Periodontal Destruction Is Associated With Coronary Artery Disease and Periodontal Infection With Acute Coronary Syndrome

Israel Gotsman,* Chaim Lotan,* W. Aubrey Soskolne,† Simona Rassovsky,† Thea Pugatsch,* Ludmila Lapidus,* Yelena Novikov,* Siham Masrawa,* and Ayala Stabholz†

Background: Coronary artery disease (CAD) is a highly prevalent disease with significant morbidity and mortality. Periodontal disease has been suggested to influence this disease and has been associated with CAD in some epidemiologic studies. However, this relation is still controversial. This study aimed to determine the relationship between periodontal disease measures and CAD and acute coronary syndromes (ACSs).

Methods: Two hundred one patients presenting with stable angina or ACS referred for coronary angiography underwent a periodontal assessment including evaluation of periodontal pathogens. Severity of CAD was determined by the number of obstructed coronary arteries.

Results: Patients with severe CAD defined by multiple vessel disease had significantly more periodontal destruction than those with mild CAD, as shown by mean clinical attachment level, a measure of chronic periodontal disease (CAL; 5.43 ± 1.8 versus 4.85 ± 1.6; $P = 0.02$), percentage of teeth with CAL ≥5 mm (82.1 ± 23.4 versus 70.4 ± 26.9; $P = 0.002$), and number of missing teeth (8.75 ± 6.6 versus 6.76 ± 6.6; $P = 0.03$). Logistic regression analysis showed that percentage of teeth with CAL ≥5 mm was significantly associated with CAD severity. Patients with ACS had significantly higher plaque scores, gingival index, and *Porphyromonas gingivalis* counts than stable patients. Logistic regression analysis showed that either plaque score or percentage of *P. gingivalis* was significantly associated with ACS.

Conclusion: Periodontal destruction measures are significantly correlated with CAD severity, whereas periodontal infectious measures are significantly associated with clinical cardiac status.
J Periodontol 2007;78:849-858.

KEY WORDS

Coronary artery disease; infection; periodontitis; *Porphyromonas gingivalis*.

* Heart Institute, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.
† Department of Periodontology, Hebrew University-Hadassah Faculty of Dental Medicine, Jerusalem, Israel.

Inflammation and the systemic immune response are believed to play a central role in the initiation and propagation of atherosclerosis.[1,2] Although many risk factors have been linked with the development of atherosclerosis, the exact role of certain chronic infections and the inflammatory response is not yet clear. Periodontitis is a chronic inflammatory disease, with a primary bacterial etiology resulting in inflammatory processes that lead to the destruction of the supporting structures of the teeth. The degree of chronic periodontal destruction is largely determined by the individual inflammatory response of the patients.[3] The main bacterial species implicated in the disease process are Gram-negative, anaerobic, and facultative microorganisms. Several studies,[4-11] including cross-sectional, case-control, and longitudinal studies, imply an epidemiologic association between cardiovascular diseases (myocardial infarction and atherosclerosis) and periodontitis. In addition, some evidence points to periodontal pathogens causing atherosclerosis in experimental animal models. *Porphyromonas gingivalis* has specifically been shown to directly induce and accelerate atherosclerosis in atherosclerotic prone mice,[12] a process that was connected with increased serum levels of proinflammatory cytokines. Data from a human clinical

doi: 10.1902/jop.2007.060301

study has shown that patients experiencing non-fatal myocardial infarction were significantly more likely to harbor *P. gingivalis* than control patients without cardiac disease.[13] In addition, numerous periodontal pathogens, including *P. gingivalis*, have been detected in human atheromatous plaques obtained during endarterectomy.[14] Despite the growing data supporting a possible relationship between periodontal disease and atherosclerosis, this question is still open.

Our study evaluated the relationship between the extent and severity of periodontal disease and the severity of coronary artery disease (CAD), as well as the clinical cardiac status of the patients undergoing diagnostic coronary angiography. Specific periodontal pathogens isolated from the periodontal pockets of these patients and serum inflammatory markers were evaluated to assess their role in coronary heart disease.

MATERIALS AND METHODS

Patients

Consecutive patients referred to the Heart Institute of the Hadassah-Hebrew University Medical Center for diagnostic coronary angiography were included in this cross-sectional study from September 2001 to April 2002. All patients signed a written informed consent form to participate in the study. The study protocol and the informed consent were approved by the Hadassah-Hebrew University Medical Center Institutional Committee for Human Studies.

Clinical Procedures

The following patient data were recorded during a personal interview: age, gender, body mass index (BMI), smoking status, the presence or absence of hypercholesterolemia (>200 mg/dl), hyperlipidemia (low-density lipoprotein [LDL] >130 mg/dl, high-density lipoprotein [HDL] < 35 mg/dl), high serum triglycerides (>200 mg/dl or lipid-lowering therapy), diabetes (fasting plasma glucose >126 mg/dl or glucose-lowering treatment), hypertension (blood pressure >140/90 mmHg measured on several occasions or antihypertensive treatment), and family history of CAD.

Clinical Status

The patients were selected according to the clinical indication for the coronary angiogram. Patients with symptoms of unstable angina or myocardial infarction made up the acute coronary syndrome (ACS) group, whereas patients who were referred for an elective diagnostic angiogram and had not undergone an acute coronary event in the preceding 6 months were defined as the stable group. Criteria for unstable angina were based on a clinical diagnosis on admission. Diagnosis of acute myocardial infarction (AMI) was based on clinical diagnosis and by compatible electrocardiographic changes and/or elevated myocardial biochemical markers. The number of patients included in each clinical group was predetermined at 100 patients. When this number was attained, recruitment of patients into the group was terminated.

