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Periodontal infection and subclinical atherosclerosis: the role of high-density lipoprotein as a modifying factor

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Abstract

Background: Periodontitis is suggested to enhance the process of vascular inflammation leading to atherosclerosis. The purpose was to study the relation between periodontal infection and subclinical atherosclerosis, and whether this relation is dependent on high-density lipoprotein (HDL) cholesterol levels.

Methods: A secondary analysis of the data of a diabetic study, confined to 60 dentate subjects who underwent clinical examinations in 1990–1992 and in 1996–1998, was carried out. Ultrasonographic measurements of carotid, aortic and femoral atherosclerosis were performed in 2000.

Results: No consistent association was found between the presence of periodontal pockets and subclinical atherosclerosis in the total population, but a fairly strong association was found among subjects with a low HDL level, whereas in subjects with a high HDL level, an opposing and less consistent association was found. Product terms indicating a possible modification by HDL were statistically significant (at $p = 0.05$ level) for total plaques with all cut-off values, for plaques in carotid arteries with cut-off values 1.2–1.4 and for intima-media thickness with cut-off values of 1.1–1.2, but not for aorta plaques.

Conclusions: HDL levels or factors closely associated with HDL levels appear to modify the association between periodontal infection and certain parameters of subclinical atherosclerosis.

Key words: atherosclerosis; cardiovascular diseases; lipoproteins; periodontal infection

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It has been suggested that periodontal infection, like a few other chronic infections, plays a role in atheroma formation, thus increasing the risk of cardiovascular diseases. An association between periodontal infection and subclinical atherosclerotic alterations has been found in several studies. For example, subjects with severe periodontitis are more likely to have a thicker carotid intima-media wall than periodontally healthy subjects (Beck et al. 2001, Cairo et al. 2008), and subjects with a high amount of periodontal pathogens have a thicker carotid intima-media thickness (IMT) than those with a low number of periodontal pathogens (Desvarieux et al. 2005). Periodontitis has also been associated with coronary artery calcification (Nakib et al. 2004) and with impaired endothelial function (Tonetti et al. 2007). In addition, alveolar bone loss has been found to be associated with carotid plaque thickness in an exposure–response manner, the effect being more evident among never-smokers (Engebretson et al. 2005).

High-density lipoproteins (HDL) have several antiatherogenic properties, such as an ability to promote the efflux of cholesterol from cells, to function as an important antioxidant by inhibiting low-density lipoprotein (LDL) oxidation, to prevent or interrupt foam

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cell formation and to retard inflammatory activity, for instance (Ansell et al. 2005). These properties may prevent the harmful effects of infections, and conversely, infections may have a more detrimental effect in the absence of functioning HDL. Therefore, considering the possible biological mechanisms by which chronic bacterial infections increase the risk of atherosclerosis, the role of high-density lipoprotein (HDL) appears to be particularly interesting.

The aim of this paper was to study whether periodontal infection, defined as the presence of deepened periodontal pockets, is related to subclinical signs of cardiovascular disease, and secondly, whether these relations are dependent on the level of HDL cholesterol.

Methods

Study population

This was a secondary analysis of the data of a diabetic study, confined to 60 dentate subjects who had >10 teeth and did not smoke.

The subjects were drawn from a population-based cohort of those inhabitants of Oulu (the provincial capital of Northern Finland) who were born in 1935 (Anttila et al. 2001). The first phase of the study was conducted in 1990–1991, when altogether 768 subjects (participation rate 78%) participated in the first clinical examination, which also included the collection of information using questionnaires, interviews and laboratory tests. A follow-up took place in 1996–1998 and ultrasound examinations were conducted in 2000. The ethical committee of the Faculty of Medicine, University of Oulu, approved the study protocol.

Ultrasound examination

The ultrasound examinations were carried out at the Department of Diagnostic Radiology, Oulu University Hospital, by a single trained radiologist (M. P.) blinded to the participants' diabetic status and oral health status. A colour Doppler ultrasound system (Toshiba PowerVision 7000 or 8000, Tokyo, Japan) with a scanning frequency of 9 MHz in the B-mode was used. The dimensions measured were the IMT of the carotid arteries, the number of atheromatous plaques and the maximal diameter of a plaque. Arterial plaque was defined as an echogenic structure encroaching into the vessel lumen with a distinct area and resulting in $>50\%$ greater IMT compared with the neighbouring sites (Giral et al. 1991). A more detailed description of the ultrasound examinations is presented in studies by Rajala et al. (2005a, b). In this paper, we used the total number of aortic, carotid and femoral plaques, the total number of plaques in the carotid arteries on both sides, the number of aorta plaques and the mean of IMT as outcome variables.

