

CHRONIC PERIODONTITIS AND CARDIOVASCULAR DISEASE: A CONTROLLED CLINICAL TRIAL

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Received October 11, 2012 – Accepted March 1, 2013

Cardiovascular disease is one of the leading causes of death worldwide. Controlled prospective studies and randomized clinical studies have shown that inflammation plays a major role in the pathogenesis of atherosclerosis, and that a chronic inflammatory systemic reaction increases the risk of cardiovascular, and cerebrovascular attacks. In recent years, many researchers have focused on defining a correlation between cardiovascular and periodontal diseases. The aim of the present study was to observe the effects of periodontal causal therapy on the level of specific inflammatory markers, such as C-reactive protein (CRP) and interleukin-6 (IL-6) levels, before and after non-surgical periodontal therapy. A total of 64 patients were enrolled in the present study. Among these, 26 patients were affected by cardiovascular disease and periodontal disease, (MCV-Perio test group), whereas 38 patients were only affected by cardiovascular disease, without periodontal disease, (MCV control group). The MCV-Perio group was then sub-divided into two additional groups: Treated MCV-Perio test group, treated with non-surgical phase I periodontal therapy; Not-Treated MCV-Perio test group, not treated with any periodontal therapy. A comprehensive periodontal treatment was carried out at baseline. The non-surgical therapy treatment was conceived according to Full Mouth Therapy (FMT) treatment protocol, consisting of various phases delivered in a very short time. Blood samples were collected at baseline and re-evaluated in order to assess periodontal and inflammation marker changes. All values were registered as an average \pm standard deviation ($\bar{x} \pm SD$) and Wilcoxon-Mann-Whitney test was used. It is interesting to observe that the serum concentrations of IL-6 and hs-CRP were higher in the group with cardiovascular and periodontal disease compared to the group with cardiovascular disease alone. Non-surgical periodontal treatment determined a dramatic improvement in the levels of the systemic inflammatory markers. The results of the present study show that non-surgical periodontal therapy performed according to the Full Mouth Therapy protocol may prove beneficial in reducing the levels of inflammatory markers typically associated with heart disease. Because the two pathologies share a certain number of common risk factors, this may be a hindrance in the correct interpretation of the results. Therefore, further evidence, represented preferably with a randomized controlled clinical design is necessary to interpret the results correctly.

Key-words: dental scaling, root planing, periodontal diseases, inflammation, periodontitis, cardiovascular disease

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1721-727X (2013)

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Cardiovascular disease is one of the leading causes of death worldwide (1), and the main cause of death in Europe and the United States (2). Only 2/3 new cases of cardiovascular disease can be attributed to the proven risk factors (age, sex, hypertension, hypercholesterolemia, diabetes, obesity, smoking, unhealthy lifestyle). Currently, therefore, the scientific community is concentrating on the identification of new hypothetical risk factors (3). Controlled prospective studies and randomized clinical studies have shown that inflammation plays a major role in the pathogenesis of atherosclerosis, and that a chronic inflammatory systemic reaction increases the risk of cardiovascular (4), and cerebrovascular, attacks (5). Among these possible risk factors, periodontal disease, namely a chronic inflammation of the supporting tooth structures due to a multifactorial etiology, might represent a predisposing inflammatory condition. For this reason, in recent years both the medical and dental communities have focused on defining a correlation between these two diseases. A careful review of the literature suggests the existence of a relationship between periodontal and cardiovascular disease. Two different mechanisms are probably implied in this relationship. According to the direct relationship, periodontal pathogens may be transferred from oral tissues to the bloodstream, (bacteremia), during periodontal therapy (6) or periodontal probing (7). Through the bloodstream, they might travel to distant sites.

The capacity of *Porphyromonas gingivalis* to invade endothelial cells in cell culture supports this hypothesis. Also, periodontal pathogens have been identified in atheromatous plaque samples obtained by endo-atherectomy of the carotid artery (8). According to this hypothesis, cytokines such as interleukins and Tumor necrosis factor- α (TNF- α), released by monocytes and endothelial cells, induce the liver to release acute-phase proteins (C-reactive protein, fibrinogen, aptoglobin, α -1 antitrypsin). Acute-phase proteins would then induce a series of responses such as: deposition of low-density lipoproteins (LDL), containing cholesterol and its esters along the vascular walls (9), platelet aggregation and activation of the coagulation cascade, which would in turn increase thrombotic risk.

