Risk factors for atherosclerosis in cases with severe periodontitis

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Abstract

Aim: Studies have reported on an association between cardiovascular disease (CVD) and periodontitis. The purpose of this case–control study was to provide an insight into this association by determining the plasma levels of some risk markers for CVD in cases with periodontitis.

Materials and Methods: Sixty-eight cases with periodontitis, mean age 53.9 (SD 7.9) years, and 48 randomly selected healthy controls, mean age 53.1 (SD 7.9) years, were investigated. Fasting blood plasma was analysed for glucose, lipids, markers systemic inflammation, cytokines and antibodies against heat shock proteins (Hsp). The associations between periodontitis and the various substances analysed in plasma were calculated using a multivariate logistic regression model, which compensated for age, gender, smoking and body mass index.

Results: The regression analyses revealed a significant association between periodontitis and high levels of C-reactive protein (CRP) [odds ratio (OR) 4.0, confidence interval (CI) 1.4–11.4] and fibrinogen (OR 8.7, CI 2.6–28.4), IL-18 (OR 6.5, CI 2.2–19.5), and decreased levels of IL-4 (OR 0.12, CI 0.0–0.5). The study showed increased levels of antibodies against Hsp65 (OR 2.8, CI 1–7.6) and 70 (OR 2.9, CI 1.1–7.8) and decreased levels of antibodies against Hsp60 (OR 0.3, CI 0.1–0.8).

Conclusions: Periodontitis was associated with increased levels of CRP, glucose, fibrinogen and IL-18, and with decreased levels of IL-4.

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Periodontitis is a chronic, tissue-destructive inflammatory state that is predominantly induced by Gram-negative bacteria that have colonized the gingival crevice. The disease degrades the attachment apparatus of the teeth, leading to tooth loosening and eventually, in its most severe form, to edentulousness. Clinical signs of the disease are often seen in early middle age. Periodontitis is a very common disease, and the National Health and Nutrition Examination Survey 1999–2000 has reported a prevalence of 4.2% among adult Americans (Borrell et al. 2005).

Cross-sectional and longitudinal epidemiological studies have shown that cases with periodontitis are at a significantly increased risk of developing cardiovascular disease (CVD) (Mattila et al. 1989, DeStefano et al. 1993, Andriankaja et al. 2007, Bahekar et al. 2007, Nibali et al. 2007a, Cairo et al. 2008). Although data have been adjusted for known risk factors such as smoking, diabetes, high blood pressure and socioeconomic factors, other confounders might still explain or at least contribute to the apparent association. Smoking is especially difficult to manage in regression models (Hujoel et al. 2002), and life-style factors such as diet and health awareness are often not included in the statistical modelling. Some researchers also claim that the findings of some epidemiologic studies of the association between oral and systemic diseases are spurious and do not reflect causality (Hujoel et al. 2006).

Levels of risk markers for CVD, such as glucose and C-reactive protein (CRP), have been reported to be elevated in patients with periodontitis (see Loos 2005 for a review). Furthermore, animal studies have demonstrated a relatively strong association between the prevalence of periodontal pathogens such as *Porphyromonas gingivalis*, bacterial products, periodontitis and the

incidence of CVD-related events. In addition, although DNA from oral bacteria has been found in atherosclerotic plaques, the contribution of these bacteria to plaque formation remains unknown. Periodontal pathogens and their products have also been reported to trigger the atherosclerotic process in animal studies (Herzberg & Meyer 1998, Dorn et al. 1999), and yet their effects in the human system remain unclear.

The release of host-derived inflammatory mediators such as cytokines from the chronically inflamed periodontal tissues into the circulation might provide a link between periodontal disease and CVD, because chronic infection and inflammation predispose individuals to the development of CVD (Espinola-Klein et al. 2002a, b, Desvarieux et al. 2005). Although only slightly elevated levels of pro-inflammatory cytokines have been reported in individuals with periodontitis, the total inflammatory burden could nevertheless have a negative impact on the atherosclerotic process (Honda et al. 2005).

Altered serological profiles of risk markers in patients with periodontitis might result from an invasion of bacteria and bacterial products from the periodontal lesion into the blood stream and the consequential induction and maintenance of a chronic inflammatory state.

The aim of this study was to determine whether plasma concentrations of established risk markers for atherosclerosis and antibodies against heat shock (stress) proteins are elevated in individuals with severe periodontitis.

Materials and methods
Cases and healthy, non-periodontal controls

The periodontitis group consisted of 68 subjects, (36 men, 32 women; group mean age 53.9 years, range 38–73 years) having a minimum of seven sites exhibiting at least 6 mm loss of clinical attachment. All individuals had been referred to a specialist clinic in periodontology due to their severe periodontitis and were undergoing treatment for this condition. All cases had periodontitis gravis et complicata (Nyman & Lindhe 2003), in which there is a horizontal loss of supporting tissue by 1/3 or more of the root length with bleeding on probing, furcation involvements of

the multi-rooted teeth and/or angular bony defects with suppuration. Cases were excluded from the study if they had a known history of CVD or took any medication.

The healthy, non-periodontal (comparison) group comprised 48 subjects (23 men, 25 women; group mean age 53.1 years, range 39–69 years), none of whom exhibited clinical signs of periodontitis or extensive gingivitis [no pathological pockets over 4 mm and bleeding on probing (BOP) ⩽ 35%]. An additional four subjects were examined; however, these individuals were found to have periodontitis and were thus excluded from the study. The pockets found in the individuals without periodontitis resulted from localized gingival oedema or gingival displacement without the concomitant migration of the epithelium, so-called pseudo pockets (Nyman & Lindhe 2003).