Clinical medical examination

The values for HDL and LDL total cholesterol and triglycerides were determined from venous blood after a fast of 10–12 h during the follow-up study in 1996–1998. Each subject's weight and height in light clothing was measured in the clinical examination. Two separate measurements of blood pressure were performed by the radiologist from the subjects' right arm in a recumbent position after the ultrasound examination. The mean value of these two measurements was used in the analyses.

Diabetes was defined as either diabetes diagnosed by a physician before the baseline or as two elevated blood glucose values (either fasting blood glucose values of $\geqslant 6.1$ mmol/l or 2-h oral glucose-tolerance test values of $\geqslant 11.1$ mmol/l) over the study period between 1992 and 1998. Impaired glucose tolerance (IGT) was defined on the basis of the test results for oral glucose tolerance in the 1996–1998 follow-up study.

Clinical oral examinations

Clinical oral examinations were performed in the first phase of the study in 1990–1991 (baseline) and in the follow-up in 1998 by two dentists (T. S. and S. A.) (Anttila et al. 2001). The periodontal status included measurement of probing pocket depth within 1 mm limits at the four surfaces of each tooth. The subjects were categorized into three categories according to the cut-off values of pocket depth used in the community periodontal index of treatment need (CPITN). Subjects were categorized into three groups: with no periodontal pockets deeper than 3 mm, subjects with at least one tooth surface with a pocket depth of 4–5 mm and subjects with at least one tooth surface with a pocket depth of $\geqslant 6$ mm. The correlation between the first clinical oral examination (1990–1991) and the follow-up (1998) in sites with a pocket depth of 4–5 mm was 0.77, and in sites with a pocket depth of 6 mm or more, it was 0.77. The distributions of sites with deepened periodontal pockets are shown in Figs 1 and 2.

Statistical methods

We estimated relative risks (RR) with 95% confidence intervals (95% CI) using generalized linear models with a log link function and a negative binomial distribution. IMT was analysed using a generalized linear regression model with an identity link function. The models were adjusted for gender and diabetic status.

Possible modification (for the four outcome variables) was studied by

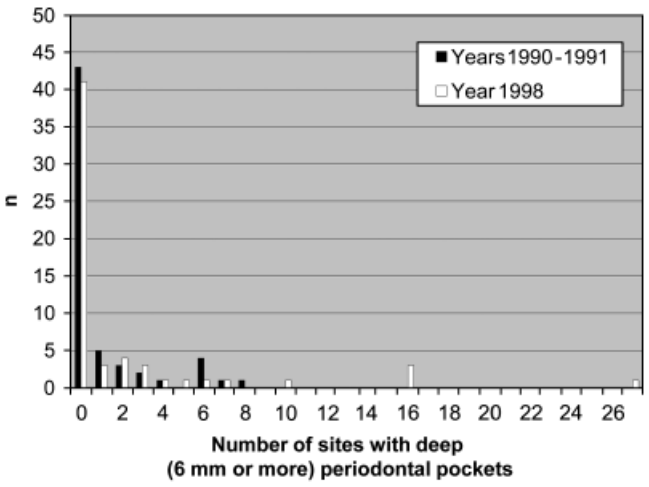

Fig. 1. Number of subjects with sites with deep (6 mm or more) periodontal pockets at baseline and follow-up.

adding product terms for the HDL variable and the explanatory variable in the models including other explanatory variables (gender and diabetic status). The data were analysed using the SAS GENMOD procedure, version 9.2.

Basic characteristics of the study population

The basic characteristics of the study subjects in relation to the presence of periodontal pockets and diabetic status are presented in Tables 1 and 2, respectively.

Results

In the total population, we did not find any consistent association between the periodontal infection and parameters characterizing subclinical atherosclerosis, except for a weak association between periodontal pocketing and aorta plaques (Tables 3 and 4).

When we studied whether HDL modified the association between periodontal infection and the different parameters characterizing subclinical atherosclerosis, we found that the pattern of association with total plaques, with plaques in carotid arteries and with aortic plaques and to a lesser extent with IMT was different among those with low HDL levels compared with those with high HDL levels.

The product terms of the HDL variable and exposure variables were statistically significant at the $p = 0.05$ level for the number of total plaques with all cut-off values used and statistically significant at the $p = 0.05$ level for plaques in carotid arteries with cut-off values 1.2–1.4.

In the association with IMT, the product term for the HDL variables and exposure variable was statistically significant at the $p = 0.05$ level with cut-off values of 1.1 or 1.2. The modifying effect of HDL in the association with the number of aorta plaques was not statistically significant at the $p = 0.05$ level (Tables 3 and 4).