Support for this indirect mechanism linking periodontal disease to cardiovascular disease is represented by the findings of clinical and epidemiological studies, which have underlined a correlation between periodontal disease and the plasmatic levels of inflammatory markers, (C-reactive protein, IL-1, IL-6, TNF- α , PAF) (16-20), an alteration pertaining to certain parameters, (fibrinogen, vWF, D-dimer), of the coagulation system and an over-expression of adhesion molecules on endothelial cells involved in the inflammatory process (ICAM-1, VCAM-1, E-selectin, P-selectin) (11). The two aforementioned mechanisms represent a biological link between periodontal disease and systemic pathology (Perio Medicine). An important contribution to this area of study was given by the work of a team of cardiologists in the late eighties. The group was interested in understanding and identifying risk factors involved in the considerable number of strokes that showed no relationship to "classic" risk factors and was able to find an association between periodontitis and acute cardiovascular episodes (12). Since then there has been a considerable amount of studies, focusing on reproducing the correlation between periodontal disease and systemic disease of interest in animal studies, and the mechanisms which have made that association possible. The feasibility of the association between periodontal disease and systemic pathology has also been investigated (13). Other studies have investigated the correlation between periodontal disease and other systemic diseases, and correlations have been found with: ischemic heart diseases, obstetric complications, (pre-term birth or low birth weight), insufficient metabolic control of type 2 diabetes and pulmonary ailments. Studies investigating the systemic effects of the resolution of the oral inflammatory processes after periodontal therapy found a significant improvement of inflammatory parameters (14). These studies suggest that periodontal disease might contribute, along with other inflammatory conditions, to the total systemic inflammatory load (15). C-reactive protein may therefore be interpreted as a key factor in predicting the long-term risk of atherosclerosis and ischemic cardiac disease (16) (Table I).

Controlling periodontal disease has also led to an improvement in altered vascular parameters (17).

Table I. *C-reactive protein blood values and cardiovascular risk.*

Hs-CRP (mg/L)	Risk Level
< 1.0	Low
$1.0 \leq x \leq 3.0$	Intermediate
> 3.0	High

The aim of the present study is to observe the effects of periodontal causal therapy on the level of specific inflammatory markers. More specifically, C-reactive protein (CRP) and interleukin-6 (IL-6) levels have been observed in two groups of patients before and after periodontal therapy.

MATERIALS AND METHODS

Patient selection

The present study was carried out at the Division of Periodontology, School of Dentistry, at the Polytechnic University of the Marche in collaboration with the National Institute of Recovery and Care of the Elderly (I.N.R.C.A, Ancona, Italy) and the "Infermi" Hospital in Rimini. This prospective multicenter study was carried out after the development of an experimental protocol. Between May 2009 and May 2010 64 patients, aged 41 to 68 years, were enrolled in the present study. Of these, 26 patients were affected by cardiovascular disease and periodontal disease, (MCV-Perio test group), whereas 38 patients were only affected by cardiovascular disease, without periodontal disease, (MCV control group) (Table II). All the periodontal patients were affected by moderate to severe chronic periodontitis.

The following inclusion criteria were respected: compensated cardiovascular disease, treated with drugs; stenosis caused by arteriosclerosis $\geq 25\%$ (according to Luepker and co-workers, 2003). The following exclusion criteria were respected: uncompensated or untreated cardiovascular disease, diabetes, acute inflammatory disease, (e.g. flu), chronic inflammatory diseases, (immune-mediated pathology, COPD, renal failure),

endocrine dysfunction, neoplastic disease; current use of antibiotics or anti-inflammatory drugs; antibiotic or anti-inflammatory therapy terminated less than 2 months before the beginning of the study; pregnancy.

The drugs used to treat the cardiovascular disease were registered in the patient files, (calcium antagonists, beta-blockers, ACE inhibitors, diuretics, statins).

As far as strictly dental exclusion criteria is concerned, all patients requiring immediate dental care or emergency treatment for acute inflammatory conditions of the teeth or oral mucosa were excluded. The same criteria were applied during the course of the study if any patient within one of the two groups developed such a condition.

