 4mm CAL for more than 30% of teeth and more than five periodontal pockets with PD>5 mm	1] Periodontal examination protocol is not mentioned 2] Clinical parameters recorded: PD, BoP and gingival recession in mm	1] Out of 20 recruited dentate patients, 19 had severe periodontitis and 1 had moderate periodontitis 2]10 patients had no natural teeth	1] Coronary atheromatous plaque samples (5) were obtained using distal protection filters during angioplasty 2] Additionally, femoral donor graft specimens (17) were collected during CABG	1] Detection of universal 16S rRNA bacterial and members of Archaea gene, universal 18S rRNA gene for ungi along with species specific primers of periodontal pathogens Pg, Td and Aa 2] Method of detection was conventional PCR	1] In recruited patients, 56.7% were smokers and ex-smokers and 46.7% were diabetic 2] >2/3 of the recruited patients had arterial hypertension (76.7%)	1] Periodontal bacteria DNA (Pg, Td, Aa) was not found in any of atheromatous plaque samples or femoral arteries. 2] Four out of twenty samples were positive for universal bacterial DNA using 16S rRNA primers. However, these bacteria were not sequenced and identified.	No	17
7. Pessi T *et al*., 2013,	Periodontitis was defined based on dental	Out of one hundred one patients, thirty were subjected	Out of thirty patients that underwent dental panoramic	1] Thrombus aspirates were collected during	1] Detection of total bacterial DNA with periodontal	1] triglycerides,	**A] Thrombus Aspirates:** 1] Total bacterial DNA in thrombi	Yes	18

	panoramic tomography: 1] Vertical bony pockets (depth >3 mm) 2] Furcation lesions (grade III; e.g. no jaw bone left at the base of the root trunk of a tooth where ≥2 roots meet)	to dental panoramic tomography	tomograpical analysis, fifteen (50%) patients showed presence of vertical pockets and nineteen (63.3%) patients had furcation involvement	primary PCI procedure 2] Peripheral arterial blood samples were used as controls	bacteria: Pg, Aa, Fn, Td, Pi, *Dialister pneumosintes* 2] Streptococcus sp.mainly *S. mitis group, S. oralis, S. sanguinis, S. gordonii, Streptococcus anginosus group, Staphylococcus aureus, S epidermidis, Parvimonas micra*, as well as *Chlamydia pneumonia* 2] Method of detection- real time PCR assay	2] LDL, 3] HDL, 4] total cholesterol, 5] smoking, 6] diabetes mellitus, 7] hypertension.	was 16 times higher (median value) than in peripheral arterial blood samples. 2] Most prevalent species was Streptococcus sp.(mainly *S. mitis* group, *S. oralis*) (72.3%). 3] Overall, periodontal pathogens were detected in 34.7% with Aa in 6 (5.9%), and Pg in 5 (5.0%) aspirates. **B] Arterial Blood Samples:**		

					2. Additionally, electron microscopy used for the detection of viable bacterial cells in 9 frozen thrombus aspirates.		16 arterial blood samples were positive for: *S. mitis (10), S. mitis and S. oralis*, (1), *S. epidermidis and S. aureus (1)*, Pg (1), Pi (1), Fn (1), and *D Pneumonistes (1)*. **C] Bacterial DNA and angiographic data:** A significant inverse association was found between *S. mitis* DNA and the number of stenotic arteries (narrowing of		

							>50% as quantified on coronary angiography. The percentage of bacterial positivity was lowest (50.0%) in individuals with 3-vessel disease compared with 1-vessel (74.5%) or 2-vessel (81.3%) disease.		
8. Mahendra J *et al.,* 2013	Chronic generalized periodontitis: CAL≥3mm and alveolar bone exceeding 1/3 of the root in	1] The protocol for periodontal examination is not mentioned 2] PD, CAL, PI, GI, Oral Hygiene Index were recorded	Periodontal examination data of the recruited patients is missing	A biopsy atheromatous plaque was procured from coronary artery during the CABG	Detection of universal 16S rRNA bacterial gene, Aa,Pg,Tf,Td, and *fim*A gene of Pg was done using conventional PCR	Not mentioned	There was statistically significant association between presence of Tf in the atherosclerotic plaque with GI	Yes	19