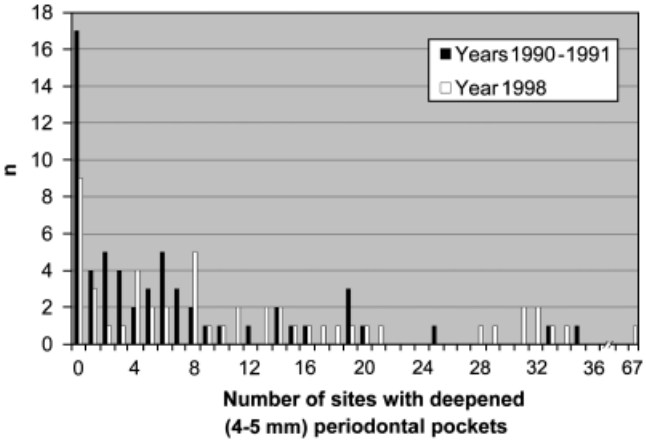

Fig. 2. Number of subjects with sites with deepened (4–5 mm) periodontal pockets at baseline and follow-up.

Discussion

The results of this preliminary study showed that among this study population, there was no consistent association between periodontal infection and subclinical parameters of atherosclerosis, except for aorta plaques, where a weak positive association was found. However, a fairly strong association between periodontal pockets and certain parameters of subclinical atherosclerosis was found among subjects with low HDL, whereas in subjects with a high HDL level, an opposing and less consistent association was found. This differing pattern in the associations between exposure and outcomes and statistically significant product terms for total plaques, for plaques in carotid arteries with cut-off values 1.2–1.4 can be interpreted in such a way that the effect of periodontal infection on parameters characterizing subclinical atherosclerosis may be dependent either on

Table 1. Characteristics of the study subjects in relation to periodontal pockets

	No deepened pockets at baseline ($n = 17$)	At least 1 pocket 4–5 mm deep at baseline ($n = 26$)	At least 1 pocket ⩾6 mm deep at baseline ($n = 17$)	Total ($n = 60$)
Intima-media thickness	0.93 ± 0.27	0.96 ± 0.15	0.93 ± 0.26	0.95 ± 0.22
Total number of plaques*	7.4 ± 7.8	6.8 ± 4.7	8.7 ± 7.1	7.5 ± 6.4
Number of carotid plaques	1.3 ± 1.4	2.0 ± 1.9	1.4 ± 1.5	1.6 ± 1.7
Number of aortic plaques	2.2 ± 2.0	2.5 ± 2.2	3.6 ± 2.5	2.8 ± 2.3
HDL cholesterol (mmol/l)	1.53 ± 0.32	1.48 ± 0.40	1.50 ± 0.56	1.50 ± 0.42
LDL cholesterol (mmol/l)	3.46 ± 1.10	4.06 ± 0.94	4.22 ± 1.29	3.94 ± 1.12
Total cholesterol (mmol/l)	5.81 ± 0.73	5.81 ± 0.98	5.64 ± 1.11	5.76 ± 0.95
Triglycerides (mmol/l)	1.40 ± 0.60	1.34 ± 0.52	1.44 ± 0.48	1.38 ± 0.53
BMI (kg/m^2)	26.7 ± 4.0	26.4 ± 3.8	27.0 ± 3.1	26.6 ± 3.6
DBP (mmHg)	82 ± 12	83 ± 10	85 ± 12	83 ± 11
SBP (mmHg)	149 ± 20	153 ± 21	154 ± 18	152 ± 20
Number of teeth at baseline	20.0 ± 5.4	23.9 ± 4.2	22.4 ± 4.0	22.3 ± 4.7
Number of pockets ⩾4 mm at baseline	0	5.2 ± 4.4	17.6 ± 14.2	7.3 ± 10.6
Number of pockets ⩾6 mm at baseline	0	0	3.5 ± 2.5	1.0 ± 2.1
Women (%)	65	65	53	62

The data present percentage or mean ± SD.

*Total number of aortic, carotid and femoral plaques.

DBP, diastolic blood pressure; SBP, systolic blood pressure; HDL, high-density lipoprotein; LDL; low-density lipoprotein; BMI, body mass index.

