

Periodontal Infections and Coronary Heart Disease

Role of Periodontal Bacteria and Importance of Total Pathogen Burden in the Coronary Event and Periodontal Disease (CORODONT) Study

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Background: Chronic inflammation from any source is associated with increased cardiovascular risk. Periodontitis is a possible trigger of chronic inflammation. We investigated the possible association between periodontitis and coronary heart disease (CHD), focusing on microbiological aspects.

Methods: A total of 789 subjects (263 patients with angiographically confirmed, stable CHD and 526 population-based, age- and sex-matched controls without a history of CHD) were included in the Coronary Event and Periodontal Disease (CORODONT) study. Subgingival biofilm samples were analyzed for periodontal pathogens *Actinobacillus actinomycetemcomitans*, *Tannerella forsythensis*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Treponema denticola* using DNA-DNA hybridization. The need for periodontal treatment in each subject was assessed using the Community Periodontal Index of Treatment Needs (CPITN). The main outcome measures included total periodontal pathogen burden, number of the vari-

ous periodontal pathogens in the subgingival biofilm, and periodontal treatment needs (according to the CPITN).

Results: In multivariable analyses, we found a statistically significant association between the periodontal pathogen burden (\log_{10} of the sum of all pathogens) (odds ratio [OR], 1.92; 95% confidence interval [CI], 1.34-2.74; $P < .001$) or the number of *A actinomycetemcomitans* in periodontal pockets (\log_{10}) (OR, 1.79-4.07; $P < .001$) and the presence of CHD. In addition, a statistically significant association between an increase in mean CPITN score by 1 and the presence of CHD (OR, 1.67; 95% CI, 1.08-2.58; $P = .02$) was observed.

Conclusions: Our findings suggest an association between periodontitis and presence of CHD. Periodontal pathogen burden, and particularly infection with *A actinomycetemcomitans*, may be of special importance.

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CARDIOVASCULAR DISEASES still represent the major cause of death in industrialized countries. Although the traditional risk factor concept has been well established, it does not fully account for the risk of cardiovascular disease.^{1,2} Inflammation plays an important role in atherothrombogenesis and its clinical complications, so recent research has aimed to identify potential causes of chronic inflammation, among them infections with various agents.^{3,4} Periodontitis is a chronic infection of the periodontium with a high prevalence in the general population and is associated mainly with gram-negative bacteria.^{5,6} Several studies have investigated the association between periodontal disease and atherosclerosis or its major clinical complication, coronary heart disease (CHD). These studies suggest that poor dental health and periodontal bone loss may be associated with CHD events, even after adjustment

for established cardiovascular risk factors.⁷⁻¹⁰ However, some studies found no such association.^{11,12} Thus, the issue of an association between periodontitis and CHD remains controversial.

Various dental procedures, routine oral hygiene efforts, and even gentle mastication may lead to the entry of bacteria or bacterial endotoxins from the oral cavity into the bloodstream; therefore, bacterial infection, antigens, endotoxins, and proinflammatory cytokines triggering a systemic response may represent the link between periodontitis and CHD.¹³⁻¹⁷ We sought to investigate the potential association between periodontitis and CHD in a large case-control study, focusing on the microbiological features of this disorder. More specifically, we wanted to determine whether the prevalence of periodontal pathogens and the pathogen burden in the subgingival biofilm are increased in patients with CHD compared with control subjects.

Table 1. 16S Ribosomal DNA- and Ribosomal RNA-Directed Probes

Species	Sequence of Probe (5' to 3')	Length (Nucleotides)	Accession No. of 16S Ribosomal RNA Sequence
<i>Actinobacillus actinomycetemcomitans</i>	CAC TTA AAG GTC CGC CTA CGT GCC	24	M75035
<i>Porphyromonas gingivalis</i>	CAA TAC TCG TAT CGC CCG TTA	21	L16492
<i>Prevotella intermedia</i>	GTT GCG TGC ACT CAA GTC CGC C	21	X73965
<i>Tannerella forsythensis</i>	CGT ATC TCA TTT TAT TCC CCT GTA	24	L16495
<i>Treponema denticola</i>	GGC TTA TTC GCA TGA CTA CCG T	22	M71236