Angiographic Status

The angiographic assessment of the severity of the coronary disease was rated according to the number of obstructed coronary arteries and the degree of coronary narrowing based on high-quality film angiograms. An experienced angiographer blinded to the study data assessed the number of coronary vessels (i.e., number of coronary vessels with >50% obstruction).

Periodontal Status

The periodontal assessment of each patient enrolled in the study was carried out in the Heart Institute using a portable dental chair or with the patient seated in bed and a portable light source. Patients admitted with ACS were examined within the first 3 days after admission, and the elective patients were examined before the angiographic study. The examination was carried out using a dental mirror and a University of North Carolina (UNC)-15 periodontal probe.‡ Each patient underwent a full periodontal evaluation by a trained dentist. All teeth excluding third molars were examined. The presence or absence of supragingival plaque was recorded at four surfaces per tooth (mesio-buccal, disto-buccal, mesio-lingual, and disto-lingual), and the plaque score was calculated (percentage of tooth surfaces with plaque). This value represents a measure of the infectious burden associated with the periodontal tissues. The gingival index[15] was measured on a scale of 0 to 3 at the buccal and lingual surfaces of each tooth. The presence or absence of bleeding on probing (BOP) was evaluated on a dichotomous scale at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) and expressed as a percentage of the total number of sites examined. Gingival index and BOP represent measures of the severity of the inflammatory burden (inflammatory response) within the gingival tissues. Probing depths (PDs) and gingival recession were measured at the same six sites per tooth as was BOP, and the clinical attachment level (CAL) was calculated as the sum of the PD and recession at each site. The number of missing and mobile teeth was recorded. Mobility was expressed as a percentage of the total number of teeth. These parameters (PD, recession, CAL, tooth mobility, and missing teeth) represent measures of cumulative periodontal tissue destruction.

Bacterial Sampling

Specific subgingival periodontal pathogens were assessed in pooled bacterial samples from the three deepest periodontal pockets of each patient. Samples were collected using paper points that were inserted to

‡ CP-15 UNC, Hu-Friedy, Chicago, IL.

the depth of the assigned pockets and left for 10 seconds. The paper points were transferred to a single tube and stored at –20°C. After all samples were collected, they were mailed at ambient temperatures to a commercial laboratory for analysis.§ Using specific probes designed by commercial laboratories, the following periodontopathic bacteria were identified and quantified: *Actinobacillus actinomycetemcomitans*, *Tannerella forsythensis*, *P. gingivalis*, and *Treponema denticola*, as well as the total bacterial load. The small subunit ribosomal RNA (ssrRNA) of each of the bacteria was detected by standard dot-blot hybridization techniques[16,17] using ^{32}P-labeled specific probes. In addition, a universal bacterial probe was used to quantify the total bacterial load.

A. actinomycetemcomitans, *T. forsythensis*, *P. gingivalis*, and total bacterial load probes[17]‖ and the probe¶ for *T. denticola* were obtained. Blots were quantified by direct counting.# Results are given as the percentage of each specific bacterial species in the total bacterial load.

Blood Profile of Acute-Phase Proteins

Venous blood was drawn, and aliquots were either analyzed for inflammatory markers or frozen and stored at –80°C until used for cytokine analysis. Systemic markers of inflammation included erythrocyte sedimentation rate, a complete differential blood count, and fibrinogen levels; C-reactive protein was measured using a standard kit;** serum cytokines, including interleukin (IL)-1β, IL-1 receptor antagonist (IL-1RA), tumor necrosis factor-alpha (TNF-α,), IL-6, IL-8 ,and IL-10, were measured in triplicate using enzyme-linked immunosorbent assay (ELISA) kits.††

Statistical Analysis

Means and percentages of major risk factors, clinical periodontal parameters, bacterial counts, inflammatory markers, and cytokine levels were calculated for both groups in each cardiac category (clinical presentation and angiographic coronary severity assessment). The significance of any difference in the means was tested using the independent sample Student *t* test. Qualitative parameters (risk factors and demographic parameters) were compared using χ^2 and Fisher exact tests. The relationship between two quantitative variables was assessed using the Pearson correlation coefficient. Data are presented as mean ± SD unless stated differently.

Logistic regression models were used to examine the effect of the independent periodontal variables on the two dependent clinical outcomes (CAD and ACS), controlling for the independent sociodemographic and risk factors. One set of models tested the effect of the independent variables on the severity of CAD (0 to 1 or 2 to 3 vessel obstruction >50%), whereas the second set examined the effect of the independent variables on the clinical cardiac status (ACS/stable).Only those variables that were significant in the univariate analysis of each cardiac category were entered into each model.

All significance levels were two-tailed, and $P \leq 0.05$ was considered statistically significant.

RESULTS

Two hundred one patients participated in the study, four of whom did not undergo angiography. Table 1 lists the cardiac status including the angiographic assessment and the clinical diagnosis of the study population at presentation. Twenty-eight patients had non-obstructive disease, with none of the vessels showing ≥50% obstruction, 17 of whom were found to have normal coronary arteries. Table 2 lists the demographic parameters and distribution of risk factors in the study population. The patients were evaluated according to coronary angiographic results and their clinical status. Based on the angiographic results, patients were divided into two groups according to the number of vessels involved. Mild CAD included patients with minimal or single-vessel disease, and severe CAD included patients with multivessel disease. Based on the clinical evaluation, patients were divided into stable and ACS groups. Most of the study participants were men (87.10%), with a mean age of 58.27 ± 10.81 years (range, 35 to 88 years). Of the 150 non-smokers, 73 were past smokers.

