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ORIGINAL ARTICLE

C-reactive protein in relation to early atherosclerosis and periodontitis

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Abstract Periodontitis may affect atherosclerosis via the chronic inflammation. We investigated high-sensitivity C-reactive protein (hsCRP) in relation to early vascular atherosclerotic changes in non-symptomatic subjects with and without long-term periodontitis. Carotid ultrasonography with calculation of common carotid artery intima-media area (cIMA) was performed, and hsCRP and atherosclerosis risk factors were analysed in randomly chosen 93 patients with periodontitis and 41 controls. The relationship between hsCRP, cIMA and atherosclerosis risk factors was evaluated with multiple logistic regression analysis. Women displayed lower hsCRP ($p<0.05$) and higher serum HDL ($p<0.001$) than men. In all patients with periodontitis, cIMA values were higher than in controls. Periodontitis appeared to be a major predictor for increased cIMA (odds ratio, 3.82; 95% confidence interval, 1.19–12.26). Neither of these factors was significantly associated with hsCRP which thus appeared not sensitive enough to be a marker for periodontitis or atherosclerosis. Hence, irrespective of low hsCRP levels, periodontitis appeared to increase the risk for atherosclerosis.

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Periodontal disease is among the most commonly occurring infections in man [1]. It is estimated that 15% to 35% of the adult population in the industrialised countries suffers from this multi-factorial illness [2]. Periodontal disease is a unique and silent infection with potentially profound effects on general health [3]. Smoking is an important risk factor for the development of periodontitis and it increases its severity although bacterial pathogens are required to initiate the disease process [4, 5].

However, it has become evident that without the inflammatory host reaction, the presence of bacterial pathogens alone is not sufficient to cause tissue destruction that occurs in the course of periodontitis [5]. It has been suggested that in individuals with poor oral health, the reaction to bacteria may lead to an excessive host response resulting in systemic inflammatory reaction [6]. Thus, chronic infection and inflammation caused by periodontitis may indeed predispose the individuals to the development of atherosclerosis since the atherosclerotic process itself has been shown to include a strong inflammatory component [7].

Atherosclerotic cardiovascular disease is the most common cause of mortality not only in men but also among women in Sweden, accounting for as much as 45% of deaths each year in the Swedish female population [8]. In addition, seen from the international perspective, there is a high mortality from coronary artery disease among Swedish women. The coronary event rate expressed as event per 100,000 women is 2.3 times higher in Sweden than, for example, in Italy [9]. Age, gender, use of tobacco, high blood pressure and high blood lipid content are the classical risk factors associated with atherosclerotic cardiovascular

disease. In addition, results from several studies published during the last two decades indicate that also oral diseases, and especially periodontitis, may possibly act as risk factors for the development of cardiovascular disease via the chronic inflammation [3, 10–13].

The hepatocyte-derived acute-phase reactant C-reactive protein (CRP) has also been a subject of research over the last two decades for its possible role in the pathogenesis of cardiovascular diseases. In prospective studies CRP has been shown to be associated with an increased risk for myocardial infarctions [14], and it appeared as a risk factor of coronary heart disease (CHD) in Japanese men [15]. A positive correlation between periodontal infections and serum level of CRP has also been shown [16]. In a systematic review, Paraskevas et al. concluded that periodontitis elicits a mild acute-phase response with elevation of CRP levels in comparison to healthy controls [17]. In addition, significantly increased levels of high-sensitivity CRP (hsCRP) have been observed in pre-eclamptic women with moderate to severe periodontitis [18] and the pathogenic mechanism involved is thought to be the effect of chronic inflammation also in pre-eclampsia. Levels of hsCRP<1.0 mg/L, 1–3 mg/L and >3.0 mg/L have been associated with lower, moderate, and higher cardiovascular risks, respectively [19]. Microchip assays have been introduced for the measurement of CRP in human saliva and it seems that chronic periodontitis may also be associated with higher levels of salivary CRP [20].