The subjects in the comparison group were randomly selected from a list of inhabitants who were born in the community of Huddinge, Sweden, between 1935 and 1965 on 1 October 2004. They were recruited during the same period as the cases and the controls originate from the same area as one of the Specialist clinics in Periodontology, and consequently from the same community as some of the cases. All controls were within the same age range as the cases. For random selection, every third person on the list was telephoned and if this individual did not answer, the next one was selected. The ages of the potential control subjects were matched with the cases and the age of the control subject was known to the person making the telephone call. All subjects had a Caucasian background. During the telephone conversation potential controls were asked whether they had any known history of CVD or were on any medication. Participants were excluded from the study if they had a known history of CVD or any other chronic disease. Cardiovascular health was assessed on the basis of medical history. None of the individuals included in this study were taking statins or any other medication for CVD.

Thirty-four of the cases and 21 of the controls were former or current smokers (13.6 ± 6.3 and 16.7 ± 8.9 cigarettes/day, respectively). All former and current smokers had a history of smoking for 10 years or more (mean 27.3 ± 11.3 years).

Clinical examination for periodontal disease

The cases underwent a comprehensive periodontal examination including radiographs. The oral health status of the control group was verified by a thorough clinical examination. A total of four dentists with extensive experience in periodontology undertook all clinical examinations. Before taking part in the study, the examiners were given detailed instructions and information with regard to the categorization of results. The measurements of the periodontal parameters, gingival probing, BOP, oral plaque, extent of furcations and mobility, were standardized between the dentists. Teeth and gingiva were evaluated, and the pocket depth was measured using a periodontal probe. Probing depth is the distance between the gingival margin and the bottom of the gingival pocket as measured from six angles for each tooth. The presence or absence of dental plaque at the gingival margin along the mesial, buccal, distal and lingual aspects was determined and the hygiene index (HI index, percentage, Love et al. 1975) was calculated. The dental plaque was made visible by gently moving the tip of the probe along the gingival margin of four sides of each tooth. Gingival pockets that were 4 mm or deeper were considered to be pathogenic. Gingival inflammation was noted as bleeding on probing, and data are expressed as the proportion of sites in the dentition that were bleeding.

Haematological, blood lipid and inflammatory indices

After overnight fasting, blood was collected into EDTA Vacutainer™ tubes (BD Inc., Franklin Lakes, NJ, USA) and haematological indices were determined using a Coulter STKS analyzer (Coulter Electronics Inc., Hialeah, FL, USA). Plasma was obtained after centrifugation at 1500 g for 10 min. and stored at $-70°C$ until analysis. Cholesterol and high-density lipoprotein (HDL) levels were determined using standard clinical chemistry procedures using a Hitachi 917 analyzer (Roche AG Diagnostics, Mannheim, Germany). The lipid profile was calculated as the ratio between total cholesterol and HDL. CRP levels were determined using a high-sensitivity commercial assay kit (DADE Behring, Deerfield, IL, USA), and haptoglobin and

fibrinogen levels were measured using a BNA nephelometer (Behringwerke AG Diagnostica, Marburg, Germany).

Interleukin (IL)-1β, IL-4, IL-5, IL-6, IL-8, IL-10, interferon (IFN)-γ and TNF-α levels were determined by Multiplex bead analysis using a Luminex 100 (Luminex Corp., Austin, TX, USA) and commercial immunoassays (Lincoplex, High-sensitivity human cytokine panel, Linco Research Inc., St. Charles, MO, USA). IL-18 levels were determined using a commercial immunoassay kit according to the manufacturers' recommended protocols (R&D Systems Europe Ltd., Abingdon, UK).

Levels of circulating immunoglobulin (Ig)A, IgG and IgM were determined using standard clinical chemistry procedures using a Roche Modular Analytics P analyzer (Roche AG Diagnostics). Glucose levels were measured similarly.

Levels of circulating antibodies against the stress proteins human Hsp60 (kindly provided by Professor Willem van Eden and Dr. Ruurd van der Zee, Utrecht University, Utrecht, the Netherlands), mycobacterial Hsp65 (kindly provided by Professor Anthony Coates, St. George's Hospital Medical School, London, UK) and human Hsp70 (kindly provided by Professor Gabriele Multhoff, Technische Universität München and HelmholtzCenter Munich, German Research Center for Environmental Health, Neuherberg, Germany) were determined as described previously (Pockley et al. 2000, Buhlin et al. 2003), except that the isotype (IgA, IgG) of the anti-heat shock protein antibodies was determined. Data are presented as the absorbance values that were generated by samples diluted 1/100. All samples were analysed at the same time and under identical experimental conditions.

Statistical methods

Statistical analyses were performed using STATISTICA 7.1 software (Statsoft Inc., Tulsa, OK, USA). The *p*-values reported are based on a univariate comparison between cases and controls, as calculated using the Student *t*-test or the Mann–Whitney *U*-test. Confidence intervals were calculated using Wald's method. The χ^2-test was used to calculate the significance of the dichotomized variables (HDL and glucose).