All patients in the test MCV-Perio group showed the following characteristics at baseline:

- absence of acute inflammatory processes of the teeth or oral mucosa
- diagnosis of generalized chronic periodontitis, ranging from moderate, (clinical attachment level $3 < \text{CAL} < 4$ mm in more than 30% of examined sites) to severe (clinical attachment level CAL 5 mm in more than 30% of examined sites)
- absence of periodontal treatment in the previous 6 months.

Therefore, of the initial 73 patients identified in the Internal Medicine Department of the aforementioned Institutes, only 64 were considered possible candidates for the study. Other information was collected and registered on a computer-generated data sheet and included: age, sex, body mass index (BMI), blood pressure, heartbeat and smoking.

The MCV-Perio group was then sub-divided into two additional groups: i) Treated MCV-Perio test group, treated with non-surgical phase I periodontal therapy; ii) Non-treated MCV-Perio test group, not treated with any periodontal therapy.

Randomization for the distribution of the patients among these groups was impossible, mainly because of ethical considerations. In fact, once the patient was diagnosed with periodontal disease, he or she was informed of the condition. The decision of whether or not to undergo non-surgical treatment was left to the patient. Participation in the study was conditional upon signing an informed consent form. The protocol has been reviewed and approved by the Ethics Committee of the University Hospital "Ospedali Riuniti" of Ancona.

Clinical periodontal evaluation

Baseline assessment and the clinical parameters considered were based on a previously published study (18): Plaque Index (PI); Gingival Index (GI); Bleeding on probing (BOP); Pocket probing depth (PD), measured between the gingival margin and the bottom of the

periodontal pocket; Clinical attachment level (CAL), measured between the CEJ and the bottom of the periodontal pocket. BOP and PD were registered at 6 sites, (mesial, medial and disto-buccal, mesial, medial and disto-lingual), with the exclusion of third molars (when present).

The aforementioned parameters were collected by a single calibrated operator. The measurements were repeated 6 weeks after the therapy and registered in the patient's periodontal chart. Every patient was also subjected to a radiographic status and a panoramic X-ray exam depending on the severity of the patient's situation. The radiographic exam was successively scanned and elaborated with a special acquisition software (Planmeca Romexis®), in order to evaluate each patient's data.

Therapeutic protocol

All patients belonging to the MCV-Perio group who received treatment were treated with non-surgical periodontal therapy (45). The treatment was conceived according to the *Full Mouth Therapy* (FMT) treatment protocol, consisting of various phases (19):

- oral hygiene instructions and patient motivation, (including tongue brushing with a special tongue-brushing instrument);

- *Full-mouth disinfection I*, meaning repeated mouth rinses with Chlorhexidine 0.20% mouthrinse 1 to 2 days before beginning therapy, (to reduce the total bacterial load) (full-mouth scaling and root planing) with manual instruments, (SG7-897; SG11-1293; SG13-1498; SAS7-897; SAS11-1293; SAS13-149, Hu-Friedy®, Chicago, USA) and full-mouth ultrasonic debridement with piezo-electric scaler, (Air Flow Master Piezon® A, P and PS debridement tips, EMS Switzerland). The therapy was always completed within 24 hours and in most of the cases divided into two very close sessions

- *Full-mouth disinfection II*, (rinse with 10 ml of Chlorhexidine 0.20% mouthrinse, for 2 min, to be repeated twice a day for 14 days following therapy. Curasept 0.12%® Curaden Healthcare, Italy). Particularly sensitive or anxious patients, received anesthesia without vasoconstrictor. Six weeks after the conclusion of therapy, patients were checked and re-evaluated.

Blood tests

After completing the patient files and registering comprehensive cardiovascular risk data, and before beginning non-surgical periodontal treatment, a blood sample was taken and sent to the laboratories of the aforementioned structure. Six weeks after therapy, a second blood sample was taken. Every blood sample (5 ml) was taken with a 5 ml disposable syringe with a n. 12 needle (0.7x30 mm, 22Gx1, 3/16) and transferred to a dry

Vacutainer 7-ml test-tube without additives. C-reactive protein levels were determined with a nefalometric method (APS, Beckman, Palo Alto, CA, USA), whereas IL-6 levels were determined with the ELISA IL-6 system (Human Biotrak Elisa System, Amersham Pharmacia Biotech, Monza-MI, Italia).