Dental Examination

Dental examinations were carried out on 201 patients, the results of which are shown in Table 3. The average number of teeth per patient was 20 ± 6.70, and the average number of sites was 119.30 ± 39.90. The study population had high bacterial plaque scores (mean plaque score = 95.61 ± 8.39%), severe gingival inflammation (mean gingival index = 1.62 ± 0.26), and a high percentage of sites that bled on probing (mean BOP score = 78.18 ± 19.60%); furthermore, the PD, CAL, and tooth mobility measures indicated severe periodontal destruction in this population.

Relationship Between Periodontal Disease and Obstructive Vessel Disease

Table 3 presents the distribution of clinical periodontal parameters according to the angiographic status of the coronary arteries and the clinical cardiac status (stable/ACS). *P* values were obtained using univariate statistical analysis. Patients with severe CAD had significantly more periodontal destruction than those

§ IAI Institut für Angewandte Immunologie, Zuchwil, Switzerland.
‖ Microprobe, Bothell, WA.
¶ IAI Institut für Angewandte Immunologie.
# Trace-96 System, Inotech, LaSalle, Canada.
** COBAS Integra, Roche Diagnostics, Basel, Switzerland.
†† R&D, Minneapolis, MN.

with mild CAD. The mean CAL, the mean recession, and the percentage of teeth with CAL of ≥5 mm were significantly higher in the severe CAD group compared to the mild CAD group ($P = 0.02$, $P = 0.001$, and $P = 0.002$, respectively). The mean PD and the percentage of teeth with PD ≥5 mm were greater in the severe CAD group than in the mild CAD group, but the difference did not reach statistical significance. In addition, the mean number of missing teeth per patient was significantly higher in patients with severe CAD ($P = 0.03$); these patients also had a higher mobility score than mild CAD patients ($P = 0.08$). The inflammatory periodontal parameters (mean gingival index, plaque score, and BOP score) were almost identical for the two groups.

When dividing the study population into those with normal coronary arteries (N = 17) and those with evidence of CAD, patients with CAD (N = 184) showed significantly more periodontal destruction. The mean CAL, the mean recession, and the percentage of teeth with CAL ≥5 mm were significantly greater among the CAD patients compared to those having normal coronary arteries ($P = 0.05$, $P = 0.001$, and $P = 0.01$, respectively). The mean PD and the percentage of teeth with PD ≥5 mm were greater in the CAD group but did not reach statistical significance. In addition, the mean number of missing teeth per patient was greater in patients with CAD ($P = 0.08$). In contrast to the measures of cumulative periodontal destruction, no significant differences were detected in the inflammatory periodontal parameters (mean gingival index, plaque, and gingival bleeding scores) between the normal coronary artery and CAD groups.

Of the significant periodontal parameters in the univariate analysis that were entered into the regression analysis model, we used only CAL ≥5 mm because of its interdependence with mean recession and mean CAL.

The only risk factors/parameters significantly associated with the severity of vessel disease as determined by regression analysis were age (odds ratio [OR] = 1.05; 95% confidence interval [CI]: 1.01 to 1.08; $P = 0.01$), hyperlipidemia (OR = 3.09; 95% CI:

Table 1.

Angiography and Clinical Status

	N Diseased Vessels (>50% Obstruction)	N Patients
Angiography (N = 197)	Non-obstructive	28
	Single	57
	Double	51
	Triple	61
	Normal coronary arteries*	17
Clinical status (N = 201)	Stable	101
	ACS	100

* The 17 patients with normal coronary arteries on angiography are included among the 28 patients with non-obstructive vessel disease.

Table 2.

Distribution of Demographic Parameters and Risk Factors According to Coronary Angiography (≥50% obstruction) and Cardiac Clinical Status (stable/ACS)

		Coronary Angiography			Clinical Status		
	All Patients	Mild CAD	Severe CAD	*P* Value	Stable	ACS	*P* Value
N	201	85	112		101	100	
Age (years; mean ± SD)	58.27 ± 10.81	54 ± 10	61 ± 10	0.0001	58 ± 11	59 ± 11	NS
Males (N [%])	175 (87.1)	74 (87)	98 (87)	NS	91 (90)	84 (84)	NS
Current smokers (N [%])	51 (25.4)	32 (38)	18 (16)	0.001	16 (16)	35 (35)	0.002
Ever smokers (N [%])	124 (62)	57 (67)	64 (57)	NS	61 (61)	63 (63)	NS
Diabetes (N [%])	52 (25.9)	18 (21)	33 (29)	NS	20 (20)	32 (32)	0.054
Hypertension (N [%])	95 (47.3)	32 (38)	60 (54)	0.03	46 (46)	49 (49)	NS
Hyperlipidemia (N [%])	127 (63.2)	41 (48)	84 (75)	0.0001	58 (58)	69 (69)	NS
Family history of coronary heart disease (N [%])	88 (43.8)	41 (48)	47 (42)	NS	59 (59)	29 (29)	0.001
BMI (mean ± SD) (N = 180)	27.72 ± 4.47	27.5 ± 3.9	27.9 ± 4.9	NS	28 ± 4.2	27.4 ± 4.7	NS

NS = not significant.

Table 3.