Associations between periodontitis and surrogate markers for the atherosclerotic process (CRP, HDL cholesterol and glucose) were calculated using a multivariate logistic regression model that was adjusted for age, gender, body mass index (BMI) and smoking. These variables were selected, as they are known risk factors for CVD. Age and smoking are also known to be important risk factors for periodontal disease. The smoking variable was also tested for interaction effects with gender, age and the primary variables (CRP, HDL cholesterol and glucose).

Using CRP as a reference variable, a power calculation based on data obtained in a previous study (Buhlin et al. 2003) showed that a similar study with 50 participants in each arm would have 80% power to find a difference of 1 mg. The power of the study was increased by including 68 cases.

The study was approved by the Regional Ethics Committee of the Karolinska Institutet, and it was conducted in accordance with the Helsinki Declaration. All participants gave their informed consent. All blood sample analyses were performed blindly.

Results

Data on patient demographics, smoking habits and clinical periodontal status are provided in Table 1. Within the cases (non-medicated), smokers exhibited a more serious periodontal status and had fewer teeth (23.9 *versus* 24.2) than their non-smoking counterparts. Cases with periodontitis tended to have a higher BMI compared with the control subjects (non-medicated), and this difference was most prominent when non-smokers in the two groups were compared ($p < 0.01$). In contrast to the BMI, the waist/hip ratio was similar among all participants.

A univariate comparison between the groups showed that HDL cholesterol levels were lower in cases ($p < 0.05$), and this difference was particularly evident in the non-smoking group ($p < 0.01$). No difference was seen among smokers. HDL cholesterol levels were below 0.9 mmol/l in nine (13%) cases with periodontitis, but in only one individual (2%) in the control group

Table 1. Pertinent characteristics of cases and control subjects (none of whom were on any medication) on the basis of smoking habits

	Cases (n = 68)	Healthy controls (n = 48)	Long-term smoking cases (n = 34)	Long-term smoking healthy controls (n = 21)	Never smoked cases (n = 34)	Never smoked controls (n = 27)
Gender (M/F)	36/32	23/25	14/20	10/11	22/12	13/14
Mean age (years)	53.9 (7.9)	53.1 (7.9)	53.4 (7.4)	52.2 (7.1)	54.5 (8.4)	53.9 (8.1)
Mean no. of teeth	**24.2 (4.6)** **p < 0.001**	27.6 (2.9)	**24.1 (5.1)** **p < 0.001**	28.2 (1.7)	**24.3 (4.2)** **p < 0.01**	27.1 (3.6)
Mean no. of cigarettes/day			13.6 (6.3)	16.7 (8.9)		
Smoking duration (years)			**31.7 (10.1)** **p < 0.001**	**20.1 (9.4)**		
Mean no. of sites over ⩽4 mm	**47 (17)** **p < 0.001**	8 (6)	**52 (15)** **p < 0.001**	9 (6)	**43 (17)** **p < 0.001**	7 (5)
Mean depth of pathological pockets (mm)	**5.8 (0.7)** **p < 0.001**	3.9 (1.0)	**5.7 (0.5)** **p < 0.001**	4.2 (0.3)	**5.8 (0.8)** **p < 0.001**	4.0 (1.3)
Mean bleeding on probing (%)	**44 (16)** **p < 0.001**	20 (11)	**47 (17)** **p < 0.001**	24 (11)	**42 (14)** **p < 0.001**	17 (10)
Mean hygiene (oral plaque) index (HI) (%)	**51 (22)** **p < 0.001**	29 (14)	**48 (21)** **p < 0.01**	32 (14)	**54 (23)** **p < 0.001**	27 (14)

Statistically significant differences between cases and controls within each category are in bold.

($p = 0.03$). This difference was also apparent in the two subgroups (smokers: 12% *versus* 0%, non-smokers: 14% *versus* 4%). Fasting plasma glucose levels were higher ($p < 0.001$) in cases with periodontitis and this difference was seen in both smokers and non-smokers (Table 2). Glucose levels were above 5.5 mmol in 35% of the cases and 4% of controls ($p < 0.001$). The levels of the acute-phase reactants CRP, fibrinogen and haptoglobin were also elevated ($p < 0.015$, $p < 0.001$, $p = 0.003$, respectively; Table 3), although CRP levels in the smoking sub-groups were not significantly different. The concentration of the anti-inflammatory cytokine IL-4 was lower in cases ($p = 0.006$). This was also seen in the smoking group, but not in non-smokers. Levels of the other two anti-inflammatory cytokines (IL-5, IL-10) in the two groups were not different (Table 3). Levels of IL-8 were also lower in cases, whereas levels of IL-18 were higher. Levels of the pro-inflammatory cytokines IL-1β and TNF-α were similar (Table 3).

Lymphocyte and monocyte numbers in a subgroup of the participants (38 cases, 49 controls) were also determined. These analyses revealed that lymphocyte ($p = 0.008$) and monocyte ($p = 0.071$) counts were higher in the patient group (data not shown).

Circulating levels of IgA, IgG and IgM in cases and controls were similar (data not shown).

Levels of antibodies against Hsp60 (IgG2) and Hsp65 (IgG1) were lower in the cases, although this difference was not apparent in the non-smoking subgroup. Levels of IgA antibodies against Hsp65 were elevated, and this was especially observed in non-smokers (Table 4).

