Periodontitis is associated with altered plasma fatty acids, a cardiovascular risk factor

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Running head: Fatty acids, periodontitis and cardiovascular disease

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ABSTRACT

In periodontitis some perturbation in lipid biomarkers, such as increased serum total cholesterol and low-density lipoprotein cholesterol has been established. Nevertheless, the relationship between fatty acids and periodontitis has been demonstrated only in a few studies and remains controversial. The aim of this investigation was to explore the effects of periodontitis on a cluster of traditional and novel cardiovascular risk factors such as plasma-lipids profile, types of plasma fatty acids, adhesion molecules and systemic inflammatory markers. At our dental school, 56 patients all over 35 years old were enrolled and invited to participate in the study. Total plasma fatty acids, saturated, n-6 polyunsaturated and monounsaturated fatty acids, peroxidability index, soluble VCAM, TNF-α, cholesterol, triacylglycerols, and VLDL-c were significantly higher in periodontitis group compared with non-periodontitis group. This close association among periodontitis and plasma fatty acid profile leads to the conclusion that there is a close association between periodontitis, plasma fatty acids profile and the increase in metabolic risk factors for CVD.

INTRODUCTION

Epidemiological studies have implicated routine chronic adult periodontitis (CP) as a risk factor for atheromatous changes in blood vessels and subsequent vascular events [1]. Cohort and case-control studies have shown that CP is associated with endothelial dysfunction [2] and atherosclerosis [3]. Also, intensive treatment of chronic periodontitis results in an improvement in hypercholesterolemia [4], but the clear molecular mechanisms linking CP to atherosclerosis remain to be defined.

It is known that the resistance to lipid oxidation within lipoproteins can be altered by the dietary fatty-acid profile and antioxidant content [5,6] . We have demonstrated that a diet rich in monounsaturated fatty acids (MUFA) in patients with peripheral vascular disease and in rabbits with experimental atherosclerosis protects LDL particles from oxidation and decreases plasma-lipid content, whereas diets rich in polyunsaturated fatty acids (PUFA), that also decrease this plasma-lipid fraction, increase LDL susceptibility to oxidation [7,8]. Furthermore, PUFA intake alters the cell-membrane fatty acid composition [9], which, in turn modulates response to infection, injury, and inflammatory events [10] and an increase in the plasma free fatty acid concentration induces oxidative stress and has a pro-inflammatory effect. This all relates to the influence of lipid metabolism in the pathogenesis of atherosclerosis.

In periodontitis, some perturbation in lipid biomarkers, for example increased total cholesterol in serum and low-density lipoprotein cholesterol, has been established. Thus, severe periodontitis is associated with a modest decrease in HDL and LDL cholesterol, and a more robust increase in plasma triacylglycerols [11,12]. Intensive periodontal therapy results in reductions of total and LDL systemic cholesterol [13]. Nevertheless, the relationship between fatty acids and periodontitis has been demonstrated in only a few studies. Some of these show that n-3 PUFA dietary supplementation modulates alveolar bone resorption

following *P. gingivalis* infection in rats and reduces the gingival tissue levels of prostaglandin E_2, platelet-activation factor, and leukotriene B_4 [14], this being a useful adjunct in the treatment of CP. On the contrary, 78 periodontitis patients with bone loss showed a higher n-6 PUFA plasma level than 27 control subjects [15].

To clarify the situation, we investigated the potential linkage between periodontitis and plasma fatty acids profile, an established cardiovascular disease (CVD) risk factor.

MATERIAL AND METHODS

Study Population and Clinical Examination

Patients attend in our Dental School, all over 35 years old, were invited to participate in the study. All patients gave voluntary written informed consent. Protocol and consent forms were approved by the Committee of Ethics and Research of Sevilla University (16-12-2006). All patients met the following inclusion criteria: had more than 20 teeth, they had not taken antibiotics or anti-inflammatory drugs in the previous 6 months, suffered no immunodeficiency, were general healthy, had undergone no previous periodontal treatment, and more of the women subjects were no pregnant or without hormonal medication. Patients were recruited over a period of 10 months and one blood sample was taken from each patient as they were recruited.