Statistical analysis

All values were registered as an average \pm standard deviation ($x \pm SD$). The analytical method used was the Wilcoxon-Mann-Whitney test, (or Mann-Whitney U test, also known as the Wilcoxon test). This test is routinely used to compare the average value between two groups that do not follow the Gaussian curve of distribution. In this specific study, it was used to determine the differences between the values before treatment and the values collected after treatment in the MCV-Perio treated group compared to the MCV-Perio at baseline. On the other hand, the student's *t*-test for coupled data was used to determine differences between the results (before and after treatment), in the MCV-Perio treated and MCV-Perio non-treated groups. Significance was established for $P < 0.05$.

RESULTS

Table II shows the demographic data of the participants in the study. Table III shows the clinical parameters relative to the two groups at baseline. For instance, it is interesting to observe that the serum concentrations of IL-6 and hs-CRP were higher in the group with cardiovascular and periodontal disease compared to the group with cardiovascular disease alone. As far as the other parameters are concerned, no statistically significant differences

Table II. Study group profile.

MCV Group		MCV-Perio Group	
38 patients		26 patients	
28 females	10 males	18 females	8 males
56 \pm 12 years		54 \pm 13 years	

Table III. *Clinical and blood parameters in the different study groups.*

Parameter	MCV Group (38) $\bar{x} \pm SD$	MCV Perio Group (26) $\bar{x} \pm SD$
BMI (Kg/m ²)	23.1 \pm 2.8	23.2 \pm 3.0
Systolic blood pressure. (mm Hg)	138.7 \pm 17.5	140.5 \pm 18.1
Diastolic blood pressure (mm Hg)	88.9 \pm 11.9	89.8 \pm 12.2
Heart frequency (bpm)	76.4 \pm 9.5	78.2 \pm 7.8
Total cholesterol (mmol/L)	4.76 \pm 0.97	4.65 \pm 0.88
Fats	1.27 \pm 0.67	1.25 \pm 0.61
HDL cholesterol (mmol/L)	1.18 \pm 0.52	1.21 \pm 0.50
LDL cholesterol (mmol/L)	2.71 \pm 0.81	2.46 \pm 0.61
Glucose (mmol/dL)	5.0 \pm 0.9	4.9 \pm 0.8
Insulin (pmol/L)	45.7 \pm 13.9	44.7 \pm 20.1
IL-6, (ng/L)	1.3 \pm 2.3	2.7 \pm 3.7*
Hs-CRP (mg/L)	1.1 \pm 1.2	2.3 \pm 2.1*
Smokers	16	9

**P* < 0.05 compared to MCV group (control)

Table IV. Laboratory and clinical changes in MCV Perio patients treated with non-surgical periodontal therapy versus MCV Perio non-treated patients.

Parameters	MCV Perio Group x ± SD			
	Non-treated patients (9)		Treated patients (17)	
	Baseline	Re-evaluation	Baseline	Re-evaluation
BMI, Kg/m ²	23.4±3.2	23.5±3.4	23.2±3.3	23.2±3.2
P.A. systolic, mm Hg	141.2±20.1	140±19.3	140.1±20.3	141.3±21.4
P.A. diastolic, mm Hg	90.2±13.8	89.9±13.3	89.2±14.1	88.7±13.7
FC, bpm	80.2±9.2	81.2±9.5	77.6±9.3	79.3±10.1
Total cholesterol, mmol/L	4.71±0.92	4.73±0.90	4.63±0.73	4.62±0.77
Triglycerides	1.28±0.71	1.27±0.57	1.22±0.78	1.23±0.64
Cholesterol HDL, mmol/L	1.22±0.63	1.23±0.39	1.20±0.63	1.19±0.41
Cholesterol LDL, mmol/L	2.69±0.72	2.59±0.54	2.38±0.71	2.42±0.57
Glucose, mmol/dL	4.9±1.0	4.9±1.2	4.9±0.8	4.8±0.7
Insulin, pmol/L	45.1±21.3	46.4±19.3	44.5±20.8	43.6±17.2
IL-6, ng/L	2.5±4.2	2.6±4.3	2.8±4.4	1.7±2.5 [§]
Hs-CRP, mg/L	2.2±2.3	2.1±2.0	2.4±2.2	1.4±1.2 [§]
N° smokers	2		7	

[§] $P < 0.05$ (baseline vs re-evaluation comparison)

Table V. Laboratory changes in MCV group versus MCV Perio-group.