The relationship between periodontal disease and hsCRP has been studied in the last five years in subjects harbouring certain bacteria [21]. Periodontitis often also coexists with general diseases such as rheumatoid arthritis and CHD [22], and with diabetes mellitus [23]. Ridker and Silwertown [24] have summarised the data supporting the role of inflammation in cardiovascular diseases. Consequently, chronic inflammation, such as in periodontal disease, may indeed play a role in atherosclerosis by influencing the risk, manifestation, and progression of vascular events [25]. So far, however, there are no reports about the relationship between the early development of atherosclerosis and levels of hsCRP in chronic periodontitis. Therefore, our aim was to study early atherosclerotic changes in carotid arteries and relate the findings to hsCRP levels in subjects who had suffered from chronic periodontitis for at least 18 years in our study cohort.

Materials and methods

Study participants

The baseline cohort was selected in 1985 using the registry file of all inhabitants of the Stockholm metropolitan area and included 3,273 individuals aged 30–40 years and born on the 20th of any month between 1945 and 1954 [4]. Subjects with three teeth (excluding third molars) with pocket depth>=5 mm as well as bleeding on probing (BOP) were diagnosed as having periodontal disease. After at least 18 years, 150 age- and gender-matched subjects with and without periodontal disease were chosen from the cohort for present cross-sectional study. The selection of the participants of the study is shown in Fig. 1. Subjects with aggressive periodontitis were excluded from the study. All subjects gave their informed consent to participate and the study plan had been approved by the Ethics Committee of the Karolinska University Hospital at Huddinge.

Clinical examinations, blood sampling and questionnaires

In all subjects, oral health parameters were recorded in the beginning and at the end of the study. Dental examinations were performed by one of the authors (B.S.) using the dental plaque index (PLI) according to Silness and Löe [26], marker for oral hygiene level, gingival index (GI) according to Löe and Silness, [27], marker for inflammation and tooth attachment loss (AL; marker of chronic periodontitis) [28]. At the time of the second oral examination at the end of the study, antecubital venous blood samples were taken after 12 h of overnight fasting for the blood analyses. Blood pressure was measured and 12-lead electrocardiogram was also recorded.

The subjects answered a questionnaire concerning health problems, medication, dental visits, use of tobacco, marital status, socioeconomic data and education. Smoking was expressed in pack years. None of the subjects participating in the study did report any known heart disease or diabetes mellitus. Body mass index values (BMI) were calculated from their anthropometric data.

Assays of hsCRP, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides in blood

HsCRP, total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, and triglycerides, were assayed in serum using routine methods at the Laboratory of Clinical Chemistry at Karolinska University Hospital, Huddinge, Sweden.

Carotid *B*-mode ultrasonography

Carotid ultrasonography was performed between 2001 and 2003. Carotid arteries were examined bilaterally with a duplex scanner (Aspen, Acuson, Mountain View, CA, USA) using a 7 MHz linear array transducer. All recordings were carried out by the same trained sonographer with the subjects in supine position, the head slightly turned away