METHODS

PATIENTS AND CONTROLS

The Coronary Event and Periodontal Disease (CORODONT) study is a case-control study in which patients and controls were recruited between October 1, 2000, and August 31, 2002. Cases consisted of 263 patients aged 43 to 73 years with clinically stable CHD who had participated in an earlier case-control study with follow-up after approximately 3 years. All patients had undergone elective coronary angiography at the University of Ulm Medical Center in 1996-1997 and had at least 1 stenosis that was 50% or more of the luminal diameter of a major coronary artery. Details of the initial study design and the methods used have been reported elsewhere.¹⁸ Patients with acute coronary syndromes or undergoing anticoagulant therapy within the previous 4 weeks were excluded.

The control group consisted of 526 subjects, individually matched for age and sex and randomly selected from the residents' registration office of the city of Ulm. Two controls for each case were chosen to increase the power of the study. Control subjects had no history of definite or suspected CHD.

None of the study participants had any of the following disorders (possibly associated with an acute-phase reaction): febrile acute infection or acute state of a chronic infection or an inflammatory disease; underlying hematologic or malignant diseases; severe liver or renal disorders; surgery within the previous 4 weeks; or oral surgery or tooth extraction, particularly within the last 7 days. Subjects with missing teeth or edentulous persons were not excluded from the study.

The participation rate was 71% in eligible patients and 67% in eligible control subjects. All subjects underwent a standardized interview performed by trained personnel. Participants were asked about their medical history, including current medication intake and specific questions related to physician-diagnosed diseases: hypertension, diabetes mellitus, and gastro-duodenal diseases. Furthermore, sociodemographic characteristics and lifestyle habits were recorded. Finally, information concerning the oral system, such as former diagnosis of periodontitis, family history of periodontitis, tooth loss due to periodontitis, gingival inflammation, and oral hygiene, was collected. Written informed consent was obtained from each subject. The study was approved by the ethical committee of the University of Ulm.

ORAL EXAMINATION

Periodontal examinations were performed by one dentist (E.K.), and a modified Community Periodontal Index of Treatment Needs (CPITN) was used.¹⁹ In each subject, the CPITN was measured at 6 sites of each tooth. The oral cavity was divided into sextants; for each sextant the highest index found was recorded, applying the following scores: 0 indicates periodontal health; 1, gingival bleeding; 2, calculus and/or overhanging res-

tations; 3, pocket depth of 4 to 5 mm; and 4, pocket depth of 6 mm or greater. Finally, the mean CPITN score of each subject (ie, mean index score of all sextants) was calculated. Additionally, the number of missing teeth and the number of toothless sextants were recorded.

LABORATORY METHODS

For detection of the periodontal pathogens *Actinobacillus actinomycetemcomitans*, *Tannerella forsythensis*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Treponema denticola*, a standardized dot blot hybridization assay with species-specific oligonucleotide probes for semiquantitative detection was performed.²⁰⁻²² Subjects with complete tooth loss were excluded from microbiological evaluation. In all other subjects, pooled subgingival biofilm samples were obtained from the 4 deepest pockets, preferably in different quadrants, of each subject. The microbiological sampling was performed using sterile absorbent paper points (ISO 40; Roeko, Langenau, Germany). After supragingival debridement, 1 paper point was inserted in every selected pocket until the perceived bottom of the pocket was reached. Every paper point remained in its pocket for 10 seconds and was then placed into a collection tube. The collection tubes, which contained the 4 paper points with the subgingival biofilm samples, were kept at -20°C until further use. For hybridization, specific oligonucleotide probes (**Table 1**) derived from 16S ribosomal RNA sequences of the bacterial species and labeled with digoxigenin-11-deoxyuridine 5-triphosphate molecules (Roche Applied Science, Mannheim, Germany) were used. Quantification was performed using DNA of the defined cell number of the respective bacterial species in decreasing concentrations and by scanning densitometry. All laboratory analyses were performed in a blinded fashion.