The regression analyses revealed that those with the highest glucose (top third) were significantly more likely to belong to the periodontitis group, odds ratio (OR) 11.6 [95% confidence interval (CI) 3.6–36.8]. A similar result was seen for CRP, OR 4.0 (CI 1.4–11.44) and for IL-18 (OR 6.5, CI 2.2–19.5). In contrast, those with lower IL-4 values were significantly more likely to belong to the periodontitis group (OR 0.1, CI 0.0–0.5).

Discussion

Although the reported association between periodontitis and CVD remains

Table 2. Mean values (SD) of plasma lipoproteins, body mass index (BMI) and glucose in smoking and non-smoking subjects with and without periodontitis

	Cases (n = 68)	Healthy controls (n = 48)	Odds ratio (95% CI) (n = 116)	Ever smoking cases (n = 34)	Ever smoking healthy controls (n = 21)	Never smoked cases (n = 34)	Never smoked controls (n = 27)
Glucose (mmol/l)	**5.28 (0.67)** **p1 < 0.001**	**4.78 (0.59)**	**11.56 (3.63–36.77)** **p2 < 0.001**	**5.21 (0.64)** **p1 = 0.028**	**4.79 (0.71)**	**5.36 (0.71)** **p1 < 0.001**	**4.77 (0.50)**
Triglycerides (mmol/l)	1.46 (0.9)	1.31 (0.45)	1.00 (0.38–2.65)	1.53 (1.11)	1.34 (0.46)	1.38 (0.64)	1.28 (0.44)
Total cholesterol (mmol/l)	5.44 (0.93)	5.49 (1.10)	1.05 (0.37–3.01)	5.64 (0.94)	5.38 (0.98)	5.25 (0.90)	5.58 (0.90)
HDL C (mmol/l)	**1.37 (0.39)** **p1 = 0.043**	**1.53 (0.41)**	0.45 (0.16–1.32)	1.48 (0.38)	1.49 (0.37)	**1.26 (0.39)** **p1 = 0.009**	**1.55 (0.44)**
Lipid profile	4.29 (1.42)	3.82 (1.14)	1.98 (0.67–5.86)	4.11 (1.40)	3.79 (0.99)	4.47 (1.44)	3.84 (1.26)
LDL C, mmol/l	3.41 (0.86)	3.37 (1.01)	2.05 (0.73–5.77)	3.47 (0.88)	3.28 (0.86)	3.35 (0.85)	3.44 (1.13)
Waist/hip ratio	0.90 (0.08)	0.89 (0.11)		0.89 (0.06)	0.92 (0.15)	0.91 (0.09)	0.87 (0.08)
BMI	25.9 (3.5)	25.0 (4.1)		24.8 (3.3)	25.3 (5.2)	**26.8 (3.5)** **p1 = 0.005**	**24.8 (2.9)**

Statistically significant differences between cases and controls within each category are in bold.

p1 indicates significance of difference between cases with periodontitis and periodontally healthy calculated with Students *t*-test, p2 indicates significance of odds ratio. Only *p*-values ⩽0.05 are reported.

Odds ratios [95% confidence interval (CI)] for the association between indicated variables and the periodontal disease group. Indicators are adjusted for gender, age, tobacco smoking and BMI. The upper third of all participants was compared with the lowest third.

Table 3. Acute phase reactants (mean and SD or median and quartile range) and cytokines from subjects with and without periodontitis, smokers and non-smokers

	Cases with severe periodontitis (n = 67)	Healthy controls (n = 47)	Odds ratio (95% CI)	Ever smoking cases (n = 34)	Ever smoking controls (n = 21)	Never smoked cases (n = 33)	Never smoked controls (n = 26)
CRP (mg/l*)	**1.35 (2.10)** $p < 0.006$	**0.92 (0.80)**	**4.03 (1.42–11.44)** $p2 = 0.009$	1.142 (1.18)	0.96 (0.81)	**2.00 (2.72)** $p < 0.008$	**0.87 (0.94)**
Fibrinogen (g/l)	**2.54 (0.36)** $p < 0.001$	**2.17 (0.39)**	**8.67 (2.64–28.44)** $p2 < 0.001$	**2.55 (0.35)** $p < 0.001$	**2.18 (0.34)**	**2.53 (0.38)** $p < 0.025$	**2.17 (0.42)**
Haptoglobin (g/l)	**1.33 (0.39)** $p = 0.003$	**1.12 (0.36)**	2.13 (0.81–5.63)	**1.36 (0.36)** $p = 0.027$	**1.12 (0.38)**	1.31 (0.42)	1.11 (0.36)
IL-1β (ng/l*)	0.00 (0.50)	0.33 (0.56)	1.22 (0.31–4.76)	0.00 (0.39)	0.00 (1.08)	0.00 (0.54)	0.33 (0.48)
IL-4 (ng/l*)	**1.44 (11.1)** $p = 0.009$	**9.70 (71.9)**	**0.12 (0.03–0.47)** $p2 = 0.002$	**1.15 (3.13)** $p = 0.008$	**9.7 (112.68)**	2.74 (21.62)	13.04 (44.06)
IL-5 (ng/l*)	0.00 (0.87)	0.15 (1.17)	0.31 (0.08–1.22)	0.00 (0.34)	0.15 (1.178)	0.13 (0.50)	0.15 (0.71)
IL-6, (ng/l*)	8.64 (19.87) *n = 55*	17.68 (33.69) *n = 30*	0.8 (0.25–2.51)	7.90 (14.1) *n = 24*	17.48 (41.47) *n = 14*	10.41 (22.47) *n = 31*	19.49 (33.69) *n = 16*
IL-8 (ng/l*)	**3.55 (3.37)** $p < 0.018$	**6.25 (16.13)**	**0.34 (0.14–0.91)** $p2 = 0.031$	2.88 (3.07)	5.18 (10.74)	3.83 (4.38)	7.00 (16.21)
IL-10 (ng/l*)	2.22 (4.08)	1.65 (4.60)	0.48 (0.14–1.64)	2.23 (3.66)	1.89 (5.51)	2.14 (4.03)	0.42 (3.59)
IL-18 (ng/l*)	**245 (122)** $p < 0.001$	**188 (99)**	**6.46 (2.15–19.46)** $p2 = 0.001$	**252 (106)** $p = 0.01$	**200 (100)**	**239 (145)** $p < 0.011$	**183 (98)**
IFN-γ (ng/l*)	14.08 (41.57) *n = 55*	10.84 (25.25) *n = 30*	0.27 (0.37–4.30)	19.12 (57.37) *n = 24*	14.12 (26.33) *n = 14*	11.82 (24.42) *n = 31*	8.87 (25.97) *n = 16*
TNF-α (ng/l*)	3.61 (2.14)	3.56 (2.08)	0.87 (0.32–2.32)	3.17 (2.24)	3.97 (2.69)	3.20 (4.06)	3.45 (2.00)