Distribution of Periodontal Clinical Parameters According to Coronary Angiography (≥50% obstruction) and Cardiac Clinical Status (stable/ACS)

		Coronary Angiography						Clinical Status		
	All Patients	Mild CAD	Severe CAD	*P* Value	NCA	CAD	*P* Value	Stable	ACS	*P* Value
N	201	85	112		17	184		101	100	
N missing teeth	8 ± 6.68	6.76 ± 6.56	8.75 ± 6.65	0.03	5.30 ± 5.71	8.25 ± 6.72	0.08	7.50 ± 6.03	8.51 ± 7.27	0.28
Plaque score (%)	95.61 ± 8.39	95.95 ± 8.26	95.21 ± 8.61	0.54	94.65 ± 11.41	95.71 ± 8.09	0.62	92.99 ± 10.35	98.26 ± 4.45	<0.0001
Mean gingival index (0-3)	1.62 ± 0.26	1.61 ± 0.26	1.62 ± 0.27	0.80	1.60 ± 0.25	1.62 ± 0.27	0.89	1.58 ± 0.29	1.66 ± 0.23	0.04
BOP score (%)	78.18 ± 19.60	78.30 ± 18.29	77.60 ± 20.73	0.81	80.65 ± 19.29	77.96 ± 19.67	0.58	76.63 ± 22.14	79.75 ± 16.62	0.22
Mean PD (mm)	4.11 ± 1.05	4.02 ± 1.01	4.16 ± 1.10	0.38	4.04 ± 1.34	4.12 ± 1.03	0.77	4.09 ± 1.24	4.12 ± 0.83	0.90
Teeth with PD ≥5 mm (%)	64.47 ± 31.64	60.33 ± 32.04	66.71 ± 31.30	0.16	55.73 ± 33.25	65.28 ± 31.46	0.24	61.20 ± 32.73	67.77 ± 30.30	0.14
Mean recession (mm)	1.09 ± 0.99	0.82 ± 0.87	1.27 ± 1.05	0.001	0.41 ± 0.40	1.15 ± 1.01	0.001	1.04 ± 0.97	1.15 ± 1.02	0.44
Mean CAL (mm)	5.20 ± 1.73	4.85 ± 1.62	5.43 ± 1.80	0.02	4.45 ± 1.57	5.27 ± 1.74	0.05	5.17 ± 1.93	5.26 ± 1.52	0.6
Teeth with CAL ≥5 mm (%)	77.51 ± 25.52	70.36 ± 26.88	82.13 ± 23.40	0.002	63.43 ± 29.48	78.81 ± 24.81	0.01	73.94 ± 27.12	81.11 ± 23.37	0.04
Teeth with mobility (%)	20.57 ± 17.53	30.64 ± 33.12	38.79 ± 31.53	0.08	19.95 ± 29.81	37.74 ± 32.79	0.032	35.39 ± 31.92	37.08 ± 33.91	0.72

NCA = normal coronary arteries.

1.56 to 6.11; $P = 0.001$), and percentage of teeth with CAL ≥5 mm (OR = 1.02; 95% CI: 1.01 to 1.04; $P = 0.001$; Table 4).

Relationship Between Periodontal Disease and Clinical Cardiac Status

Patients with ACS had a significantly higher mean gingival inflammation index and bacterial plaque score compared to the stable group ($P = 0.04$ and $P < 0.0001$, respectively; Table 3); the gingival bleeding score showed a similar trend but did not reach statistical significance. Of the periodontal parameters representing cumulative destruction of the attachment apparatus, only the percentage of teeth with CAL ≥5 mm was significantly higher in the ACS group ($P = 0.04$).

To examine the effect of the independent variables on the severity of ACS, two regression analysis models were used to eliminate the interdependence between plaque score and the specific counts of the bacteria in the plaque. In the first model, the effect of the percentage of teeth with CAL ≥5 mm, the plaque score, and the gingival index on ACS were examined controlling for diabetes, smoking, and family history of cardiac disease, whereas in the second model, the effect of the specific bacterial counts on ACS was examined controlling for the same variables. The first model showed that diabetes (OR = 2.59; 95% CI: 1.19 to 5.63; $P = 0.02$), current smoking (OR = 4.08; 95% CI: 1.81 to 9.19; $P = 0.001$), and the periodontal variable, plaque score (OR = 1.14; 95% CI: 1.07 to 1.23; $P < 0.0001$), remained significantly associated with clinical cardiac status. Mean gingival index was not significant in this model (Table 5). When the specific

Table 4.

Regression Analysis of the Associations Between Periodontal Disease and CAD

Sociodemographics and Risk Factors	OR	95% CI
Age	1.05	(1.01, 1.08)
Smoking		
Non-smokers	1.00	(0.17, 0.84)
Smokers	0.37	
Hyperlipidemia		
No	1	(1.56, 6.11)
Yes	3.09	
Hypertension		
No	1	(0.62, 2.42)
Yes	1.23	
Periodontal parameters		
Percent teeth with CAL:		
<5 mm	1	(1.01, 1. 04)
≥5 mm	1.03	
Missing teeth	0.98	(0.73, 1.03)

bacterial counts replaced the plaque score in the second model, the model tended to be unstable, with a large SE for smoking. Because the effects of either mean gingival index and percentage of teeth with CAL ≥5 mm on ACS were not statistically significant (P >0.1) after adjusting for sociodemographics and risk factors, a multivariate model that did not incorporate these two variables was constructed to evaluate the effect of the three specific bacterial counts. In this model, the bacterial counts remained significantly associated with ACS (*P. gingivalis*: OR = 1.52; 95% CI: 1.27 to 1.83; P <0.0001; *T. denticola*: OR = 0.65; 95% CI: 0.52 to 0.81; P <0.0001; *T. forsythensis*: OR = 0.82; 95% CI: 0.68 to 0.99; P <0.04; Table 5).