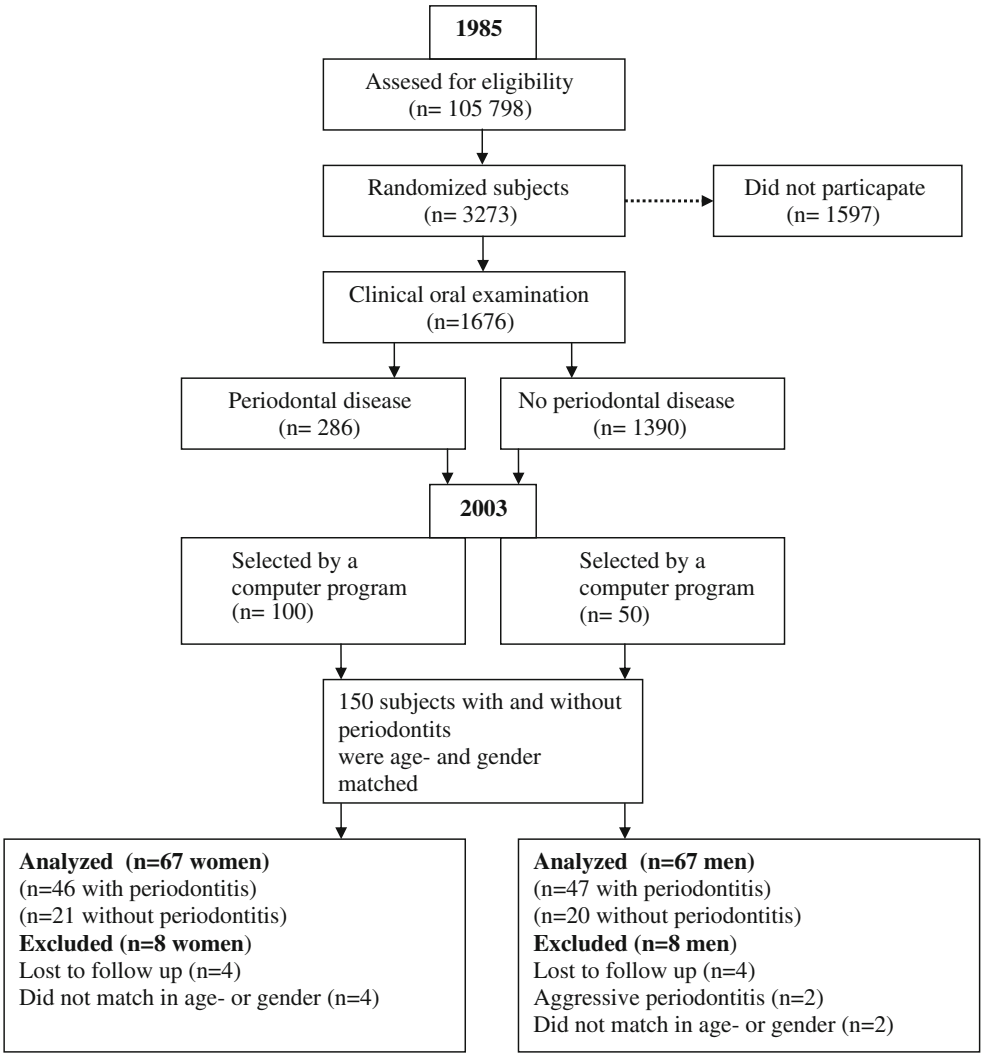

Fig. 1 The selection of the participants of the study

from the sonographer. The scans were videotaped for subsequent analyses by a computer system with automated tracing of echo interfaces [29]. Measurements of distances between the wall echoes within a 10-mm long section of the common carotid artery (CCA) were made in late diastole defined by a simultaneous electrocardiographic recording. The far wall of CCA, 0.5 to 1.0 cm proximal to the proximal delimitation of the carotid bulb, was used for measurements of the intima-media thickness and lumen diameter. The intima-media thickness was defined as the distance between the leading edge of the lumen-intima echo and the leading edge of the media-adventitia echo. The lumen diameter was defined as the distance between the leading edge of the intima-lumen echo of the near wall and the leading edge of the lumen-intima echo of the far wall. The mean values of the intima-media thickness and lumen diameter within each 10-mm long section were calculated unless presence of plaques was observed in the region of interest, in which cases the measurements of the intima-media thickness were abandoned. Carotid plaque was defined as a localised intima-media thickening of greater than 1 mm and at least a 100% increase in thickness compared with adjacent wall segments. In order to compensate for the stretching effect of arterial distension (secondary to increased arterial pressure) on the wall thickness, the cross-sectional intima-media area (cIMA) was calculated by using the formula 3.14 [(lumen diameter/2+intima-media thickness)^2−(lumen diameter/2)^2] [30]. The differences between repeated measurements of intima-media thickness and lumen diameter, using the automated analysing system, were 3.2% and 0.6% (coefficient of variation), respectively (with an intima-media thickness of 0.48 to 1.04 mm and a lumen diameter of 4.34 to 7.91 mm).