*Variable not normally distributed, expressed as median and quartile range.

$p1$ indicates significance of difference between cases with periodontitis and periodontally healthy calculated with Students t-test or Mann–Whiney U-test. $p2$ indicates significance of odds ratio. Only p-values $\leqslant 0.05$ are reported. Statistically significant differences between cases and controls within each category are in bold.

Odds ratios [95% confidence interval (CI)] for the association between indicated variables and the periodontal disease group. Indicators are adjusted for gender, age, tobacco smoking and body mass index. The upper third of all participants was compared with the lowest third.

Table 4. Median values (quartile range 75–25%) of heat shock protein antibodies in smoking and non-smoking subjects with and without periodontitis

	Cases with severe periodontitis (n = 64)	Healthy controls (n = 48)	Odds ratio (95% CI)	Ever smoking cases (n = 30)	Ever smoking healthy controls (n = 21)	Never smoked cases (n = 34)	Never smoked controls (n = 26)
IgA anti-Hsp60*	0.107 (0.09)	0.101 (0.101)	1.25 (0.48–3.25)	0.089(0.05)	0.158 (0.156)	0.134 (0.127)	0.100 (0.06)
IgG1 anti-Hsp60*	**0.018 (0.03)** $p<0.05$	**0.008 (0.02)**	2.64 (0.98–7.15)	**0.015 (0.03)**	0.014 (0.03)	**0.020 (0.04)** $p<0.05$	**0.008 (0.02)**
IgG2 anti-Hsp60*	**0.011 (0.01)** $p<0.05$	**0.015 (0.02)**	**0.29 (0.10–0.82)** $p2 = 0.019$	**0.009 (0.009)** $p<0.01$	**0.014 (0.02)**	0.013 (0.02)	0.015 (0.02)
IgA anti-Hsp65*	**0.130 (0.13)** $p<0.01$	0.103(0.13)	2.60 (0.97–6.99)	**0.127(0.13)**	0.131 (0.17)	**0.140 (0.17)** $p<0.01$	**0.098 (0.01)**
IgG1 anti-Hsp65*	**0.09 (0.08)** $p<0.005$	**0.019 (0.02)**	**2.77 1.02–7.57** $p2 = 0.046$	**0.04 (0.05)** $p<0.05$	**0.016 (0.04)**	0.040 (0.06)	0.03 (0.03)
IgG2 anti-Hsp65*	0.02 (0.04)	0.025 (0.04)	0.62 (0.23–1.66)	0.017 (0.05)	0.026 (0.03)	0.016 (0.03)	0.023 (0.05)
IgA anti-Hsp70*	0.09 (0.08)	0.07 (0.06)	**2.88 (1.07–7.80)** $p2 = 0.037$	0.09 (0.07)	0.01 (0.08)	**0.10 (0.08)** $p<0.05$	**0.06 (0.06)**
IgG1 anti-Hsp70*	**0.004 (0.02)** $p<0.05$	**0.002 (0.01)**	**3.42 (1.23–9.54)** $p2 = 0.019$	0.003(0.02)	0.004 (0.008)	**0.007 (0.024)** $p<0.05$	**0.00 (0.006)**
IgG2 anti-Hsp70*,†	0.00 (0.01)	0.00 (0.00)	2.10 (0.35–2.65)	0.00 (0.014)	0.00 (0.00)	0.00 (0.024)	0.00 (0.005)

*Data are absorbance values generated by 1/100 dilution of sample.

†Upper half compared with lower half.

Statistically significant differences between cases and controls within each category are in bold.

$p1$ indicates significance of difference between patients with periodontitis and periodontally healthy calculated with Mann–Whiney *U*-test. $p2$ indicates significance of odds ratio. Only *p*-values ≤ 0.05 are reported. Statistically significant differences between cases and controls within each category are in bold.