A baseline periodontal examination was performed, and full medical and dental histories were collected by a single examiner. Periodontal data were recorded by a single trained dental examiner. Periodontal clinical measurements included the presence or absence of supragingival dental plaque and gingival bleeding on probing. The periodontal probing depth (PD) and the recession of the gingival margin (GM) relative to the cementoenamel junction at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual) were also recorded. Clinical attachment level (CAL) was calculated by adding

recession to PD. PD and CAL were recorded to the nearest higher millimeter by means of the North Carolina periodontal probe (Hu-Friedy®, Chicago, IL, USA), 15 mm in length and 0.35 mm in diameter. According to the criteria established by Machtei et al. (16), the clinical entity of periodontitis is based on the presence of CAL $\geq$ 6 mm in two or more teeth and one or more sites with PD $\geq$ 5 mm. The score for full-mouth gingival bleeding on probing (the number of sites with gingival bleeding on probing divided by the total number of sites per mouth, multiplied by 100), and the score for full-mouth plaque (the number of sites with detectable supragingival dental plaque divided by the total number of sites per mouth, multiplied by 100) were calculated for each patient and were compared between the two groups. Two groups of patients were established one with periodontitis (n=30) and another without periodontitis (n=26).

Dietary assessment

The habitual diet of the subjects was daily checked with 24-hours dietary recalls using food records of measured and weighed food intake and all recipes of homemade dishes during one week. Especifically, three recall days were registered at the day of recruitment by a dietitian. Another four days (including one weekend day) were registered by the patient, starting the first day after recruitment, with further supervision by the dietitian. The content of macronutrients and selected micronutrients in the diet was calculated using the computer program ALIMENTACIÓN Y SALUD 0698.046 (BitASDE General Médica Farmacéutica, Valencia, Spain).

Sampling

Venous blood samples (5mL) were collected into evacuated glass tubes containing K_3·EDTA (Venoject, Terumo, Leuven, Belgium) after a 12-h fasting period by venipuncture.

All samples were immediately centrifuged at 1000 x *g* for 15 minutes at 4ºC and plasma was divided into aliquots of 250µl for immediate analysis or for storage at -80 °C during 25 days, until assayed.

Biochemical Analysis

Glucose, plasma triacylglycerols, total cholesterol, LDL-c, HDL-c and VLDL-c cholesterol levels were measured using enzymatic methods in an automatic analyser (Roche-Hitachi Modular PyD Autoanalyzer, Roche Laboratory Systems, Mannheim, Germany).

Total lipids from 0.1 mL of plasma were extracted and quantified as previously described [17]. A gas-liquid chromatograph (Model HP-5890 Series II, Hewlett Packard, Palo Alto, CA, USA) equipped with a flame ionization detector was used to analyse the fatty acids. Chromatography was performed using a capillary column 60 m long, 0.32 mm internal diameter and 0.20 µm thickness, impregnated with Sp™ 2330 FS (Supelco Inc. Bellefonte, Palo Alto, CA, USA). Peroxidability index (PI) is the Σ (% dienoic acid x 1) + (% trienoic acid x 2) + (% tetraenoic acid x 3) + (% pentaenoic acid x 4) + (% hexaenoic acid x 5). Oxidized, reduced and total Coenzyme Q_{10}, and vitamin E were assayed by HPLC-EC as previously described [18]. The HPLC system comprised of a Beckman Model 126 pump, a Rheodyne model 7125 valve fitted with a 20 µl loop, a stainless steel column 15 cm long 4.6 mm internal diameter, packed with 3 µm ODS Supelcosil from Supelchem, an ESA Coulochem model 5100 A electrochemical detector and a model 5011 Analytical cell. Briefly, 50 µl of the sample were precipitated with 150 µl of isopropanol and vortexed for 60 sec. After centrifugation at 12,000 x *g* for 10 min. in a bench-top centrifuge for Eppendorf vials, 20 µl of supernatant were injected into the HPLC.

Plasma TNF-α and soluble VCAM (sVCAM) were assessed by quantitative enzyme-linked immunosorbent assays (BIOSOURCE Europe S.A) according to the manufacturer's instructions.