Table 2. Characteristics of the study population in relation to diabetic status

	DM* subjects ($n = 13$)	IGT† subjects ($n = 33$)	NGT‡ subjects ($n = 14$)	Total ($n = 60$)
Mean IMT (mm)	0.90 ± 0.25	0.97 ± 0.22	0.93 ± 0.20	0.95 ± 0.22
Total number of plaques§	6.5 ± 5.4	7.4 ± 5.5	8.6 ± 8.9	7.5 ± 6.4
Number of carotid plaques	1.5 ± 1.7	1.7 ± 1.6	1.5 ± 2.0	1.6 ± 1.7
Number of aortic plaques	3.2 ± 2.8	2.6 ± 1.8	2.6 ± 2.7	2.8 ± 2.3
HDL cholesterol (mmol/l)	1.52 ± 0.58	1.50 ± 0.39	1.49 ± 0.37	1.50 ± 0.42
LDL cholesterol (mmol/l)	3.90 ± 1.25	4.01 ± 1.05	3.81 ± 1.21	3.94 ± 1.12
Total cholesterol (mmol/l)	5.82 ± 1.10	5.70 ± 0.87	5.84 ± 1.03	5.76 ± 0.95
Triglycerides (mmol/l)	1.64 ± 0.60	1.29 ± 0.45	1.37 ± 0.59	1.38 ± 0.53
BMI (kg/m2)	28.6 ± 4.2	26.1 ± 3.2	26.1 ± 3.7	26.6 ± 3.6
DBP (mmHg)	87 ± 10	82 ± 11	84 ± 11	83 ± 11
SBP (mmHg)	160 ± 24	150 ± 19	148 ± 16	152 ± 20
Number of teeth at baseline	21.2 ± 4.7	23.1 ± 4.9	21.6 ± 4.4	22.3 ± 4.7
Number of pockets $\geqslant$4 mm at baseline	11.5 ± 17.5	6.5 ± 8.3	5.1 ± 5.4	7.3 ± 10.6
Number of pockets $\geqslant$6 mm at baseline	1.5 ± 2.5	0.9 ± 2.1	0.7 ± 1.6	1.0 ± 2.1
Women (%)	62	64	57	62

The data present percentage or mean ± SD.
*Diabetes mellitus.
†Impaired glucose tolerance.
‡Normoglycaemia.
§Total number of aortic, carotid and femoral plaques.
DBP, diastolic blood pressure; SBP, systolic blood pressure; HDL, high-density lipoprotein; BMI, body mass index; IGT, impaired glucose tolerance.

HDL levels or factors closely associated with HDL levels. The differing pattern was also found in the association between periodontal infection and IMT as well as between periodontal infection and aorta plaques with different cut-off values. However, the pattern of the association with IMT was not consistent with different cut-off values, whereas the pattern with aorta plaques was fairly consistent, although the modifying effect of HDL on aorta plaques was not statistically significant.

The results of this study conform well with experimental animal studies where a combination of risk factors, namely simultaneous presence of infection and a high-fat diet, has been shown to accelerate atheroma formation (Richardson et al. 1997, Li et al. 2002, Jain et al. 2003, Lalla et al. 2003). This study did not address the mechanisms by which HDL may modify the association between periodontal infection and atherosclerotic alterations. However, several well-known biological properties of HDL may be responsible for totally or partially blocking the possible effect of infection on atheroma formation, such as prevention or interruption of oxidation of LDL, prevention of foam cell formation and affecting inflammatory activity (Ansell et al. 2005).

An alternative explanation is that subclinical atherosclerotic alteration is a joint effect of low HDL (or a factor related to it) and other factors associated with the presence of deepened periodontal pockets. This would mean that periodontal infection is only a marker of such factors – these factors could be other infections, or innate or acquired susceptibility to infections, for instance.

Validity considerations

The subjects in this study were quite homogeneous due to several restrictions: our study subjects were never smoking members of the Oulu – 55 cohort, which means that age, ethnic origin, smoking and regional differences related to access to dental care, for example, most likely do not confound the association. Moreover, by confining the analyses to subjects who had >10 teeth, we were able to reduce heterogeneity in the study population and thus reduce the risk of confounding.

Because this was a secondary analysis of a diabetes study, the study population consisted of subjects with a differing diabetic status: diabetic patients, subjects with IGT and healthy subjects. Because of the small number of subjects, no profound adjustment could be made, and so diabetic status was the only covariate in addition to gender in our regression models. This means that the possibility of residual confounding is not totally excluded.

The overall participation rate in the radiographic measurements that were performed in 2000 was 95%. This fairly high participation rate means that bias from non-participation most likely does not essentially affect the results. However, due to the way in which the data were collected, there was no information about any death cases in the cohort members, but it can be expected that a high proportion of subjects with a high cardiovascular risk died before the latter phases of the study. This kind of survival bias may have an effect that is difficult to estimate.

We used a negative binomial distribution in the analyses, because the model fit was better than in the models with a Poisson's distribution. The small data size means that there is a risk for overparametrization (too many parameters in relation to the number of subjects), and in order to rule out this possibility, we also performed an analysis with no covariates. The results of these complementary analyses (data not shown) were not essentially different from those presented.