Odds ratios [95% confidence interval (CI)] for the association between indicated variables and the periodontal disease group. Indicators are adjusted for gender, age, tobacco smoking and body mass index. The upper third of all participants was compared with the lowest third.

controversial, a mechanistic or a causal relationship cannot be excluded. This study has shown an association between levels of three prominent risk factors for CVD (HDL cholesterol, hyperglycaemia and CRP) and periodontitis. The observed association between periodontitis and low HDL cholesterol level (OR 3.22) is important, as the mortality in individuals with HDL cholesterol levels below 0.9 mmol/l is higher than that in individuals with levels over 0.9 mmol/l, regardless of the total cholesterol levels (Goldbourt et al. 1997). In this study, the HDL cholesterol concentration was ≤ 0.9 mmol/l in nine (13%) of the cases, but only one (2%) of the individuals in the comparison group. This finding is in agreement with earlier studies (Buhlin et al. 2003, Nibali et al. 2007b). However, the observation that there were no differences in HDL cholesterol in the smoking groups indicates that periodontal inflammation might have an impact only on non-smokers. One explanation for the relationship between periodontitis and low-HDL cholesterol levels might be that chronic inflammation in the periodontium leads to the release of lipopolysaccharide and pro-inflammatory cytokines such as IL-1β and TNF-α, which have the capacity to influence lipid metabolism (Iacopino & Cutler 2000).

Fasting glucose levels were 0.5 mmol/l higher in the patient group, and regression analysis showed that those with the highest glucose levels belonged to the periodontitis group (OR 15.5). The American Diabetes Association defines pre-diabetes as fasting plasma glucose levels above 5.5 mmol/l (100 mg/dl). On the basis of this definition, significantly more cases were pre-diabetic (35% *versus* 4%). Although diabetes is an established risk factor for periodontitis (Khader et al. 2006), it is not currently known whether pre-diabetes or impaired glucose tolerance also increases the risk. Several studies have also indicated that periodontitis *per se* is a risk factor for diabetes; however, conclusive evidence has yet to appear (Janket et al. 2005). Diagnosed diabetes was an exclusion criterion, and yet 10 cases with periodontitis and one control had a fasting glucose of 6.1 mmol/l or more. This is in line with a recent study by Borrell et al. (2007), which showed that the risk of undiagnosed diabetes increases with the presence of periodontitis. In line with previous findings from our group (Buhlin et al. 2003), the

cases also had a tendency to weigh more in the current study, although the difference was significant only for non-smokers.

Increased levels of CRP, fibrinogen and haptoglobin and the elevated number of lymphocytes indicated a more pronounced systemic inflammation in the patient group. The mean CRP was 0.9 mg/l higher in cases, and differences of this magnitude have been shown to influence the risk for CVD (Ridker et al. 2002). Higher CRP levels in cases with periodontitis have been shown in several earlier studies (Slade et al. 2000, Noack et al. 2001, Buhlin et al. 2003). Even though the elevated levels of CRP are relatively small, this could still be of clinical importance, as slightly elevated levels of CRP can be considered as an independent risk factor for developing CVD. This is especially so, given the chronicity of generalized periodontitis and the consequential exposure to its systemic inflammatory consequences for a prolonged period of time (de Ferranti & Rifai 2002). Using surrogate markers for atherosclerosis (e.g. HDL cholesterol and CRP) also has limitations; however, endpoints for CVD such as stroke or death are not practical and feasible in a case–control study such as this due to time constraints.

In studies involving associations, it is important to assess confounding and effect modification factors (Hyman 2006), and it is for this reason that those with systemic diseases were excluded from this study. Smoking habit is another confounding factor that is also an effect modification factor, and subjects were therefore divided into different smoking status groups. A case–control study such as this has its weaknesses; this study was not blinded and the cases originate from four different areas, whereas the controls originate from only one of these. These shortcomings resulted from the practicalities of undertaking the study during ordinary dental care visits. This could mean that there is some selection bias, as has been discussed by Lopez et al. (2007). However, there is always a possibility that other factors or patterns could influence the findings, and associations between common diseases should be therefore interpreted with caution.

Regarding circulating levels of cytokines, the most pronounced difference between cases and controls related to the

anti-inflammatory cytokines, IL-4, IL-5 and IL-10. These cytokines were significantly lower in the patient group. The clinical implications of these findings are difficult to interpret; however, an inability to resolve inflammation has been suggested as being a pathogenic trait of cases with periodontitis (Johnson et al. 2004). This proposition is supported, in part at least, by recent findings that indicate that circulating levels of putative anti-inflammatory stress proteins Hsp10 and Bip (also known as grp78) are significantly lower in individuals with periodontitis (Shamaei-Tousi et al. 2007).

Levels of the more archetypical pro-inflammatory cytokines IL-1β and TNF-α were not elevated in the patient group, whereas levels of IL-18 were. Although some studies have shown an association between IL-18 and CVD (Blankenberg et al. 2003, Tiret et al. 2005), a recent study from Germany failed to show an independent association (Koenig et al. 2006). Whether the alleged association between IL-18 and CVD is causal or just a reflection of the vascular disease has not been established. There have been no previous reports on plasma IL-18 levels in periodontitis; however, increased levels of IL-18 have been shown to be present in inflamed periodontal tissues (Johnson & Serio 2005) and in gingival crevicular fluid from sites showing tissue destruction (Orozco et al. 2006).

Conclusions

This study shows that cases with periodontitis have aberrant levels of some serological risk factors for CVD. Glucose, CRP and IL-18 were significantly higher, and HDL cholesterol significantly lower in the cases. These differences might contribute to the heightened risk for CVD in cases with periodontitis, which has been reported in earlier epidemiological studies.