Parameters	MCV Group	MCV Perio group	MCV-Perio Group			
			Non-treated (9)		Treated (17)	
			Baseline	Re-evaluation	Baseline	Re-evaluation
IL-6 (ng/L)	1.3±2.3	2.7±3.7	2.5±4.2	2.6±4.3	2.8±4.4	1.7±2.5
Hs-CRP (mg/L)	1.1±1.2	2.3±2.1	2.2±2.3	2.1±2.0	2.4±2.2	1.4±1.2

were noted between the two groups (Table III). Table IV shows the MCV-Perio values divided into the two sub-groups. Periodontal therapy determined a dramatic improvement in the levels of the systemic inflammatory markers, obtained through the

resolution of the inflammatory oral foci (Table V).

DISCUSSION

Periodontal disease is responsible for most tooth

loss in adults. For several decades this oral disease has received considerable attention and a new understanding of it is emerging. The microbial causes of periodontal disease, the mechanisms through which periodontal tissues are destroyed, the effect of the host on periodontal disease expression, and the impact periodontal disease has on overall health have been subjects of intense study (20-22). Understanding the complex interaction between chronic infections, such as periodontal disease and systemic conditions such as cardiovascular disease, has led to a new way of thinking about the importance of periodontal disease in overall health. Upon observing the results of the present study it is possible to notice that there were no differences between the values of the non-treated MCV-Perio group and the treated MCV-Perio group at baseline, before non-surgical periodontal therapy was performed. On the other hand, 6 weeks after treatment a significant decrease in the levels of IL-6 and hs-CRP in the treated MCV-Perio group was noted. Non-surgical periodontal therapy has not influenced the other registered values. In the non-treated MCV-Perio group, the results showed no alteration 6 weeks after treatment. The base hypothesis was that periodontal therapy contributes to the resolution of the inflammatory oral foci thus decreasing the level of inflammatory systemic markers in the blood. The results of the present study have confirmed this hypothesis (Table V). Consequently, periodontal disease may be considered a cause, among other inflammatory conditions, contributing to the systemic inflammatory load. This aspect in particular underlines the importance of C-reactive protein as a risk predictor for the future development of arteriosclerosis and ischemic cardiac disease (Table I). Although many variables have been taken into consideration, it is important to note that none of the participants changed their lifestyle, meaning their diet regimen, physical activity, drug assumption, habits and so forth, during the course of the study. This means that controlling chronic inflammation of the oral cavity and decreasing its bacterial load may effectively contribute to the reduction of the systemic inflammatory conditions as other Authors have previously observed. This effect may have an even greater importance in those categories of patients who are at an intermediate or high risk of developing heart disease. The periodontal

pathogens and their metabolic by-products, together with the inflammatory reaction they bring about, represent a chronic inflammatory stimulus. This can be considered the principal cause of a series of alterations of the vascular wall, which may in turn cause arteriosclerosis in predisposed subjects. The levels of C-reactive protein, IL-6 and other markers, have been suggested as measures to determine the entity of the inflammatory reaction and, consequently, predict cardiovascular risk. C-reactive protein is a marker of general inflammation and has been used for several years to divide patients among different risk categories (23). This method has also been recommended by the American Heart Association (24). The results of the present study show that non-surgical periodontal therapy performed according to the Full Mouth Therapy protocol may prove beneficial in reducing the levels of inflammatory markers typically associated with heart disease. Because the two pathologies share a certain number of common risk factors, this may be a hindrance in the correct interpretation of the results.

Most of the patients included in the present study were subsequently subjected to resective and/or regenerative periodontal surgery. Some of them needed plastic periodontal surgery. It would be very interesting to evaluate the effect of periodontal surgery on cardiovascular disease. However, it would have been non-ethical to wait a long time before correctly treating the patients who were included in the non-treated MCV-Perio test group. In conclusion, this study may be improved by a longer evaluation and follow-up time in a future paper. In fact, further evidence, represented preferably by randomized clinical trials (RCT) performed on a large scale and with a rigorous protocol, is necessary to obtain definitive results regarding the periodontal-cardiovascular disease relationship.

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