Statistical Analyses

Data were expressed as means ± standard error. Comparisons between periodontitis and control groups were analysed by the student's T-test when the variations were homogeneous analysed by Kolmogorov-Smirnov; if not, the Mann-Whitney U Test was applied instead. Correlations between variables were assessed using Pearson's correlation coefficients. Analyses were performed using the software program SPSS version 15.0 (Statistical Package for Social Sciences, SPSS Inc. Chicago, IL, USA). A *P* value of less than 0.05 was considered significant.

RESULTS

Fifty-six patients met the inclusion criteria and accepted to participate in the study; 30 were diagnosed as CP. No statistical differences in age were detected between periodontitis and non periodontitis groups. Table 1 summarizes the results of the periodontal examination with significant differences in all the parameters studied ($P<0.001$ for GM, PD, CAL, dental plaque and gingival bleeding determinations) and not significant differences were found for age, height, weight and dietary intake (energy and specific nutrients) among the considered groups.

Periodontitis group showed significant differences in all plasma-lipid parameters, which for cholesterol, triacylglycerols, LDL-c, VLDL-c, and cholesterol/HDL-c index were higher than in the non-periodontitis group, except for the HDL-c level, which was lower (Table 2). Plasma-glucose level was not significantly different between groups.

Oxidative stress status was measured in plasma using the following parameters: vitamin E, oxidized and reduced coenzyme Q_{10} (Table 2) and PI (Table 3). The determination of the PI was significantly lower in control compared with periodontitis patients. Although, plasma vitamin E levels did not differ between experimental groups, significant differences were found for total and oxidized coenzyme Q_{10}, the highest values being found for periodontitis group.

Patients with periodontitis showed significantly higher values of plasma TNF-α and soluble VCAM (Table 2).

Plasma fatty acid content (in mg/dl) is presented in Table 3. Myristic (C14:0), palmitic (C16:0), palmitoleic of the n-9 series (C16:1n-9), stearic (C18:0), linoleic (C18:2n6), arachidonic (C20:4n6) acid, total saturated fatty acids (SFA), total polyunsaturated fatty acids (PUFA), total monounsaturated (MUFA) as well as total fatty acids amount were significantly higher in periodontitis group. When plasma fatty acids were presented in terms of g/100g, the proportion of most fatty acids and indices were mostly unaffected (results not shown), with the exception of myristic, palmitic, palmitoleic of the n-9 series and SFA, that were higher ($P < 0.05$ or less) for periodontitis group in comparison with non periodontitis group (0.9 ± 0.1 *vs.* 0.5 ± 0.1 for myristic; 25.5 ± 0.6 *vs.* 22.3 ± 0.5 for palmitic; 2.3 ± 0.3 *vs.* 1.3 ± 0.1 for palmitoleic of the n-9 series and 36.2 ± 0.7 *vs.* 32.7 ± 0.5 for SFA).

Finally, some noteworthy and significant correlations among parameters of cardiovascular risk (triacylglycerols, LDL-c, HDL-c, fatty acids) with periodontal data (GM, PD and CAL) are in Table 4. Palmitic acid (C16:0) and saturated fatty acids (measured as mg/dl), as well as total and reduced coenzyme Q showed a significant correlation for GM, PD and CAL parameters. Meanwhile, LDL-c, triacylglycerols and HDL-c, as well as PUFA, n-6 PUFA, and total fatty acids (measured as mg/dl) were significantly correlated with two of these periodontitis parameters.

DISCUSSION

CVD remains one of the leading causes of morbidity and mortality worldwide [19]. Periodontal disease is a group of chronic inflammatory diseases characterized by the breakdown of the tooth supporting tissues and the impaired host inflammatory immune response. This condition is due fundamentally to an ecological imbalance between the normal microbial biofilm on theeth and the host tissues [20]. There is increasing evidence linking chronic periodontitis (CP) to systemic diseases [21], in particular CVD and atherosclerosis [1, 3, 22, 23], but biological parameters supporting this association remain controversial. Rencently [24], a link between CVD and CP has been established through metabolic syndrome. Since both states share a common feature of metabolic syndrome, which is inflammation, several related mechanisms have been postulated to explain this link. However, nowadays it is not clear to what extent, if any, a relationship exists between the two diseases.