In regression analysis, where the outcome distribution follows a Poisson's distribution or a negative binomial distribution, the interpretation of the risk estimate is that it is the ratio between the rate (the rate of diseased teeth in relation to the total number of teeth) of the index category and the corresponding rate of the reference category of the determinant. Because, under suitable conditions, the ratios of rates and proportions approximate risk and because

Table 3. Multivariable regression models for total number of plaques, number of plaques in carotid arteries and number of plaques in aorta among the total study population and in high-density lipoprotein (HDL) subgroups. Adjusted* relative risks (RR) and 95% confidence intervals (CI)

Periodontal condition at baseline	RR (95% CI)		
	total number of plaques†	plaques in the carotid arteries	plaques in the aorta
Total population ($n = 60$)			
No deepened pockets	1.0	1.0	1.0
4–5-mm-deep pockets	0.9 (0.6–1.5)	1.6 (0.8–3.1)	1.2 (0.7–1.9)
6 mm or deeper pockets	1.1 (0.7–2.0)	1.0 (0.5–2.2)	1.6 (0.9–2.8)
		Analyses using different cut-off values	
		Cut-off value 1.1	
Subjects with HDL <1.1 ($n = 14$)			
No deepened pockets	1.0	1.0	1.0
4–5-mm-deep pockets	1.0 (0.3–3.3)	2.5 (0.4–50.5)	1.0 (0.3–4.3)
6 mm or deeper pockets	3.3 (0.9–11.4)	4.5 (0.7–89.5)	2.5 (0.7–10.3)
Subjects with HDL ≥ 1.1 ($n = 46$)			
No deepened pockets	1.0	1.0	1.0
4–5-mm-deep pockets	1.0 (0.6–1.6)	1.7 (0.9–3.4)	1.2 (0.7–2.2)
6 mm or deeper pockets	0.7 (0.4–1.3)	0.7 (0.3–1.5)	1.3 (0.7–2.4)
Statistical significance for the product term	0.01	0.05	0.26
		Cut-off value 1.2	
Subjects with HDL <1.2 ($n = 15$)			
No deepened pockets	1.0	1.0	1.0
4–5-mm-deep pockets	1.0 (0.3–3.3)	2.5 (0.4–49.4)	1.1 (0.3–4.7)
6 mm or deeper pockets	3.3 (0.9–11.4)	4.5 (0.7–88.9)	2.5 (0.7–10.4)
Subjects with HDL ≥ 1.2 ($n = 45$)			
No deepened pockets	1.0	1.0	1.0
4–5-mm-deep pockets	1.0 (0.6–1.7)	1.8 (0.9–3.5)	1.2 (0.7–2.1)
6 mm or deeper pockets	0.7 (0.4–1.3)	0.7 (0.3–1.5)	1.3 (0.7–2.4)
Statistical significance for the product term	0.01	0.04	0.36
		Cut-off value 1.3	
Subjects with HDL <1.3 ($n = 18$)			
No deepened pockets	1.0	1.0	1.0
4–5-mm-deep pockets	1.0 (0.3–3.2)	2.2 (0.3–43.1)	1.1 (0.3–4.7)
6 mm or deeper pockets	2.8 (0.8–9.1)	3.7 (0.6–73.0)	1.9 (0.5–8.1)
Subjects with HDL ≥ 1.3 ($n = 42$)			
No deepened pockets	1.0	1.0	1.0
4–5-mm-deep pockets	1.0 (0.6–1.8)	1.9 (1.0–3.7)	1.2 (0.7–2.2)
6 mm or deeper pockets	0.7 (0.4–1.3)	0.7 (0.3–1.6)	1.4 (0.8–2.7)
Statistical significance for the product term	0.01	0.04	0.75
		Cut-off value 1.4	
Subjects with HDL <1.4 ($n = 22$)			
No deepened pockets	1.0	1.0	1.0
4–5-mm-deep pockets	1.7 (0.6–4.4)	4.3 (0.8–81.6)	2.3 (0.7–8.5)
6 mm or deeper pockets	4.6 (1.7–12.0)	7.1 (1.3–133.0)	4.1 (1.4–15.3)
Subjects with HDL ≥ 1.4 ($n = 38$)			
No deepened pockets	1.0	1.0	1.0
4–5-mm-deep pockets	1.0 (0.6–1.6)	1.7 (0.9–3.2)	1.0 (0.6–1.9)
6 mm or deeper pockets	0.6 (0.3–1.1)	0.6 (0.2–1.3)	1.1 (0.6–2.2)
Statistical significance for the product term	<0.01	0.01	0.14
		Cut-off value 1.5	
Subjects with HDL <1.5 ($n = 30$)			
No deepened pockets	1.0	1.0	1.0
4–5-mm-deep pockets	1.2 (0.6–2.4)	1.7 (0.6–4.8)	1.5 (0.7–3.3)
6 mm or deeper pockets	2.3 (1.1–4.9)	1.6 (0.6–4.6)	2.1 (1.0–4.8)
Subjects with HDL ≥ 1.5 ($n = 30$)			
No deepened pockets	1.0	1.0	1.0
4–5-mm-deep pockets	0.8 (0.4–1.6)	1.5 (0.7–3.6)	0.9 (0.5–1.9)
6 mm or deeper pockets	0.6 (0.3–1.2)	0.7 (0.2–1.8)	1.2 (0.6–2.6)
Statistical significance for the product term	0.03	0.39	0.58