STATISTICAL ANALYSIS

All data were double entered into a database by 2 independent persons to ensure data quality. For quantitative variables, the median or the mean and standard deviation were calculated. For further analyses, logarithmic transformation of the number of pathogens was performed. Values below the detection limit were set to 500 beforehand. For categorical variables, relative frequencies are reported.

For all potential periodontal and microbiological risk factors, crude and adjusted odds ratios (ORs) with their 95% confidence intervals (CIs) and the respective *P* values were calculated by means of conditional logistic regression.²³ Multivariable logistic regression analyses for common effects of the various periodontal pathogens were not performed because of multicollinearity. In multivariable analyses, adjustment was made for age, sex, body mass index (calculated as weight in kilograms divided by the square of height in meters), smoking behavior (never, ex-smoker, or current smoker), alcohol consumption (grams per day), history of diabetes mellitus, history of hyper-

Table 2. Characteristics of the Study Population*

Characteristic	Cases (n = 263)	Controls (n = 526)
Male†	87	87
Age, mean (SD), y†	61.0 (7.1)	61.0 (7.1)
BMI, mean (SD)	28.3 (3.5)	26.7 (3.7)
Smoking status		
Current	14	15
Past	60	45
Never	26	40
Alcohol consumption		
Daily	30	33
No alcohol	13	9
Occasionally	57	58
History of myocardial infarction	68	0
History of hypertension	60	36
History of hyperlipoproteinemia	78	35
History of diabetes mellitus	14	6
School education <10 y	71	44
Physical activity‡	84	67
Total cholesterol, mean (SD), %	202.2 (45.2)	223.1 (34.1)
Statin intake	73	4

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).

*Data are given as percentage of subjects unless otherwise indicated.

†Matching variables.

‡Less than 2 hours per week.

tension and hyperlipoproteinemia, years of formal education (<10 years or ≥10 years), leisure-time physical activity (≤2 h/wk or >2 h/wk), and intake of statins (yes or no). A 2-sided $P < .05$ was considered statistically significant. Data processing and all statistical analyses were performed using the SAS software package (version 8.2 for Windows NT; SAS institute Inc, Cary, NC).

RESULTS

CHARACTERISTICS OF THE STUDY POPULATION

Demographic characteristics and classic risk factors in cases and controls are presented in **Table 2**. Age and sex (matching variables) were identical in both groups. Body mass index was higher in cases than in controls. A history of hypertension, hyperlipoproteinemia, diabetes, low school education, low physical activity, and past smoking was more prevalent in cases than in controls. Sixty-eight percent of the patients reported a previous myocardial infarction. No differences were seen in alcohol consumption habits and the percentage of current smokers, whereas never smokers were more frequent among controls. As expected, the percentage of subjects taking statins was higher in cases.

PERIODONTAL EVALUATION

Slightly more cases than controls showed periodontal pockets of 4 mm or greater (CPITN score of 3 or 4, 95.5% and 92.8%, respectively). The number of subjects showing pockets of 6 mm or greater (CPITN score of 4) was almost identical in cases and controls (57.2% and 57.9%,

respectively). The mean (SD) CPITN score in cases (2.8 [0.8]) was slightly higher compared with controls (2.7 [0.9]). However, conditional logistic regression analysis revealed that an increase of the mean CPITN score by 1 was statistically significantly associated with stable CHD (OR, 1.23; 95% CI, 1.02-1.49; $P = .03$). This was even more pronounced after adjustment for potential confounders (OR, 1.67; 95% CI, 1.08-2.58; $P = .02$).

Although in 8% of cases a complete loss of teeth was observed, this was present in only 2% of controls. Partial tooth loss was found in 27% of cases compared with only 19% of controls. Also, the mean number of toothless sextants was more than twice as high in cases (1.3) compared with controls (0.6).