Statistical methods

All results are expressed as mean±SD. Statistically significant differences were determined either by Student's unpaired *t* test or analysis of variance. Since data was not distributed normally, we used logistic regression instead of

linear regression model, even if the dependent cIMA was a continuous variable. Chi-square tests were used when analysing non-parametric data. Multiple logistic regression analysis of hsCRP, with backwards elimination of non-significant variables was performed with dichotomized median split. The criteria for variable entry was p=<0.05 and for retention p=<0.10. All variables were included in the model. To reach 96% power in the hsCRP values for men and women at least 20 subjects were needed in each gender group with or without chronic periodontitis. The power calculation and data analyses were performed using the SPSS® software package, version 16.1 (SPSS Inc. Chicago, IL, USA). All p values are two-tailed, and confidence intervals were calculated at the 95% level.

Results

The characteristics of the subjects are given in Table 1. Women did not differ from men regarding age, smoking, BMI and the prevalence of hypertension. However, the percentage of individuals with higher education was greater among women when compared with men. Women had significantly lower hsCRP values (Fig. 2) and significantly higher HDL cholesterol values than men (Fig. 2). In general, men with chronic periodontitis had higher PLI and GI values and higher AL scores than women. Carotid artery ultrasonography showed that both the women and men with chronic periodontitis had significantly higher cIMA values as compared with the subjects without periodontitis. In contrast to cIMA, hsCRP could not discriminate the patients from controls, neither in men nor in women. These results are also given in Table 1.

Multiple logistic regression analysis identified age (odds ratio (OR), 2.83 with 95% confidence interval (CI), 1.14–7.04), low level HDL cholesterol (OR, 5.12; 95% CI, 1.19–22.03) and high BMI (OR, 2.76; 95% CI, 1.06–7.23) as the major predictors of high hsCRP values, respectively. Other factors considered in the model, including chronic periodontitis and cIMA, exerted no significant influence as seen in Table 2.

Table 1 Characteristics of participants in the study

	Women			Men		
	Chronic periodontitis (number, mean±SD (n=46[a]))	Controls (number, mean±SD (n=21))	p	Chronic periodontitis (number, mean±SD (n=47))	Controls (number, mean±SD (n=20))	p
Age (years)	54.7±29	535±2.7	NS	54.4±2.6	53.4±3.0	NS
Education (high school (%))	33.8	29.2	<0.01	50.8	27.7	<0.05
Smoking (pack years)	16.6±15.3	1.4±3.9	<0.001	10.9±15.5	2.0±6.4	<0.05
Hypertension (%)	24.6	7.7	NS	13.4	2.9	NS
Body mass index, BMI (kg/m^2)	24.9±4.3	23.1±2.6	NS	25.9±4.4	24.4±3.2	NS
hsCRP (mg/L)	1.9±1.4	1.6±1.2	NS	3.4±4.7	3.0±7.6	NS
Plasma lipids (mmol/L)						
Total cholesterol	5.8±1.0	5.5±0.6	NS	5.9±0.9	5.5±1.0	NS
LDL	3.6±0.9	3.3±0.7	NS	3.9±0.8	3.5±0.7	NS
HDL	1.6±0.4	1.7±0.4	NS	1.3±0.3	1.3±0.3	NS
TG	1.4±1.3	1.0±0.6	NS	1.7±1.1	1.4±0.8	NS
Periodontal parameters						
PLI	0.4±0.5	0.2±0.2	<0.05	0.8±0.7	0.2±0.2	<0.001
GI	1.1±0.9	0.3±0.3	<0.001	1.5±1.0	0.2±0.7	<0.001
BOP (%)	36.0±22.7	20.4±13.5	<0.01	39.5±25.6	13.9±16.7	<0.001
PD (mm)	2.7±0.8	2.1±0.3	<0.001	2.9±0.6	1.9±0.2	<0.001
AL (mm)	3.2±0.9	2.3±0.5	<0.001	3.7±1.5	1.9±0.6	<0.001
Carotid artery examination						
cIMA dx (mm^2)	13.9±3.2	11.4±2.4	<0.05	14.5±2.9	12.2±2.9	=0.05
cIMA sin (mm^2)	12.2±2.9	10.9±1.9	<0.01	15.2±4.1	12.2±2.8	<0.01