	at least 30% of the entire dentition						(P= 0.03) and PD (P= 0.01).		
9. CA Calandrini *et al.,* 2014,	1] Initial periodontitis- 1 to 30% periodontal sites with >3 mm, ≤5 mm; 2] Moderate and advanced periodontitis- CAL of >3 mm, ≤5 mm, and >5 mm, in >30% of the periodontal sites.	Full mouth periodontal examination with clinical parameters recorded: PD, CAL, PI, BoP(%)	1] From 35 recruited participants six (17.1%) had no natural teeth 2] In remaining 29 patients, 7(20%) individuals were periodontally-healthy; 1 (2.9%) had initial periodontitis; 12 (34.3%) had moderate periodontitis and 9 (25.7%) had advanced periodontitis	During endarterectomy, atheromatous plaque samples were collected	Detection of universal 16S rRNA bacterial gene using conventional PCR, followed by Sanger sequencing of PCR-amplicon	Not mentioned	1] Clinical data: There was no significant association (P<0.05) between atheromatous plaque bacteria and clinical parameters. 2] Bacterial analysis: Taxa Proteobacteria 78.3% and Firmicutes 21.7% were observed. Aa was identified in 20% of samples.	No	20

10. Serra e Silva Filho W *et al.,* 2014	Generalized moderate to severe chronic periodontitis: PD ≥ 5mm on at least four teeth	Full mouth periodontal examination performed with clinical parameters recorded: PD, BoP, PI and number of missing teeth	All eighteen recruited patients had generalised moderate to severe periodontitis	All patients were diagnosed with coronary artery atherosclerosis (OCAA). The balloons used during percutaneous transluminal coronary angioplasty procedure were collected	Detection of universal 16S rRNA bacterial gene using conventional PCR, followed by sequencing of PCR-amplicon	1] Three subjects withType-2 diabetes mellitus (presenting HbA1c, 7%) 2] 3 had osteoporosis, 3] 10 were smokers	1] Genera identified in coronary balloons were- *Alloprevotella , Acinetobacter Pseudomonas, Enterobacter, Sphingomonas* and *Moraxella* 2] From periodontal bacteria, Pg was the most prevalent (67%) microorganism	Yes	21
11. Hansen GM *et al*., 2015	Not mentioned	A telephonic questionnaire was administered to determine whether	Out of 45 recruited participants 39.5% had periodontitis diagnosed by their own dentists	1] Coronary angioplasty balloons and segment of catheter (as a control) was	Detection by universal 16S rRNA bacterial gene using conventional PCR	Not mentioned	No bacterial DNA was detected from any of the collected samples	No	22

		periodontitis was diagnosed by a treating dentist at the time of PCI		collected during primary PCI 2] Additionally, blood samples from femoral artery was used as control					
12. Szulc M *et al*., 2015	1] Moderate periodontitis- at least a pocket with ≥ 5mm 2] Severe periodontitis- at least a pocket with ≥ 7mm	Full mouth periodontal examination with clinical parameters recorded: API, BoP, and PD	Out of 91 recruited patients, 32 had periodontitis with coronary artery disease	Atheromatous plaque samples were collected using sterile paper points were inserted into the coronary vessel during CABG procedure	Detection of Pg DNA using conventional PCR	Not mentioned	Out of 32 coronary atheromatous plaque samples, 3 (9.4%) were positive for Pg DNA	No	23
16. Mahendra J *et al.,* 2015	Generalized chronic periodontitis- CAL having	Full mouth periodontal examination with clinical parameters	1] Prevalence of periodontitis in 51 MI patients is not mentioned	In 51 MI patients, a surgeon excised a small bit (0.5–1 cm) of culprit coronary artery during CABG	Detection of Pg, Tf, Cr, Ec, Pg, Pg (*fim*A), Td and *Prevotella*	Not mentioned	1] Out of 51 tested specimens, prevalence of Pg 20(39.21%) was highest, followed	Yes	24