*Adjusted for diabetic status and gender.
†Total number of aortic, carotid and femoral plaques.

all these measures are relative risk measures, we called our risk estimate 'relative risk' (RR). This emphasizes that risk is not related to specific time periods but only to the referent category.

Because of the small number of subjects, the estimates were subject to a large random variation and consequently CIs were wide. A small data size also means that controversies may exist in the results. An example of such a seemingly controversial observation was that when HDL was higher than the cut-off values, subjects with deep pockets ($\geqslant$6 mm) had a lower

Table 4. Results of regression analyses. Regression coefficients and 95% confidence intervals (CI) between periodontal pockets and intima-media thickness (IMT) of the carotid arteries*

Periodontal condition at baseline	Regression co-efficient (95% CI) total population ($n = 60$)	*p*-value
No deepened pockets	0	
4–5-mm-deep pockets	0.04 (− 0.08–0.16)	0.51
6 mm or deeper pockets	− 0.02 (− 0.15–0.12)	0.81
Stratified analyses using different cut-off values		
Cut-off value 1.1		
Subjects with HDL < 1.1 ($n = 14$)		
No deepened pockets	0	
4–5-mm-deep pockets	− 0.14 (− 0.41–0.14)	0.32
6 mm or deeper pockets	0.03 (− 0.26–0.32)	0.86
Subjects with HDL ≥ 1.1 ($n = 46$)		
No deepened pockets	0	
4–5-mm-deep pockets	0.06 (− 0.06–0.18)	0.32
6 mm or deeper pockets	− 0.08 (− 0.21–0.06)	0.24
Statistical significance for the product term	0.04	
Cut-off value 1.2		
Subjects with HDL < 1.2 ($n = 15$)		
No deepened pockets	0	
4–5-mm-deep pockets	− 0.14 (− 0.41–0.13)	0.30
6 mm or deeper pockets	0.03 (− 0.26–0.32)	0.86
Subjects with HDL ≥ 1.2 ($n = 45$)		
No deepened pockets	0	
4–5-mm-deep pockets	0.06 (− 0.06–0.19)	0.32
6 mm or deeper pockets	− 0.08 (− 0.21–0.06)	0.24
Statistical significance for the product term	0.03	
Cut-off value 1.3		
Subjects with HDL < 1.3 ($n = 18$)		
No deepened pockets	0	
4–5-mm-deep pockets	− 0.16 (− 0.43–0.13)	0.27
6 mm or deeper pockets	− 0.08 (− 0.37–0.22)	0.60
Subjects with HDL ≥ 1.3 ($n = 42$)		
No deepened pockets	0	
4–5-mm-deep pockets	0.07 (− 0.06–0.20)	0.33
6 mm or deeper pockets	− 0.07 (− 0.22–0.07)	0.29
Statistical significance for the product term	0.11	
Cut-off value 1.4		
Subjects with HDL < 1.4 ($n = 22$)		
No deepened pockets	0	
4–5-mm-deep pockets	0.07 (− 0.14–0.28)	0.49
6 mm or deeper pockets	0.17 (− 0.05–0.39)	0.11
Subjects with HDL ≥ 1.4 ($n = 38$)		
No deepened pockets	0	
4–5-mm-deep pockets	0.03 (− 0.11–0.16)	0.68
6 mm or deeper pockets	− 0.15 (− 0.31– − 0.00)	0.05
Statistical significance for the product term	0.02	
Cut-off value 1.5		
Subjects with HDL < 1.5 ($n = 30$)		
No deepened pockets	0	
4–5-mm-deep pockets	0.05 (− 0.11–0.22)	0.52
6 mm or deeper pockets	0.10 (− 0.08–0.28)	0.27
Subjects with HDL ≥ 1.5 ($n = 30$)		
No deepened pockets	0	
4–5-mm-deep pockets	0.02 (− 0.13–0.19)	0.75
6 mm or deeper pockets	− 0.14 (− 0.32–0.04)	0.13
Statistical significance for the product term	0.12	

*Adjusted for diabetic status and gender.

likelihood of having total plaques in the aorta, carotid and femoral arteries, plaques in carotid arteries and high IMT than periodontally healthy subjects. However, this finding could at least partly be explained by the differences in the HDL levels; for unknown reasons, HDL levels among subjects whose HDL was higher than the cut-off values were consistently higher among those who had deepened periodontal pockets than periodontally healthy ones. One interpretation for this is that high HDL is truly able to prevent the effect of potentially harmful microbes, whereas an alternative/complementary explanation is that the findings of this study can partly be attributed to the individual variation in the HDL levels instead of any true biological interaction between infection and HDL.