Data from the present study indicate that there is an association between periodontitis and plasma-lipid profile, with significant differences appearing in total cholesterol, triacylglycerols, and VLD-c, compared with control group. These results agree with those from other authors [11] in the sense that periodontitis is often associated with enhanced concentration of proatherogenic plasma lipids. Our results did not reveal significant differences for LDL-c and HDL-c, as described also by Rufail *et al.* [12]. It should be noted that other authors have not found significant differences for total cholesterol and/or triacylglycerols [25, 26]. These controversial results could be due to the fact that these studies differed in the parameters employed to define periodontal disease [16].

Besides measuring the proportions of fatty acids in total plasma, as usually has traditionally been done, we also determined their concentrations. This is a methodology that is being used more frequently every day, in studies ranging from obesity [27], aging [9], cystic

fibrosis [28], vascular risk factors analysis [29], to studies on the smoking impact on health [30]. In the present study, the importante of the quantitative analysis of fatty acids resides in the fact that periodontal disease has been related with marked dyslipidaemia [3, 4]. On the other hand, dyslipidaemia, that may affect plasma fatty acid concentration, might be hidden since an absence of changes in the proportion of fatty acids does not rule out important alterations in their concentrations [31]. Plasma fatty acids, an important cardiovascular biomarker, showed an altered profile, particularly when meassured in concentration, in periodontitis patients (Table 3), which reported higher amount of total fatty acids, saturated fatty acids, MUFA, and n-6 PUFA than did control. Dietary fat may affect immune response and determines lipoprotein susceptibility to oxidation, which affects on the activation of adhesion molecules and other inflammatory factors [32]. We have previously demonstrated that patients with peripheral vascular disease fed a diet rich in saturated fatty acids and n-6 polyunsaturated fatty acids, but not those fed on n-3 PUFA, had increased CVD risk factors due to increased oxidized lipoprotein and vascular inflammation [17, 33]. Essential fatty acids are precursors of inflammation mediators such as eicosanoids, which regulate the production of inflammatory cytokines and the expression of some major inflammation genes. Total n-3 PUFA was associated with lower levels of pro-inflammatory markers (IL-6, TNF-α, C reactive protein), higher levels of anti-inflammatory markers (Soluble IL-6r, IL-10, TGF-α), and lower levels of some markers of endothelial activation (sVCAM-1 and sICAM-1) [19]. In the present study, n-6 PUFA (mainly arachidonic and linoleic acids) and saturated fatty acids in periodontitis group were higher than in the control group. This plasma fatty acid profile increases CVD risk and might be responsible, at least in part, for the higher values in TNF-α and sVCAM-1 found in these patients. These results are consistent with those from Lichtenstein *et al.* [34], which describe the effects of dietary interventions with fatty acids on biomarkers of inflammation in randomized clinical trials. This suggests that periodontitis

itself, maybe, is accompanied by a proatherogenic plasma fatty acid profile. Periodontitis is a chronic, inflammatory, destructive disease that affects the supporting tissues of the teeth and is associated with enhanced concentration of inflammatory markers including TNF-α. The present study shows higher TNF-α in periodontitis than in control group, which agrees with many authors [e.g. 35]. Soluble vascular cell-adhesion molecule (sVCAM)-1 has a role in the pathogenesis of atherosclerosis mainly in the early phase of the development of the atherosclerotic lesion. In this sense, patients with periodontitis had higher sVCAM concentrations, which agree with other studies [36,37]. Although it has been reported that periodontal therapy does not reduce plasma sVCAM-1, studies in human subjects have shown that by increasing plasma n-3 PUFA, sVCAM-1 is reduced [32]. This means that changes in the plasma fatty acid profile could reduce the CVD risk in patients with periodontitis.