	≥30% sites with ≥5 mm	recorded: PI, OHI, PD, CAL	2] The mean CAL in MI group was 5.61±1.20 and mean PD was 6.01±8.8, which was significantly more than 51 healthy-cardiac control participants		*nigrescens* by a conventional PCR		by Td in 18(35.29%). 2] 18 out of 20 Pg-positive specimens were positive for presence of fimA gene. 3] No atherosclerotic plaque samples were positive for Aa.		
17. Mahalakshmi K *et al.,* 2017	Chronic periodontitis - more than 3 teeth with PD > 4 mm and bleeding on probing	1] Periodontal examination protocol is not mentioned 2] Periodontal parameters recorded: PD, CAL and BoP	1] Prevalence of periodontitis in 65 Ischemic heart disease (IHD) patients is not mentioned. 2] In IHD group, mean PD (PD)	1] Vascular tissues from IHD patients were collected. 2] No details of the type of procedure is mentioned.	1] Detection of Aa, Tf, Pg, Td, Ec, Cr, Pi, and *Prevotella nigrescens using conventional PCR.* 2] Additionally, samples were	Not mentioned	1] Out of 65 atheromatous plaque samples, prevalence of Td was highest in 34(64.60%) samples, followed by Pg in	Yes	25

			was 5.27 ± 1.00 mm and mean CAL was 6.28 ± 1.32mm, which was significantly (p<0.0001)less than systemically healthy individuals (n=59) with periodontitis-control group.		analysed for 5 virulence genes (*P. gingivalis Type II fimA, P. gingivalis prtC, T.forsythia bspA, T.forsythia prtH, T.denticola fhbB)* using conventional PCR.		34(52.30%) samples. 2] 43(50.8%) samples demonstrated co-prevalence of Td and Pg. 3] Out of 65 atheromatous plaque samples, 59.1% were positive for T. forsythia bspA gene and 46.8% were gingivalis Type II fimA gene.		

Abbreviations: Pocket Depth/Probing Depth- PD, Clinical attachment level/loss- CAL, Plaque index- PI, Gingival index- GI, Bleeding on probing:BoP, Sulcular bleeding index-SBI, BMI-Body Mass Index, Oral Hygiene Index- OHI. MI- Myocardial infarction, low density lipoprotein-LDL, high-density lipoprotein- HDL, Coronary artery bypass grafting (CABG), Approximal plaque index- API, Polymerase Chain Reaction- PCR, Quantitative polymerase Chain Reaction- qPCR, millimetres- mm, *Porphyromonas gingivalis*- Pg, *Aggregatibacter actinomycetemcomitans*- Aa, *Tannerella forsythia*- Tf, *Treponema denticola*- Td, *Fusobacterium nucleatum*- Fn, *Campylobacter rectus*- Cr, *Prevotella intermedia*- Pi, *Eikenella corrodens*- Ec, *Chlamydia pneumonia*- Cp

		Number of studies	Effect size	CI 95 % Lower CI	CI 95 % Upper CI	I^2 heterogeneity
1	All studies	14	0.301	-0.198	0.390	19.274
	Microorganism wise					
2	Studies positive for *P. gingivalis*	10	0.397	0.237	0.552	0.000
3	Studies positive for *A. actinomycetemcomitans*	6	0.179	-0.398	0.282	16.031
	Influence of different factors					
4	Methods of atheromatous plaque collection	14	0.378	0.217	0.540	0.000
5	Microbial detection techniques	14	0.394	0.242	0.547	70.543

		Number of studies	Effect size	CI 95 % Lower CI	CI 95 % Upper CI	I^2 heterogeneity
1	All studies	14	0.301	-0.198	0.390	19.274
	Microorganism wise					
2	Studies positive for *P. gingivalis*	10	0.397	0.237	0.552	0.000
3	Studies positive for *A. actinomycetemcomitans*	6	0.179	-0.398	0.282	16.031
	Influence of different factors					
4	Methods of atheromatous plaque collection	14	0.378	0.217	0.540	0.000
5	Microbial detection techniques	14	0.394	0.242	0.547	70.543