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References

Andriankaja, O. M., Genco, R. J., Dorn, J., Dmochowski, J., Hovey, K., Falkner, K. L. & Trevisan, M. (2007) Periodontal disease and risk of myocardial infarction: the role of gender and smoking. *European Journal of Epidemiology* **22**, 699–705.

Bahekar, A. A., Singh, S., Saha, S., Molnar, J. & Arora, R. (2007) The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: a meta-analysis. *American Heart Journal* **154**, 830–837.

Blankenberg, S., Luc, G., Ducimetiere, P., Arveiler, D., Ferrieres, J., Amouyel, P., Evans, A., Cambien, F. & Tiret, L. (2003) PRIME Study Group. Interleukin-18 and the risk of coronary heart disease in European men: the Prospective Epidemiological Study of Myocardial Infarction (PRIME). *Circulation* **108**, 2453–2459.

Borrell, L. N., Burt, B. A. & Taylor, G. W. (2005) Prevalence and trends in periodontitis in the USA: the [corrected] NHANES, 1988 to 2000.[erratum appears in *Journal of Dental Research.* 2006;85:287]. *Journal of Dental Research* **84**, 924–930.

Borrell, L. N., Kunzel, C., Lamster, I. & Lalla, E. (2007) Diabetes in the dental office: using NHANES III to estimate the probability of undiagnosed disease. *Journal of Periodontal Research* **42**, 559–565.

Buhlin, K., Gustafsson, A., Pockley, A. G., Frostegård, J. & Klinge, B. (2003) Cardiovascular disease, periodontitis and the monocyte relationship. *European Heart Journal* **25**, 1365–1366.

Cairo, F., Castellani, S., Gori, A. M., Nieri, M., Baldelli, G., Abbate, R. & Pini-Prato, G. P. (2008) Severe periodontitis in young adults is associated with sub-clinical atherosclerosis. *Journal of Clinical Periodontology* **35**, 465–472.

DeStefano, F., Anda, R. F., Kahn, H. S., Williamson, D. F. & Russell, C. M. (1993) Dental disease and risk of coronary heart disease and mortality. *British Medical Journal* **306**, 688–691.

Desvarieux, M., Demmer, R. T., Rundek, T., Boden-Albala, B., Jacobs, D. R. Jr., Sacco, R. L. & Papapanou, P. N. (2005) Periodontal microbiota and carotid intima-media thickness: the Oral Infections and Vascular Disease Epidemiology Study (INVEST). *Circulation* **111**, 576–582.

Dorn, B. R., Dunn, W. A. & Progulske-Fox, A. (1999) Invasion of human coronary artery cells by periodontal pathogens. *Infection and Immunity* **67**, 5792–5798.

Espinola-Klein, C., Rupprecht, H. J., Blankenberg, S., Bickel, C., Kopp, H., Rippin, G., Victor, A., Hafner, G., Schlumberger, W. & Meyer, J. (2002a) AtheroGene investigators. Impact of infectious burden on extent and long-term prognosis of atherosclerosis. *Circulation* **105**, 15–21.

Espinola-Klein, C., Rupprecht, H. J., Blankenberg, S., Bickel, C., Kopp, H., Victor, A., Hafner, G., Prellwitz, W., Schlumberger, W.

& Meyer, J. (2002b) Impact of infectious burden on progression of carotid atherosclerosis. *Stroke* **33**, 2581–2586.

de Ferranti, S. & Rifai, N. (2002) C-reactive protein and cardiovascular disease: a review of risk prediction and interventions. *Clinica Chimica Acta* **317**, 1–15.

Goldbourt, U., Yaari, S. & Medalie, J. H. (1997) Isolated low HDL cholesterol as a risk factor for coronary heart disease mortality. A 21-year follow-up of 8000 men. *Arteriosclerosis, Thrombosis, and Vascular Biology* **17**, 107–113.

Herzberg, M. C. & Meyer, M. W. (1998) Dental plaque, platelets, and cardiovascular diseases. *Annals of Periodontology* **3**, 151–160.

Hujoel, P. P., Cunha-Cruz, J. & Kressin, N. R. (2006) Spurious associations in oral epidemiological research: the case of dental flossing and obesity. *Journal of Clinical Periodontology* **33**, 520–523.

Hujoel, P. P., Drangsholt, M., Spiekerman, C. & DeRouen, T. A. (2002) Periodontitis–systemic disease associations in the presence of smoking – causal or coincidental. *Periodontology 2000* **30**, 51–60.

Honda, T., Oda, T., Yoshie, H. & Yamazaki, K. (2005) Effects of *Porphyromonas gingivalis* antigens and proinflammatory cytokines on human coronary artery endothelial cells. *Oral Microbiology and Immunology* **20**, 82–88.

Hyman, J. (2006) The importance of assessing confounding and effect modification in research involving periodontal disease and systemic diseases. *Journal of Clinical Periodontology* **33**, 102–103.

Iacopino, A. M. & Cutler, C. W. (2000) Pathophysiological relationships between periodontitis and systemic disease. Recent concepts involving serum lipids. *Journal of Periodontology* **71**, 1375–1384.

Janket, S. J., Wightman, A., Baird, A. E., Van Dyke, T. E. & Jones, J. A. (2005) Does periodontal treatment improve glycemic control in diabetic patients? A meta-analysis of intervention studies. *Journal of Dental Research* **84**, 1154–1159.