Relationship Between Periodontal Pathogens and Cardiac Disease

Table 6 presents the distribution of the bacterial pathogens (mean percentage of each species in the total bacterial count) according to the severity of coronary vessel obstruction and the clinical cardiac status. *P. gingivalis*, *A. actinomycetemcomitans*, *T. forsythensis*, and *T. denticola* were detected in 86%, 25%, 90%, and 85% of the population, respectively. Patients with ACS harbored a significantly higher percentage of *P. gingivalis* than patients in the stable category (P <0.0001). Further analysis of the subset of patients that were positive for *P. gingivalis* showed a significantly higher bacterial percentage in the ACS group compared to the stable group (9.63 ± 7.30 versus 1.91 ± 1.46, respectively; P<0.001). Among patients that were positive for *A. actinomycetemcomitans*, higher, but not significant, counts were observed in the ACS compared to the stable group (0.34 ± 0.57 versus 0.24 ± 0.26, respectively; P= 0.59). The counts of the other two examined pathogens (*T. denticola* and *T. forsythensis*) were significantly higher in the stable group.

The percentage of *P. gingivalis* was significantly higher in patients with CAD compared to patients with normal coronary arteries (P <0.001); this relation remained consistent for the subset of patients that were positive for *P. gingivalis* (CAD = 5.99 ± 6.62; normal coronary arteries = 1.76 ± 1.42; P<0.001). All pathogens examined were positively associated with the severity of the vessel disease, but none were statistically significant.

Inflammatory Markers and Serum Cytokine Levels

Table 7 lists the distribution of the acute-phase proteins, including markers of inflammation and cytokines, between the acute and stable patient groups. All inflammatory markers and cytokines were elevated in patients with ACS, with differences significant for all markers except IL-8 and IL-10. No significant differences were detected in the levels of inflammatory markers in patients grouped according to their coronary artery disease status.

Significant correlations of IL-6 with both plaque score (R = 0.25; P = 0.007) and mean gingival index (R = 0.22; P = 0.01) were detected. In addition, significant but weak correlations of TNF-α with mean CAL (R = 0.19; P = 0.04), mean recession (R = 0.19; P = 0.03), and percent tooth mobility (R = 0.21; P = 0.02) were found.

Table 5.

Regression Analysis of the Associations Between Periodontal Disease and Clinical Cardiac Status

	Model 1		Model 2	
Sociodemographics and Risk Factors	OR	95% CI	OR	95% CI
Diabetes				
No	1	(1.19, 5.63)	1	0.98, 9.33
Yes	2.59		3.03	
Smoking				
Non-smokers	1	(1.81, 9.19)	1	(2.27, 23.23)
Smokers	4.08		7.26	
Family history				
No	0.27	(0.14, 0.53)	0.20	(0.08, 0.49)
Yes	1		1	
Periodontal parameters:				
Percent teeth with CAL ≥5 mm	0.99	(0.98, 1.01)	–	–
Mean GI	0.57	(0.10, 3.01)	–	–
Plaque score	1.14	(1.07, 1.23)	–	–
Bacterial counts	–	–		
P. gingivalis			1.52	(1.27, 1.83)
T. forsythensis			0.82	(0.68, 0.99)
T. denticola			0.65	(0.52, 0.81)

DISCUSSION

Our results, as presented here, are in accordance with a growing body of data that strongly established an epidemiologic association between two chronic inflammatory diseases: atherosclerosis and periodontitis. Matilla et al.[5] were the first to report an association between dental infections and severe coronary atherosclerosis diagnosed by angiography; however, their dental examination was limited to pan tomography radiographs, which interpreted all oral pathologies without specific focus on periodontal conditions. Abou-Raya et al.[18] evaluated 50 patients angiographically and reported a strong

Table 6.

Distribution of Bacterial Pathogens (per pooled sample) According to Coronary Angiography and Clinical Status

	Coronary Angiography						Clinical Status		
	Mild CAD	Severe CAD	*P* Value	NCA	CAD	*P* Value	Stable	ACS	*P* Value
N	83	104		16	175		91	100	
P. gingivalis	4.34 ± 5.14	5.13 ± 7.02	0.38	1.54 ± 1.45	5.13 ± 6.47	<0.0001	1.78 ± 1.49	7.60 ± 7.58	<0.0001
*A. actinomycetemcomitans**	0.07 ± 0.19	0.06 ± 0.24	0.84	0.11 ± 0.17	0.06 ± 0.22	0.41	0.91 ± 0.20	0.04 ± 0.23	0.13
*T. forsythensis**	4.23 ± 3.32	4.56 ± 3.54	0.51	4.14 ± 3.37	4.38 ± 3.45	0.79	4.99 ± 3.82	3.78 ± 2.95	0.01
*T. denticola**	3.38 ± 3.69	4.16 ± 5.63	0.25	4.85 ± 5.05	3.66 ± 4.82	0.35	5.92 ± 6.16	1.80 ± 1.46	<0.0001
Total bacterial load†	37.79 ± 24.77	33.81 ± 22.99	0.26	34.76 ± 21.76	35.39 ± 23.86	0.92	37.90 ± 25.47	33.00 ± 21.71	0.15

NCA = normal coronary arteries.
* These bacteria are presented as mean percentages of the total bacterial load.
† Total bacterial load is the mean of the numbers of bacteria per sample × 10^6.

Table 7.