MICROBIOLOGICAL EVALUATION

The prevalence of the various periodontal pathogens was not statistically significantly different between cases and controls (85% and 81% for *A actinomycetemcomitans*, 76% and 84% for *P gingivalis*, 62% and 81% for *T forsythensis*, 80% and 89% for *P intermedia*, and 60% and 76% for *T denticola*, respectively). However, patients showed a considerably higher total periodontal pathogen burden in their subgingival biofilm compared with controls (**Table 3**). Using conditional logistic regression analysis, total pathogen burden (increase in \log_{10} of the sum of all pathogens) was strongly associated with CHD (unadjusted OR, 1.22; 95% CI, 1.06-1.41; $P = .006$), and this was even more pronounced in multivariable analyses (OR, 1.92; 95% CI, 1.34-2.74; $P < .001$) (Table 3). The median pathogen burden was more than twice as high in cases compared with controls. Although a higher number of *A actinomycetemcomitans* could be found in subgingival plaque samples of cases compared with controls, no or only minor differences were detected in the other tested pathogens (Table 3). After multivariable adjustment, a statistically significant association between a single periodontal pathogen and CHD could be found only for *A actinomycetemcomitans* (\log_{10}) (OR, 2.70; 95% CI, 1.79-4.07; $P < .001$) and *P intermedia* (OR, 1.43; 95% CI, 1.00-2.03; $P = .049$) (Table 3). Other periodontal pathogens failed to show an association with CHD (Table 3).

To further evaluate whether either the mean CPITN score or the microbiological parameters *A actinomycetemcomitans*, *P intermedia*, or total periodontal pathogen burden exerts the strongest effect in multivariable analyses, each of the microbiological variables was entered simultaneously with the mean CPITN score in a conditional logistic regression model. These analyses revealed that the total periodontal pathogen burden and in particular the number of *A actinomycetemcomitans* were stronger predictors for CHD than the clinical parameter mean CPITN score, which was no longer statistically significant when entered simultaneously in a model with microbiological parameters (**Table 4**).

COMMENT

In this large case-control study, we evaluated the potential association between periodontitis and CHD, focus-

Table 3. Association Between Periodontal Pathogens and Coronary Heart Disease by Conditional Logistic Regression Analysis*

Variable	Median Log Value		Unadjusted		Adjusted†	
	Cases	Controls	OR (95% CI)	P	OR (95% CI)	P
				Value		Value
Total periodontal pathogen burden	79 × 10 ³	38 × 10 ³	1.22 (1.06-1.41)	.006	1.92 (1.34-2.74)	<.001
<i>Actinobacillus actinomycetemcomitans</i>	30 × 10 ³	8 × 10 ³	1.66 (1.42-1.95)	<.001	2.70 (1.79-4.07)	<.001
<i>Porphyromonas gingivalis</i>	5 × 10 ³	8 × 10 ³	0.89 (0.75-1.05)	.16	1.36 (0.94-1.95)	.10
<i>Tannerella forsythensis</i>	1 × 10 ³	5 × 10 ³	0.66 (0.54-0.81)	<.001	0.95 (0.65-1.39)	.79
<i>Prevotella intermedia</i>	10 × 10 ³	10 × 10 ³	0.99 (0.84-1.16)	.86	1.43 (1.00-2.03)	.049
<i>Treponema denticola</i>	1 × 10 ³	5 × 10 ³	0.66 (0.55-0.81)	<.001	0.94 (0.66-1.34)	.74

Abbreviations: CI, confidence interval; OR, odds ratio.

*For an increase in periodontal pathogens of log₁₀, which is the amount of pathogens logarithmically transformed to base 10.

†Adjusted for age, sex, body mass index (calculated as weight in kilograms divided by the square of height in meters), smoking, alcohol consumption, diabetes mellitus, hypertension, hyperlipoproteinemia, level of education, physical activity, and statin intake.

ing on the microbiological characterization of periodontitis. We found a strong and statistically significant association between periodontal pathogen burden and CHD. Among the various periodontal pathogens assessed, a statistically significant association with CHD could be established for *A actinomycetemcomitans* and *P intermedia*. We also found a statistically significant association between mean CPITN and CHD. However, microbiological parameters such as total periodontal pathogen burden and especially the amount of *A actinomycetemcomitans* in the periodontal pockets seemed to be stronger risk factors for CHD than clinical parameters.