Recently, a new association between lipid peroxidation, oxidative stress and periodontitis has been suggested and it is also related to the clinical periodontal status [24]. On the other hand, the plasma fatty acid profile plays an important role in the lipid-peroxidation process, which is intimately related also with inflammation [24]. It has been reported that high plasma PUFA levels increase lipid peroxidation, and it is well correlated with PI. Here, patients with periodontitis showed higher PI, which implies that a putative decrease in double bound-rich fatty acids, mainly arachidonic acid, could lower lipid peroxidation susceptibility in these patients, thereby reducing CVD risk. However, this plasma fatty acid profile can not be due to an increase in PUFA intake because as it has been described above, dietary intake of this fatty acid fraction is the same between both experimental groups (table 1).

Plasma antioxidants limit LDL oxidation but the presence of co-antioxidants is required for effectively scavenging free radicals since some antioxidant molecules *per se* would act paradoxically as pro-oxidants once activated in the lipid moiety. Results in our

periodontitis patients suggest that even if total α-tocopherol amount does not change, a similarly large extent of oxidative damage occurs. In fact, endogenouns synthesis of the co-antioxidant coenzyme Q_{10} was induced, probably to attenuate plasma peroxidation. Several studies have proved that an induction in coenzyme Q_{10} synthesis is due in part to an increase in oxidative stress [38,39].

Evidence suggests an association between inflammatory markers, such as interleukins, oxidative stress-related parameters and others and CVD events. Taking into account the new cardiovascular risk markers, some studies suggest an association between periodontal disease and CVD. It has been hypothesized that CVD may be triggered by systemic mechanisms, in addition to local inflammatory factors [24], and chronic periodontal infection is one of the possibilities to be considered. Here, this hypothesis is fully supported by reported results on oxidative stress status, fatty acid profile, inflammatory interleukines and adhesion molecules. Indeed, the high correlations found between plasma triacylglycerols, LDL-c, saturated fatty acids, polyunsaturated fatty acids, total amount of fatty acids and coenzyme Q_{10} with some of periodontal data as PD, GM and CAL, leads to the conclusion that there is a close association between periodontitis, plasma fatty acids profile and the increase in metabolic risk factors for CVD.

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Table 1. Estimated daily nutrient intakes and periodontal data from periodontitis patients and control groups.

	PERIODONTITIS (n=30)	NON PERIODONTITIS (n=26)
	Nutrient intake data	
Age (years)	47± 1.8	41.8± 1.8
Height (cm)	169± 1.8	164± 1.6
Weight (kg)	79± 2.6	71± 2.9
Total energy (kcal/d)	2782± 102	2641± 91
Carbohydrates (g/d)	275± 14	271± 11
% energy	39.2± 1.2	41± 1.5
Protein (g/d)	129± 6.5	130± 5.2
% energy	18.5± 0.6	19.6± 0.6
Total fat (g/d)	122± 5.6	115± 5.3
% energy	39.7± 2.6	39.4± 1.6
SFA (g/d)	35,6± 2,2	36,8±2,9
MUFA (g/d)	56,7± 2,6	51,3± 2,9
PUFA (g/d)	13,5± 1,2	16,3± 2,4
Cholesterol (mg/d)	262± 33	257± 26
Dietary fiber (g/d)	21± 2.5	25± 1.6
	Periodontal data	
GM	-0.75± 0.12‡	-0.15± 0.04
PD	3.0± 0.09‡	2.30± 0.10
CAL	3.7± 0.2‡	2.46± 0.1

Table 1 continued

Dental Plaque	42.0±3.7‡	29.15± 3.7
Gingival Bleeding	63.6± 4.4‡	47.93±4.0

Values are expressed as $\bar{x}$ ± s.e.m.

Significantly different periodontitis *vs* controls: ‡P<0.001.

CAL: clinical attachment level; GM: recession of the gingival margin; MUFA: monounsaturated fatty acids; PD: periodontal probing depth; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.

Table 2. Biochemical parameters, TNF-α, sVCAM and selected antioxidants in plasma of periodontitis and control groups.