Distribution of Inflammation Markers and Cytokines Between the Stable and ACS Groups Based on Clinical Cardiac Status

	Normal Value	All Patients	ACS Patients	Stable Patients	*P* Value*
C-reactive protein (mg/l)	<0.5	2.06 ± 0.39 (158)	3.97 ± 0.81 (71)	0.51 ± 0.06 (87)	0.0001
Erythrocyte sedimentation rate (mm/h)	<20	23 ± 2 (187)	32 ± 4 (87)	15 ± 1 (100)	0.0001
Fibrinogen (mg/dl)	150-400	324 ± 12 (162)	355 ± 22 (78)	296 ± 11 (84)	0.02
White blood cells (×10^3/mm^3)	5-10	8.8 ± 0.2 (198)	10.3 ± 0.4 (97)	7.4 ± 0.2 (99)	0.0001
Platelets (×10^3/mm^3)	140-440	227 ± 5 (198)	238 ± 7 (97)	217 ± 6 (99)	0.02
IL-1β (pg/ml)	<1.9	0.50 ± 0.09 (118)	1.00 ± 0.21 (44)	0.21 ± 0.05 (74)	0.001
IL-6 (pg/ml)	<10	9.90 ± 1.13 (119)	19.7 ± 2.18 (45)	3.9 ± 0.55 (74)	0.0001
IL-8 (pg/ml)	<70	12.47 ± 1.35 (119)	15.1 ± 2.98 (45)	10.8 ± 1.18 (74)	0.18
TNF-α (pg/ml)	<20	6.56 ± 0.43 (119)	7.73 ± 0.85 (45)	5.9 ± 0.46 (74)	0.05
IL-1RA (pg/ml)	<500	748 ± 75 (86)	1019 ± 128 (45)	451 ± 33 (41)	0.0001
IL-10 (pg/ml)	<9	7.63 ± 4.04 (40)	13.4 ± 7.94 (20)	1.89 ± 0.65 (20)	0.16

Data given as mean ± SE; parentheses denote the sample size (n).
* *P* value by *t* test between stable and acute coronary syndromes.

independent association between pan tomographic index representing the severity of the dental disease and CAD. Although Malthaner et al.[19] found that chronic periodontal indices were significantly associated with angiographic CAD, after adjustment for confounding factors, the significance was less pronounced. Further support for the association between atherosclerosis and periodontitis can be found in two recent studies[20,21] showing that subclinical CAD was significantly associated with chronic periodontitis severity after adjustment for major risk factors for atherosclerosis. Several studies[22-25] published in the last few years did not report significant correlations between periodontitis and CAD. However, in contrast to our study, those results were based on either self-reported periodontitis or on prospective data of patients with dental disease who had experienced their first cardiac event.

In our patients, the severity of CAD, as diagnosed by angiography, was significantly associated with the parameters of periodontal disease that best represent the cumulative destructive outcome of long-standing periodontitis (i.e., missing teeth, recession,

CAL). This point was further strengthened by the finding that all patients with CAD showed significantly more periodontal destruction than those with normal coronary arteries, even though the group with normal arteries was small (recession, CAL). The regression analysis, adjusting for major cardiac risk factors, showed that periodontal destruction as indicated by the percentage of teeth with CAL ≥5 mm was a significant independent factor associated with the severity of CAD, whereas the markers of periodontal infection (plaque score and specific periodontal pathogens counts) were clearly associated with acute coronary disease in our patients. However, in a recent publication, Desvarieux et al.[21] showed that the same periodontal pathogens examined in our study were directly related to subclinical atherosclerosis in a population with no history of cardiovascular disease. Their findings are in contrast to ours and probably reflect the difference in the study populations.

The association between CAD and the destruction of the periodontal attachment apparatus is certainly interesting. Both diseases are chronic inflammatory processes; this, in turn, may imply that the two either share a common pathogenesis based on similar genetic and/or yet unknown environmental factors or that non-specific activation of the inflammatory cascade in chronic periodontitis may play an important role in the development of CAD.

The correlation found between the measures of periodontal infectious/inflammatory burden (plaque score, gingival index, *P. gingivalis*, *T. denticola*, and *T. forsythensis*) and the acute presentation of cardiac symptoms can be more easily explained. It has been suggested that the local inflammatory process associated with the periodontium may trigger an immune activation as observed by the high levels of cytokines and increase the likelihood of plaque rupture leading to ACS.[26-28] It was also proposed that periodontal pathogens (*P. gingivalis*) may directly or indirectly affect the atheromatous plaque leading to rupture and embolization.[29] In a recent study, Bazile et al.[30] showed in 80 patients that only the periodontal inflammatory indices gingival index and BOP were associated with AMI. The above data support the hypothesis that there is a relationship between the infective/inflammatory load and the occurrence of acute cardiac symptoms.

A possible confounder that was not accounted for in the logistic regression analysis was the possibility that the hospitalization of the ACS patients for up to 3 days before measuring the plaque levels would bias the plaque score outcome to the detriment of these patients. However, because of the extremely high plaque scores for the whole study population (mean plaque score, ~96%), the influence of this confounder would be minimal.