CLINICAL ORAL CONDITIONS AND CHD

Results from several studies²⁴⁻²⁶ have suggested an association between the prevalence of periodontal disease, as measured by CPITN, and CHD. Two meta-analyses and 1 systematic review concluded that periodontitis was moderately but significantly associated with CHD and stroke.²⁷⁻²⁹ These findings are in accordance with the results of the present study. Even though it has been suggested that the CPITN may underestimate the extent and severity of periodontitis among elderly individuals, as examined in this study, a statistically significant relationship was found between mean CPITN score and risk of CHD,^{5,6,19,30} thus indicating that the real association may be even stronger. The mean CPITN values and the risk estimates found in the present study were in the same range as those reported in the literature.^{31,32}

The association between tooth loss and CHD observed in the present study is also in agreement with other studies,^{9,33-35} which reported a relationship between tooth loss and other atherosclerosis end points. Periodontal disease represents the main cause for tooth extraction after the age of 40 years. It has been suggested that changes in dietary behavior due to reduced chewing ability and increased intake of soft high-calorie food with larger amounts of carbohydrates and fat may underlie this relationship.³²⁻³⁷ However, periodontitis itself was shown to be accompanied by a proatherogenic lipid profile.^{25,38} Furthermore, since clinical signs of periodontitis represent the result of an interaction between infectious bur-

Table 4. Association Among Periodontal Pathogens, CPITN, and Coronary Heart Disease Simultaneously Assessed in the Same Basic Model*

Variable	OR (95% CI)	P Value
Total periodontal pathogen burden, log	1.83 (1.23-2.71)	.003
CPITN score, mean	1.15 (0.70-1.89)	.58
<i>Actinobacillus actinomycetemcomitans</i> , log	2.68 (1.74-4.14)	<.001
CPITN score, mean	1.02 (0.62-1.70)	.93
<i>Prevotella intermedia</i> , log	1.25 (0.85-1.84)	.26
CPITN score, mean	1.48 (0.92-2.39)	.10

Abbreviations: CI, confidence interval; CPITN, Community Periodontal Index of Treatment Needs¹⁹; OR, odds ratio.

*Adjusted for age, sex, body mass index (calculated as weight in kilograms divided by the square of height in meters), smoking, alcohol consumption, diabetes mellitus, hypertension, hyperlipoproteinemia, level of education, physical activity, and statin intake. For an increase in mean CPITN score of 1 unit and an increase in periodontal pathogens of log₁₀, pathogen numbers were logarithmically transformed to base 10.

den and host response, it has been hypothesized that the presence of oral pathogens may better explain the possible relationship between periodontitis and CHD.

MICROBIOLOGICAL ASPECTS OF PERIODONTITIS AND ASSOCIATION WITH CHD

The negative impact of an oral infection on systemic health results from the entry of oral pathogens or their products into the bloodstream.^{32,39} The incidence of bacteremia after dental procedures such as tooth extraction or periodontal treatment has been well documented.^{32,40} Interestingly, even normal mastication and tooth brushing induce bacteremia.⁴¹ The exposure time to bacteremia during a 1-month period was shown to be 1000 times greater from routine chewing and tooth brushing than from dental extraction.⁴¹ Furthermore, the inflammatory activity of periodontitis may increase the presence of bacteria in the bloodstream.^{39,42,43} The loss of epithelial integrity in periodontal pockets (approximately 8-20 cm² of periodontal pocket tissue) creates ample oppor-