[a] In three cases, measurements of the B-mode variables listed in the table could not be successfully performed because of a local plaque formation or other technical reasons

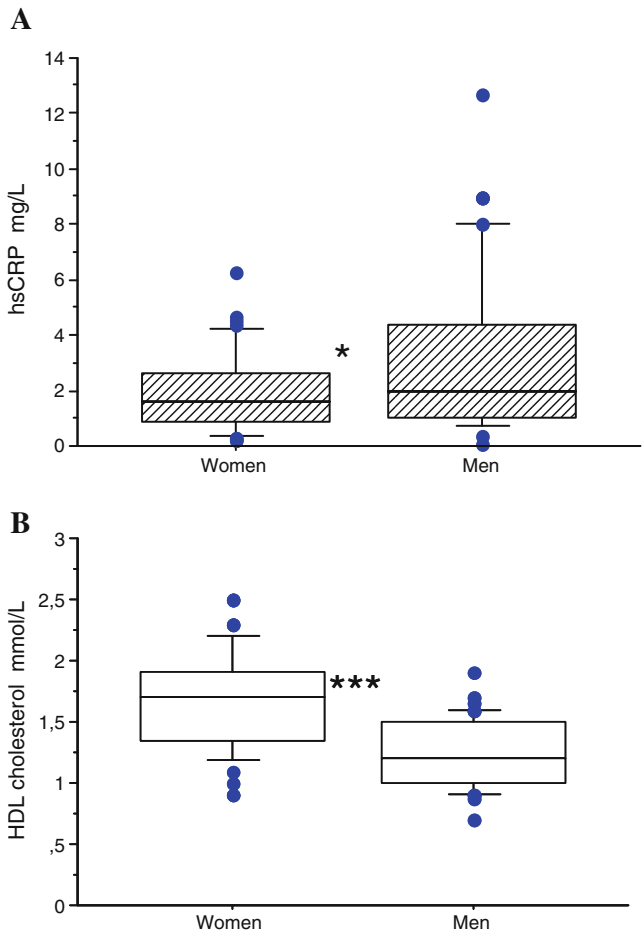

Fig. 2 hsCRP and HDL cholesterol in serum in women and men. **a** hsCRP in serum from women and men with chronic periodontitis. Results are expressed as mg/L, *$P<0.05$ versus women. **b** Concentration of HDL cholesterol in serum from women and men with chronic periodontitis. Results are expressed as mmol/L, ***$P<0.001$ versus men

Education (low) (OR, 4.30; 95% CI, 1.39–13.30), gender (men) (OR, 3.44; 95% CI, 1.19–9.95), chronic periodontitis (OR, 3.82; 95% CI, 1.19–12.26) and high BMI (OR, 3.20; 95% CI, 1.09–9.38), respectively, were identified as the major independent predictors of cIMA using the multiple logistic regression analysis. Other factors considered in the model exerted no significant influence (Table 2).