As shown in our study and reported previously,[31-33] the proinflammatory cytokine IL-6, a key factor in the inflammatory process, was elevated in patients with ACS and correlated with the measures of periodontal inflammation (plaque score and gingival index). This association supports the hypothesis that increased serum IL-6 levels (and other inflammatory mediators) that result from periodontal infections may trigger the rupture of atheromatous plaques, resulting in ACS.[34]

The fact that the mean percentage of *P. gingivalis* in the ACS group was four-fold higher than that in the stable group ($P<0.0001$) lends further support to the role of this periodontal pathogen in the development of ACS. The method by which this periodontal pathogen may induce acute coronary symptoms has been discussed by a number of authors. In animal models, Herzberg and Meyer[35,36] and Herzberg[37] found a clear relationship between specific oral pathogens and their thrombogenic effect. In recent studies, Kuramitsu et al.[29,38] reported that *P. gingivalis* exhibits properties that could play a role in CVD by mediating LDL oxidation, foam cell formation, and rupture of the atherosclerotic plaque. In addition, preliminary data from a clinical study involving periodontal pathogens and myocardial infarction showed a significantly higher presence of *P. gingivalis* and *B. forsythus* (renamed *T. forsythensis*) in 97 participants who experienced non-fatal MI compared to 233 control patients who had no heart problems.[13]

It was also suggested that the number and species of periodontal bacteria may be directly involved in CAD by contributing to the development of microthrombosis in the atheromatous plaque[35-37] or by activating the inflammatory cascade.[34] In our patients, the mean *P. gingivalis* count per patient in the severe CAD group was higher than in the mild CAD group; in fact, in patients with CAD, the *P. gingivalis* load was significantly higher than in those with normal coronary arteries. Recently, a number of authors have analyzed the relationship between known periodontal pathogens and the development of atherosclerosis in apolipoprotein E-null mice. Their results support the hypothesis that *P. gingivalis* is indeed involved in the pathogenesis of developing atheromatous plaques in vessel walls.[12,39] In addition, a recent clinical paper[40] showed a significant association between periodontal pathogen burden and the presence of CHD.

All patients had poor oral hygiene (mean plaque score = 95.61%) and advanced periodontal destruction as defined by percentage of teeth with PD or CAL ≥5 mm. The 100% prevalence of severe periodontal destruction was an unexpected finding that did not allow for the stratification of the patients into groups with different disease severities. This prevalence of severe periodontal destruction is above what would be expected in a normal, age-matched, adult

population.[41] Although the extremely high prevalence of periodontitis and poor oral hygiene strongly suggests a relationship with CAD, we cannot rule out that this was caused by the highly selected study population.

An additional finding was the significant negative association of *T. denticola* and *T. forsythensis* counts with ACS. This negative association is contrary to recent data reported by Renvert et al.[42] These contradictory results are difficult to explain. They may simply be chance findings or related to differences in the methodology (e.g., probes) and/or the nature of the study populations. On the other hand, these two bacteria may have a direct role in protecting the patients from an acute episode or may indirectly act by inhibiting microorganisms that are believed to have a positive causal relationship with ACS (e.g., Cytomegalovirus, *C. pneumonia*, *P. gingivalis*).

CONCLUSIONS

The severity of chronic periodontal disease was shown to be a significant independent factor associated with the severity of CAD. Periodontal bacterial load in general, and in *P. gingivalis* in particular, is significantly associated with acute cardiac symptoms.

ACKNOWLEDGMENTS

This work was supported by a non-restricted grant from the Phillip Morris External Research Program, Phillip Morris, Richmond, VA. The authors thank Prof. Orly Manor, The Hebrew University-Hadassah School of Public Health, for help with the logistic regression analysis.