Besides random occurrence, controversies in the results may also be attributed to the biological properties of HDL. For example, it has been suggested that HDL may, under certain circumstances, transform into a dysfunctional form of HDL (Nichols et al. 2005), meaning that a high level of HDL does not necessarily guarantee that it functions properly. In addition, when comparing the different outcomes, it appears that the relation between periodontal infection and aortic plaques is less modified by the level of HDL than plaques in carotid arteries or the total number of aortic, carotid and femoral plaques. This may be related to the differences in arterial cholesterol metabolism (Schwenke & Carew 1988) and/or local differences in the structure or the function of endothelial cells (Fujimoto & Singer 1986).

The small number of subjects also means that the clinically recommended cut-off value for HDL indicating an elevated cardiovascular disease risk (about 1.0 mmol/l) could not be used. Because of the lack of knowledge of an accurate cut-off value – in relation to the combined effect of periodontal infection and unfavourable lipid composition – and in order to exclude the possibility of a random finding when studying the modification, we used a different cut-off value from 1.1 to 1.5, the latter being the median value in these data.

Our inferences about the possible modifying effect of HDL are not based solely on statistical significance but mostly on the fact that associations between exposure and outcomes among the subgroups of HDL followed a different pattern. In the interpretation of the results from the statistical point of view, it must be kept in mind that the *p*-value is a confounded measure, determined by two compounds: the consistency of the association and the size of the study population. This means that it is not meaningful to base the interpretation merely on *p*-values, because even essential differences may not be statistically significant when data sizes are small, as is the case in the present study.

On the other hand, the pattern of the relation can be different and not consistent despite a statistically significant interaction, as it was seen in the case of IMT. Moreover, it must be added that the correspondence between statistical interaction (product term) in multiplicative models and biological interaction is poor; in fact, a biological interaction may be present even in the absence of a statistically significant interaction (actually in any *p*-value level obtained from a multiplicative model).

Periodontal infection was measured by means of the presence of deepened periodontal pockets. This variable was categorized as no deepened periodontal pockets, periodontal pockets with a pocket depth of 4–5 mm and periodontal pockets with a pocket depth of 6 mm or more. In relation to periodontal pocket depth, this categorization corresponds to the cut-off value used in the CPITN, which is used mostly in assessing treatment need but also in research. Despite this robust measurement of exposure, the subjects in these categories represent fairly different subjects on average in relation to periodontal condition (mean number of sites with deepened periodontal pockets) as shown in the descriptive table (Table 1). Moreover, it must be kept in mind that periodontal pocket depth is only one indicator of periodontal infection, although reflecting the current status more so than disease history.

In this study, the correlations between the baseline measurements and the follow-up study were fairly high: in shallow pockets (pocket depth of 4–5 mm), the correlation was 0.77 and was the same in deep pockets (6 mm or deeper). These high correlations between different time-points indicate that there were on average no essential changes in the periodontal condition of the subjects during the follow-up.

Lastly, it must be emphasized that despite the longitudinal aspects of this study, this is not a true longitudinal study in the sense that we used the presence of subclinical atherosclerotic alterations instead of the occurrence of such alterations as outcomes.

Concluding remarks

The results of this study suggest that the association of periodontal infection and subclinical atherosclerosis is dependent on HDL levels or factors closely associated with HDL levels. Despite a seemingly strong association with some of the parameters describing subclinical atherosclerosis, the findings of this pilot study should be interpreted cautiously, because chance related to the small study size and biases including confounding may account for the findings.

References

Ansell, B. J., Watson, K. E., Fogelman, A. M., Navab, M. & Fonarow, G. C. (2005) High-density lipoprotein function. *Journal of the American College of Cardiology Foundation* **46**, 1792–1798.

Anttila, S. S., Knuuttila, M. L. & Sakki, T. K. (2001) Relationship of depressive symptoms to edentulousness, dental health, and dental health behavior. *Acta Odontologica Scandinavica* **59**, 406–412.

Beck, J. D., Elter, J. R., Heiss, G., Couper, D., Mauriello, S. M. & Offenbacher, S. (2001) Relationship of periodontal disease to carotid artery intima-media wall thickness: the atherosclerosis risk in communities (ARIC) study. *Arteriosclerosis Thrombosis and Vascular Biology* **21**, 1816–1822.

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.