tunity for bacteremia.⁴⁴ Therefore, it can be assumed that chronic periodontitis repeatedly results in systemic exposure to periodontal pathogens and/or their products, as supported by higher serum IgG titers. Moreover, recent data demonstrated the capability of *A actinomycetemcomitans*, *P gingivalis*, and *P intermedia* to invade the coronary endothelium and the presence of viable *A actinomycetemcomitans* and *P gingivalis* in human atherosclerotic plaque.⁴⁵⁻⁴⁹ In addition, *A actinomycetemcomitans* may gain access to the circulation even through intact oral tissue.⁴⁹ In the present study, however, no differences in the prevalence of periodontal pathogens could be observed between cases and controls. Most subjects tested positive for all species investigated, albeit at low levels. Similar observations were made by Papapanou et al⁴⁶ in periodontally healthy subjects. Therefore, to merely dichotomize subjects into those with and without oral pathogens, without quantification of the actual bacterial load, may not be adequate.⁴⁶ Accordingly, the results of the quantitative microbiological analysis in the present study showed a markedly higher total periodontal pathogen burden and a higher number of *P intermedia* and in particular *A actinomycetemcomitans* in the subgingival biofilm of patients with CHD compared with controls, thus corroborating our hypothesis.

IS THERE A PARTICULAR ROLE FOR *A ACTINOMYCETEMCOMITANS*?

The potentially eminent role of *A actinomycetemcomitans* may, at least in part, be explained by its specific colonization pattern. *Actinobacillus actinomycetemcomitans* was shown to be one of the first colonizers on supragingival tooth surfaces in early plaque development, suggesting that this species is able to colonize an even healthy and clean oral cavity. The relationship between *A actinomycetemcomitans*-associated infections and age indicates that this pathogen is acquired at a young age and that the disease also starts early.⁵⁰ Comparative statistical analyses in the present study further revealed that the microbiological parameters, total periodontal pathogen burden, and especially the amount of *A actinomycetemcomitans* seem to be of greater importance as potential risk factors for CHD than the clinical parameter CPITN score. These results are supported by a recent study⁵¹ showing that elevated serum anti-*A actinomycetemcomitans* antibody levels predict stroke.

MECHANISTIC ASPECTS LINKING PERIODONTAL PATHOGENS TO CHD

Periodontal pathogens may increase the risk of CHD through various mechanisms (eg, by platelet activation and aggregation).⁵² Experimental studies suggest the potential of periodontal pathogens or their respective products, such as lipopolysaccharide, to activate mononuclear phagocytes. The potencies of lipopolysaccharide isolates seem to rank as follows: *P gingivalis* less than *P intermedia* less than *A actinomycetemcomitans*. Low concentrations of lipopolysaccharide from *A actinomycetemcomitans* stimulate human macrophages to profoundly increase secretion of interleu-

kins 1 α and 1 β and tumor necrosis factor, all representing cytokines involved in the inflammatory response in atherothrombogenesis.^{53,54} Furthermore, it has been demonstrated that macrophages can accumulate cholesterol-rich lipids such as oxidized low-density lipoprotein and convert to large foam cells on interaction with periodontal pathogens.⁵²

STRENGTHS AND LIMITATIONS OF THE STUDY

Our study has several strengths. We included a large number of consecutive patients with angiographically defined and clinically stable CHD. We used population-based controls from the same catchment area as the cases, individually matched for age and sex, and a sampling ratio of 1:2 was intended to ensure adequate power. To the best of our knowledge, the present study is the first to thoroughly assess the relationship between colonization of the periodontal pockets with periodontal pathogens and presence of CHD. Furthermore, we tried to reduce potential confounding by simultaneously controlling for a large number of indicators of health and socioeconomic status.

The present study also has potential limitations that should be considered. First, a case-control design does not allow assessment of the causal role of periodontal pathogens in the initiation or progression of the atherothrombotic process. However, the primary aim of our study was to investigate the potential association between periodontitis and CHD, focusing on microbiological aspects after carefully controlling for covariates. Furthermore, subclinical CHD in controls cannot be ruled out completely, since no electrocardiogram or coronary angiogram was obtained. However, the prevalence of CHD in an asymptomatic middle-aged population appears to be low (2%-4%),⁵⁵ and selection of controls among subjects who undergo coronary angiography for various reasons would possibly introduce a more severe bias.

In summary, we found a statistically significant association between periodontitis and the presence of CHD, even after controlling for a variety of potential confounders. Microbiological parameters, such as total periodontal pathogen burden and especially the amount of *A actinomycetemcomitans* in the periodontal pockets, seem to be of greater importance as potential risk factors for CHD than the clinical parameter CPITN score.

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