REFERENCES

1. Ross R. Atherosclerosis — An inflammatory disease. *N Engl J Med* 1999;340:115-126.
2. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;105:1135-1143.
3. Offenbacher S. Periodontal diseases: Pathogenesis. *Ann Periodontol* 1996;1:821-878.
4. Mattila KJ, Nieminen MS, Valtonen VV, et al. Association between dental health and acute myocardial infarction. *BMJ* 1989;298:779-781.
5. Mattila KJ, Valle MS, Nieminen MS, Valtonen VV, Hietaniemi KL. Dental infections and coronary atherosclerosis. *Atherosclerosis* 1993;103:205-211.
6. Mattila KJ, Valtonen VV, Nieminen M, Huttunen JK. Dental infection and the risk of new coronary events: Prospective study of patients with documented coronary artery disease. *Clin Infect Dis* 1995;20:588-592.
7. DeStefano F, Anda RF, Kahn HS, Williamson DF, Russell CM. Dental disease and risk of coronary heart disease and mortality. *BMJ* 1993;306:688-691.
8. Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Periodontal disease and cardiovascular disease. *J Periodontol* 1996;67(Suppl.):1123-1137.
9. Joshipura KJ, Rimm EB, Douglass CW, Trichopoulos D, Ascherio A, Willett WC. Poor oral health and coronary heart disease. *J Dent Res* 1996;75:1631-1636.
10. Paunio K, Impivaara O, Tiekso J, Maki J. Missing teeth and ischaemic heart disease in men aged 45-64 years. *Eur Heart J* 1993;14(Suppl. K):54-56.
11. Rutger Persson G, Ohlsson O, Pettersson T, Renvert S. Chronic periodontitis, a significant relationship with acute myocardial infarction. *Eur Heart J* 2003;24: 2108-2115.
12. Li L, Messas E, Batista EL Jr., Levine RA, Amar S. *Porphyromonas gingivalis* infection accelerates the progression of atherosclerosis in a heterozygous apolipoprotein E-deficient murine model. *Circulation* 2002; 105:861-867.
13. Genco RJ, Wu TJ, Grossi S, Falkner KL, Zambon JJ, Trevisan M. Periodontal microflora related to the risk of myocardial infarction: A case control study. *J Dent Res* 1999;78:457.
14. Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. *J Periodontol* 2000;71:1554-1560.
15. Löe H, Silness J. Periodontal disease in pregnancy (I). Prevalence and severity. *Acta Odontol Scand* 1963;21: 533-551.
16. Khandjian EW. UV crosslinking of RNA to nylon membrane enhances hybridization signals. *Mol Biol Rep* 1986;11:107-115.
17. Dix K, Watanabe SM, McArdle S, et al. Species-specific oligodeoxynucleotide probes for the identification of periodontal bacteria. *J Clin Microbiol* 1990;28: 319-323.
18. Abou-Raya S, Naeem A, Abou-El KH, El BS. Coronary artery disease and periodontal disease: Is there a link? *Angiology* 2002;53:141-148.
19. Malthaner SC, Moore S, Mills M, et al. Investigation of the association between angiographically defined coronary artery disease and periodontal disease. *J Periodontol* 2002;73:1169-1176.
20. Beck JD, Elter JR, Heiss G, Couper D, Mauriello SM, Offenbacher S. Relationship of periodontal disease to carotid artery intima-media wall thickness: The atherosclerosis risk in communities (ARIC) study. *Arterioscler Thromb Vasc Biol* 2001;21:1816-1822.
21. Desvarieux M, Demmer RT, Rundek T, et al. Periodontal microbiota and carotid intima-media thickness: The Oral Infections and Vascular Disease Epidemiology Study (INVEST). *Circulation* 2005;111:576-582.
22. Mattila KJ, Asikainen S, Wolf J, Jousimies-Somer H, Valtonen V, Nieminen M. Age, dental infections, and coronary heart disease. *J Dent Res* 2000;79:756-760.
23. Hujoel PP, Drangsholt M, Spiekerman C, DeRouen TA. Periodontal disease and coronary heart disease risk. *JAMA* 2000;284:1406-1410.
24. Howell TH, Ridker PM, Ajani UA, Hennekens CH, Christen WG. Periodontal disease and risk of subsequent cardiovascular disease in U.S. male physicians. *J Am Coll Cardiol* 2001;37:445-450.
25. Khader YS, Albashaireh ZS, Alomari MA. Periodontal diseases and the risk of coronary heart and cerebrovascular diseases: A meta-analysis. *J Periodontol* 2004; 75:1046-1053.
26. Beck JD, Offenbacher S, Williams R, Gibbs P, Garcia R. Periodontitis: A risk factor for coronary heart disease? *Ann Periodontol* 1998;3:127-141.
27. Loos BG, Craandijk J, Hoek FJ, Wertheim-van Dillen PM, van der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *J Periodontol* 2000; 71:1528-1534.

28. Wu T, Trevisan M, Genco RJ, Falkner KL, Dorn JP, Sempos CT. Examination of the relation between periodontal health status and cardiovascular risk factors: Serum total and high density lipoprotein cholesterol, C-reactive protein, and plasma fibrinogen. *Am J Epidemiol* 2000;151:273-282.
29. Kuramitsu HK, Qi M, Kang IC, Chen W. Role for periodontal bacteria in cardiovascular diseases. *Ann Periodontol* 2001;6:41-47.
30. Bazile A, Bissada NF, Nair R, Siegel BP. Periodontal assessment of patients undergoing angioplasty for treatment of coronary artery disease. *J Periodontol* 2002; 73:631-636.
31. Ide M, Jagdev D, Coward PY, Crook M, Barclay GR, Wilson RF. The short-term effects of treatment of chronic periodontitis on circulating levels of endotoxin, C-reactive protein, tumor necrosis factor-alpha, and interleukin-6. *J Periodontol* 2004;75:420-428.
32. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 2000;101:1767-1772.
33. Ebersole JL, Cappelli D. Acute-phase reactants in infections and inflammatory diseases. *Periodontol 2000* 2000;23:19-49.
34. Haynes WG, Stanford C. Periodontal disease and atherosclerosis: From dental to arterial plaque. *Arterioscler Thromb Vasc Biol* 2003;23:1309-1311.
35. Herzberg MC, Meyer MW. Effects of oral flora on platelets: Possible consequences in cardiovascular disease. *J Periodontol* 1996;67(Suppl.):1138-1142.
36. Herzberg MC, Meyer MW. Dental plaque, platelets, and cardiovascular diseases. *Ann Periodontol* 1998;3:151-160.
37. Herzberg MC. Coagulation and thrombosis in cardiovascular disease: Plausible contributions of infectious agents. *Ann Periodontol* 2001;6:16-19.
38. Kuramitsu HK, Kang IC, Qi M. Interactions of *Porphyromonas gingivalis* with host cells: Implications for cardiovascular diseases. *J Periodontol* 2003;74:85-89.
39. Lalla E, Lamster IB, Hofmann MA, et al. Oral infection with a periodontal pathogen accelerates early atherosclerosis in apolipoprotein E-null mice. *Arterioscler Thromb Vasc Biol* 2003;23:1405-1411.
40. Spahr A, Klein E, Khuseyinova N, et al. 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. *Arch Intern Med* 2006;166:554-559.
41. Papapanou PN. Periodontal diseases: Epidemiology. *Ann Periodontol* 1996;1:1-36.
42. Renvert S, Pettersson T, Ohlsson O, Persson GR. Bacterial profile and burden of periodontal infection in subjects with a diagnosis of acute coronary syndrome. *J Periodontol* 2006;77:1110-1119.

Correspondence: Dr. Ayala Stabholz, Department of Periodontology, Hebrew University-Hadassah Faculty of Dental Medicine, P.O. Box 12272, Jerusalem, Israel 91120. Fax: 972-2-6536762; e-mail: astabh@cc.huji.ac.il.

Accepted for publication November 26, 2006.