Desvarieux, M., Demmer, R. T., Rundek, T., Boden-Albala, B., Jacobs, D. R., 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.

Engebretson, S. P., Lamster, I. B., Elkind, M. S. V., Rundek, T., Serman, N. J., Demmer, R. T., Sacco, R. L., Papapanou, P. N. & Desvarieux, M. (2005) Radiographic measures of chronic periodontitis and carotid artery plaque. *Stroke* **36**, 561–566.

Fujimoto, T. & Singer, S. J. (1986) Immunocytochemical studies of endothelial cells in vivo, I: the presence of desmin only, or of desmin plus vimentin, or vimentin only, in the endothelial cells of different capillaries of the adult chicken. *Journal of Cell Biology* **103**, 2775–2786.

Giral, P., Pithois-Merli, I., Filitti, V., Levenson, J., Plainfosse, M. C., Mainardi, C. & Simon, A. C. (1991) Risk factors and early extracoronary atherosclerotic plaques detected by three-site ultrasound imaging in hypercholesterolemic men. *Archives of Internal Medicine* **151**, 950–956.

Jain, A., Batista, E. L. Jr., Serhan, C., Stahl, G. L. & Van Dyke, T. E. (2003) Role for periodontitis in the progression of lipid deposition in an animal model. *Infection and Immunity* **71**, 6012–6018.

Lalla, E., Lamster, I. B., Hofman, M. A., Bucciarelli, L., Jerud, A. P., Tucker, S., Lu, Y., Papapanou, P. N. & Schmidt, A. M. (2003) Oral infection with a periodontal pathogen accelerates early atherosclerosis in apolipoprotein E-null mice. *Arteriosclerosis Thrombosis and Vascular Biology* **23**, 1405–1411.

Li, L., Messas, E., Batista, E. L. Jr., Levine, R. A. & Amar, S. (2002) Porphyromonas gingivalis infection accelerates the progression of atherosclerosis in a heterozygous apolipoprotein E – deficient murine model. *Circulation* **105**, 861–867.

Nakib, S. A., Pankow, J. S., Beck, J. D., Offenbacher, S., Evans, G. W., Desvarieux, M. & Folsom, A. R. (2004) Periodontitis and coronary artery calcification: the Atherosclerosis Risk in Communities (ARIC) Study. *Journal of Periodontology* **75**, 505–510.

Nichols, S. J., Zheng, L. & Hazen, S. L. (2005) Formation of dysfunctional high-density lipoprotein by myeloperoxidase. *Trends in Cardiovascular Medicine* **15**, 212–219.

Rajala, U., Laakso, M., Päivänsalo, M., Pelkonen, O., Ruokonen, A., Suramo, I. & Keinänen-Kiukaanniemi, S. (2005a) Associations of microalbuminuria and blood pressure with carotid, aortic and femoral atheromatous plaques in elderly Finns. *Diabetes Research and Clinical Practise* **69**, 262–271.

Rajala, U., Laakso, M., Päivänsalo, M., Suramo, I. & Keinänen-Kiukaanniemi, S. (2005b) Blood pressure and atherosclerotic plaques in carotid, aortic and femoral arteries in elderly Finns with diabetes mellitus or impaired glucose tolerance. *Journal of Human Hypertension* **19**, 85–91.

Richardson, M., De Reske, M., Delaney, K., Fletch, A., Wilcox, L. H. & Kinlough-Rathbone, R. L. (1997) Respiratory infection in lipid-fed rabbits enhances sudanophilia and the expression of VCAM-1. *American Journal Pathology* **151**, 1009–1017.

Schwenke, D. C. & Carew, T. E. (1988) Quantification in vivo of increased LDL content and rate of LDL degradation in normal rabbit aorta occurring at sites susceptible to early atherosclerotic lesions. *Circulation Research* **62**, 699–710.

Tonetti, M. S., D'Aiuto, F., Nibali, L., Donald, A., Storry, C., Parkar, M., Suvan, J., Hingorani, A. D., Vallance, P. & Deanfield, J. (2007) Treatment of periodontitis and endothelial function. *New England Journal of Medicine* **356**, 911–920.

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Clinical Relevance

Scientific rationale for the study: Several studies have shown that periodontal infection, like other chronic infections, may enhance the process leading to atherosclerotic alterations. Whether this process is dependent on extraneous factors, such as lipid profile, is poorly known.

Principal findings: A fairly strong association between periodontal pockets and certain parameters of subclinical atherosclerosis was found among subjects with low HDL whereas in subjects with a high HDL level, an opposing and less consistent association was found.

Practical implications: The results of this preliminary study showed that HDL level or related factors may enhance the development of atherosclerotic vascular diseases. Additional research is needed to determine which factors modify the association between periodontal infection and atherosclerosis.