Discussion

The results clearly showed that atherosclerotic changes in carotid arteries linked with chronic periodontitis. It should be emphasised that the subjects had had no symptoms of cardiovascular disease. Our second observation showed no association between the calculated carotid intima-media area, hsCRP values and chronic periodontitis which was contrary to our expectation. Logistic regression analysis showed that hsCRP was only associated with age, BMI and HDL cholesterol. Thus, hsCRP cannot be used to determine the levels of chronic inflammation caused by periodontal disease.

The strength of our study was indeed the long follow-up period regarding verified periodontitis, i.e. at least 18 years, of our subjects. Subsequently our results confirm earlier data suggesting that periodontal disease seems to be involved in the development of cardiovascular disease [3, 10–13, 31]. Periodontitis may also be linked with premature death as shown from the same cohort used in the present study [32].

The results from our study further showed that women with chronic periodontitis despite low hsCRP concentrations and high level HDL cholesterol may still be at risk for future cardiovascular events as indicated by the significantly increased cIMA values when compared with the healthy women. This was apparent even though the high level of HDL cholesterol might exert important anti-atherosclerotic effects by suppressing the increase of hsCRP [33]. Furthermore, in a recently published study by Halvordsen et al. [34] it was found in ultrasound images of 3,205 subjects with carotid plaque that CRP did not discriminate

Table 2 The results of multiple logistic regression analysis of the relationship between hsCRP (median, 1.600 mg/L) and cIMA (median, 12.57 mm2) as dependent variables and several independent variables (chronic periodontitis, age, gender, BMI, heredity for atherosclerotic disease, hypertension, smoking, education, plasma cholesterol, LDL cholesterol, HDL cholesterol, cIMA sin and cIMA d*x*/2)

Dependent variable	Explaining variable	*p*	Odds ratio	95% confidence interval
hsCRP	Age	0.026	2.83	1.14–7.04
	HDL cholesterol (less than 1 mmol/L)	0.028	5.12	1.19–22.03
	BMI	0.039	2.76	1.06–7.23
cIMA sin, d*x*/2	Education (low)	0.011	4.30	1.39–13.30
	Gender (men)	0.023	3.44	1.19–9.95
	Chronic periodontitis	0.024	3.82	1.19–12.26
	BMI	0.034	3.20	1.09–9.38

inflammatory plaques (echolucent plaque) from echogenic plaques. In contrast to the observed gender differences in the present study, with higher hsCRP values in men than in women, McCawley and Matrisian [35] reported significantly higher hsCRP values in women than men. Furthermore, Sander et al. [36] reported from a prospective study in 3,387 subjects including 2,001 women that hsCRP was independently associated with early carotid atherosclerosis progression in women, but not in men. Halvorsen et al. further reported that the association between measures of carotid atherosclerosis and inflammatory markers is sex dependent [34]. They highlighted the importance of sex-specific analysis in future studies. Nevertheless, there still seems to be controversy in these issues and more studies are called for the final conclusion. Weakness in our study was the small number of patients and controls albeit their periodontal long-term status was very well known.

To the best of our knowledge the present study showed for the first time that irrespective of a low hsCRP level, periodontitis might be a notable risk marker for future cardiovascular disease in particularly in women as indicated by the currently observed increased cIMA values. hsCRP in blood did not seem to be sensitive enough to elucidate the atherogenetic response to chronic periodontitis since there occurred no significant differences in hsCRP values between the diseased and control subjects whereas significant differences were found between the patients with chronic periodontitis and controls in the cIMA. Furthermore, the logistic regression analysis revealed that gender (men) and chronic periodontitis were two of the independent predictors for increased carotid intima-media thickness. Therefore we agree with earlier reports that sex differences indeed should be taken into account in studies of cardiovascular diseases in patients with chronic periodontitis [34].

In conclusion, chronic periodontitis associated with early atherosclerotic changes in subjects with no symptoms of cardiovascular disease. hsCRP did not appear to be a sufficiently sensitive marker of atherogenic process or periodontitis. Irrespective of low hsCRP levels, periodontitis might still present a risk for atherosclerotic disease in particular in women.

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