	PERIODONTITIS (n=30)	NON PERIODONTITIS (n=26)
Glucose (mg/dl)	97.7± 4.3	89.2± 2.5
Cholesterol (mg/dl)	217.7± 7.6*	193.8± 7.9
Triacylglycerols (mg/dl)	144.6± 13.9‡	92.3± 11.4
HDL-cholesterol (mg/dl)	54.3± 3.9	60.3± 3.7
LDL-cholesterol (mg/dl)	135.4± 8.3	113.4± 7.3
VLDL-cholesterol (mg/dl)	28.4± 2.5‡	18.3± 2.3
Total Cholesterol /HDL-c	4.58± 0.3*	3.5±0.3
TNF-α (pg/ml)	35.4± 5.2*	14.1±8.9
sVCAM (ng/ml)	421.6± 8.1‡	388.8±9.8
Vitamin E (µM)	7.6± 0.6	7.6± 0.5
Coenzyme $Q_{10}H_2$ (µM)	0.81± 0.01	0.66± 0.07
Coenzyme Q_{10} (µM)	0.14± 0.01‡	0.09± 0.01
Total Coenzyme Q_{10} (µM)	1.04± 0.08*	0.7± 0.08

Values are expressed as $\bar{x}$ ± s.e.m

Significantly different periodontitis *vs* controls: *$P<0.05$; ‡$P<0.001$.

TNF-α: tumour necrosis factor alpha; sVCAM: soluble vascular cell-adhesion molecule.

Table 3. Plasma fatty acid profile in periodontitis patients and in control groups.

Fatty acids (mg/dl)	PERIODONTITIS (n=30)	NON PERIODONTITIS (n=26)
C14:0	2.6± 0.3*	1.4± 0.2
C16:0	77.1± 3.8*	53.4± 3.4
C16:1n9	4.2± 1.1‡	3.2± 0.3
C18:0	24.4± 1.1‡	18.1± 1.1
C18:1n9	46.8± 2.4*	37.3± 2.5
C18:2n6	99.3± 3.7*	85.4± 5.4
C20:3n6	2.7± 0.1	2.2± 0.2
C20:4n6 (AA)	16.1± 0.7*	13.7± 0.7
C24:0	3.4± 0.4	3.1± 0.4
C24:1n9	5.4± 0.3‡	4.1± 0.3
C22:6n3 (DHA)	9.9± 0.7	8.4± 0.7
SFA	109.1± 5.1‡	78.5± 5.0
MUFA	60.1± 3.8‡	45.7± 2.9
PUFA n-6	118.2± 4.1*	101.4± 5.9
PUFA n-3	12.4±1.0	11.9± 1.2
PUFA	130.6± 4.6*	113.4± 6.7
MUFA/PUFA	0.5± 0.04	0.4± 0.03
AA/DHA	1.8± 0.1	1.8± 0.1
n-6/n-3	11.6± 1.0	9.5± 0.6
TFA	300± 10.6‡	237.6±13.0
Plasma PI	67.5± 2.5†	57.6± 3.2

Table 3 continued

Values are expressed as $\bar{x}$ ± s.e.m.

Significantly different periodontitis *vs* controls: $^{*}P<0.05$; $^{\dagger}P<0.01$; $^{\ddagger}P<0.001$.

PI= (% dienoic acid x 1) + (% trienoic acid x 2) + (% tetraenoic acid x 3) + (% pentaenoic acid x 4) + (% hexaenoic acid x 5).

MUFA: monounsaturated fatty acids; PI: peroxidizability index; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; TFA: total fatty acids.

Table 4. Selected correlations among parameters of cardiovascular risk with periodontal data

Cardiovascular risk factors	**Correlation (Pearson)**			***p***		
	GM	PD	CAL	GM	PD	CAL
Triacylglycerols	-0.51		0.38	0.0001		0.003
LDL-c	-0.32		0.29	0.02		0.03
HDL-c	0.32			0.01		
Palmitic acid (16:0)	-0.46	0.34	0.46	0.0001	0.009	0.0001
SFA	-0.39	0.32	0.41	0.003	0.013	0.001
PUFA n-6		0.36	0.34		0.05	0.009
PUFA		0.32	0.31		0.016	0.02
TFA	-0.28		0.26	0.03		0.04
Oxidised Coenzyme Q	-0.30	0.56	0.50	0.02	0.0001	0.0001
Total Coenzyme Q	-0.27	0.30	0.33	0.04	0.02	0.01

CAL: clinical attachment level; GM: recession of the gingival margin; PD: periodontal probing depth; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; TFA: total fatty acids.