Johnson, R. B. & Serio, F. G. (2005) Interleukin-18 concentrations and the pathogenesis of periodontal disease. *Journal of Periodontology* **76**, 785–790.

Johnson, R. B., Wood, N. & Serio, F. G. (2004) Interleukin-11 and IL-17 and the pathogenesis of periodontal disease. *Journal of Periodontology* **75**, 37–43.

Khader, Y. S., Dauod, A. S., El-Qaderi, S. S., Alkafajei, A. & Batayha, W. Q. (2006) Periodontal status of diabetics compared with nondiabetics: a meta-analysis. *Journal of Diabetes and its Complications* **20**, 59–68.

Koenig, W., Khuseyinova, N., Baumert, J., Thorand, B., Loewel, H., Chambless, L., Meisinger, C., Schneider, A., Martin, S., Kolb, H. & Herder, C. (2006) Increased concentrations of C-reactive protein and IL-6 but not IL-18 are independently associated with incident coronary events in middle-aged men and women: results from the MONICA/KORA Augsburg case-cohort 0study, 1984–

2002. *Arteriosclerosis, Thrombosis, and Vascular Biology* **26**, 2745–2751.

Loos, B. G. (2005) Systemic markers of inflammation in periodontitis. *Journal of Periodontology* **76**, 2106–2115.

Lopez, R., Scheutz, F., Errboe, M. & Baelum, V. (2007) Selection bias in case–control studies on periodontitis: a systematic review. *European Journal of Oral Sciences* **115**, 339–343.

Love, W. D., Ramirez, J. M. & Fultz, R. P. (1975) An oral hygiene measurement system for possible research and clinical use. *Journal of Public Health Dentistry* **35**, 227–230.

Mattila, K. J., Nieminen, M. S., Valtonen, V. V., Rasi, V. P., Kesaniemi, Y. A., Syrjala, S. L., Jungell, P. S., Isoluoma, M., Hietaniemi, K. & Jokinen, M. J. (1989) Association between dental health and acute myocardial infarction. *British Medical Journal* **298**, 779–781.

Nibali, L., D'Aiuto, F., Griffiths, G., Patel, K., Suvan, J. & Tonetti, M. S. (2007a) Severe periodontitis is associated with systemic inflammation and a dysmetabolic status: a case–control study. *Journal of Clinical Periodontology* **34**, 931–937.

Nibali, L., Ready, D. R., Parkar, M., Brett, P. M., Wilson, M., Tonetti, M. S. & Griffiths, G. S. (2007b) Gene polymorphisms and the prevalence of key periodontal pathogens. *Journal of Dental Research* **86**, 416–420.

Noack, B., Genco, R. J., Trevisan, M., Grossi, S., Zambon, J. J. & De Nardin, E. (2001) Periodontal infections contribute to elevated systemic C-reactive protein level. *Journal of Periodontology* **72**, 1221–1227.

Nyman, S. & Lindhe, J. (2003) Examination of patients with Periodontal Disease. In: Lindhe, J. (ed). *Clinical Periodontology and Implant Dentistry*, pp. 403–413. Copenhagen: Blackwell Munksgaard.

Orozco, A., Gemmell, E., Bickel, M. & Seymour, G. J. (2006) Interleukin-1beta, interleukin-12 and interleukin-18 levels in gingival fluid and serum of patients with gingivitis and periodontitis. *Oral Microbiology and Immunology* **21**, 256–260.

Pockley, A. G., Wu, R., Lemne, C., Kiessling, R., de Faire, U. & Frostegård, J. (2000) Circulating heat shock protein 60 is associated with early cardiovascular disease. *Hypertension* **36**, 303–307.

Ridker, P. M., Rifai, N., Rose, L., Buring, J. E. & Cook, N. R. (2002) Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *New England Journal of Medicine* **347**, 1557–1565.

Shamaei-Tousi, A., D'Aiuto, F., Nibali, L., Steptoe, A., Coates, A. R., Parkar, M., Donos, N. & Henderson, B. (2007) Differential regulation of circulating levels of molecular chaperones in patients undergoing treatment for periodontal disease. *PLoS One* **2**, e1198.

Slade, G. D., Offenbacher, S., Beck, J. D., Heiss, G. & Pankow, J. S. (2000) Acute-phase inflammatory response to periodontal disease in the US population. *Journal of Dental Research* **79**, 49–57.

Tiret, L., Godefroy, T., Lubos, E., Nicaud, V., Tregouet, D. A., Barbaux, S., Schnabel, R.,

Bickel, C., Espinola-Klein, C., Poirier, O., Perret, C., Munzel, T., Rupprecht, H. J., Lackner, K., Cambien, F. & Blankenberg, S. (2005) AtheroGene Investigators. Genetic analysis of the interleukin-18 system highlights the role of the interleukin-18 gene in cardiovascular disease. *Circulation* **112**, 643–650.

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Clinical Relevance

Scientific rationale for study: Studies have shown an association between CVD and periodontitis. Individuals with periodontitis have been investigated to determine whether plasma concentrations of risk markers for atherosclerosis are elevated.

Principal findings: The levels of some inflammation markers together with glucose were higher in cases, whereas levels of some anti-inflammatory markers were lower. Differences in the concentrations of HDL cholesterol were also observed.

Practical implications: Given that some of the differences found are indicative of an increased risk for CVD, these findings may, in part, support the proposition that there could be an association between these diseases.