Assessment of C-Reactive Protein Levels in Periodontal Patients Using a Standard Laboratory Procedure

Vittorio Checchi[1,*], Giuseppe Daprile[2], Karin Genovesi[3] and Luigi Checchi[2]

[1]*Department of Restorative Dentistry, School of Dentistry, University of Bologna, Italy*

[2]*Department of Periodontology, School of Dentistry, University of Bologna, Italy*

[3]*Private practice, Ancona, Italy*

Abstract: *Background:* the aims of the randomized clinical trial were (i) to verify the association between periodontal disease and C-reactive protein (CRP) and (ii) to evaluate a possible reduction of serum CRP levels after non-surgical periodontal treatment.

Methods: Thirty-two subjects, 18 affected by chronic periodontitis, and 14 periodontally healthy patients, aged between 21 and 65 (41± 13) were included. Clinical and radiographic examinations were used for each patient to obtain three dental indices that were used to evaluate severity of periodontal disease and changes after treatment. Periodontal patients were randomly assigned to one of two groups for different treatments: special oral hygiene instructions alone or in combination with scaling and root planing. Blood samples were taken for measurement of CRP levels and eritrosedimentation rate before and after treatment

Results: a reduction of clinical index CPSS was observed for both groups of periodontal patients after treatment but there were no statistically significant differences for CRP and ESR at baseline and between baseline and re-examination. Non statistically significant differences of CRP values between periodontal patients and healthy controls were found

Conclusions: CRP values don't seem to change after non-surgical treatment of periodontitis, even in presence of a reduction of clinical indices.

Keywords: Periodontal disease, C-reactive protein, Inflammation, Nephelometric method.

INTRODUCTION

Several studies over the last years have shown a possible association between periodontal and cardiovascular diseases [1-6]. This can be due to common risk factors such as age, smoking, and diabetes. It can also be due to the action of Gram-negative bacteria and their products which, in the case of periodontal inflammation, may spread through the circulation and reach organs like the heart. Finally, inflammatory mediators such as TNFα, IL-1, IL-6, IFN-γ and PGE $_2$ can reach the bloodstream from inflamed periodontal sites [7]. Of these mediators, IL-6, released by activated macrophages, induces production of C-reactive Protein (CRP) by liver hepatocytes. CRP represents a marker of inflammation, normally present in small quantities, but it can increase dramatically within 72 hours following tissue injury. This protein can act as an opsonine, binding specific cell membrane components of microorganisms and stimulating the activation of complement fragments to enhance opsonization and elimination of bacteria. In addition, CRP can interact with natural killer cells and monocytes and may increase their tumoricidal activity [8].

In many studies CRP appears to be a risk predictor for cardiac and cerebrovascular events [1, 2, 6, 9]. When associated with smoking, hypertension or high plasma cholesterol levels, it acts as a "risk multiplier". Concentrations >3 mg/l seem to increase the risk of myocardial infarction or stroke within 8 years by three or four times, both in medically healthy subjects and in subjects with previous episodes [10-12].

Hence, the aims of our clinical trial were (i) to verify whether levels of C-reactive protein are different between subjects affected by periodontal disease and healthy subjects using a standard laboratory procedure, and (ii) to evaluate a potential reduction of serum CRP levels after two different kinds of non-surgical periodontal therapies.

MATERIALS AND METHODS

In order to set the sample dimension, several studies were examined with the aim to detect a value of minimum difference between periodontal and healthy subjects (Table **1**). On the basis of the similar

*Address correspondence to this author at the Department of Restorative Dentistry, School of Dentistry, University of Bologna, Via San Vitale, 59, 40125 Bologna – Italy: Tel: +390512088132;
E-mail: vittorio.checchi@unibo.it

Table 1:   Median Values Reported in Literature for CRP (mg/dl) in Periodontal Patients

Authors	Number of Patients (N)	CRP
Ebersole *et al.* 1997	40 periodontal patients	0,91 in periodontal patients;
	35 controls	0,21 in controls.
Beck *et al.* 2000	1550 patients with at least 10% of sites with probing depth ≥4 mm;	0,41 in patients with at least 10% of sites with probing depth≥4mm ;
Slade *et al.* 2000	9145 patients without pockets;	0,29 in patients without pockets;
	1817 edentulous.	0,40 in edentulous patients.
Loos *et al.* 2000	54 patients with generalized periodontitis;	0,15 in patients with generalized periodontitis;
	43 controls.	0,09 in controls.
Mattila *et al.*2002	35 with adult periodontitis.	0,11 at baseline in periodontal patients;
		0,03 of median decrease after treatment:
D'Aiuto *et al.* 2004	94 patients with severe generalized periodontitis	at baseline with periodontal patients;
		0,05 of median decrease after treatment.
Ide *et al.* 2004	23 patients with generalized periodontitis.	0,40 at baseline in periodontal patients;
		0,37 after treatment.

\CRP, C-reactive protein.

experimental design, the difference reported by Ebersole *et al.* (1997) of approximately 1 mg/dl between periodontal patients and controls was used, establishing an alpha error of 0,05 and a power of 0,80 for the significance test, so the number of subjects enrolled was 14 for each group.

The 42 initial subjects, 28 with chronic periodontal disease, and 14 periodontally healthy, were aged between 21 and 65 years. The subjects were enrolled by a single operator among the patients examined at the Department of Periodontology of the School of Dentistry, University of Bologna, Italy, between September 2003 and June 2004.

The exclusion criteria comprised: coronary heart disease, myocardial infarction within the last 24 months, systemic illnesses or infections, antibiotic treatment, use of aspirin or treatment with any medication known to affect the serum level of inflammatory markers in the preceding two months [4, 9, 13].

The 28 periodontal patients were randomly assigned to two treatment subgroups and the allocation sequence was made by computer. However, 10 subjects did not come back for periodontal revaluation, so the number of periodontal patients for our analyses was reduced to 10 for Group 1 and 8 for Group 2. However, no adverse effect motivated the dropouts apart an inadequate compliance to the scheduled

treatment. Group I drop out rate was 29% (4\14) and 43% in Group II (6\14); the difference of 14% is slightly higher than 10%, considered the acceptable drop out selective rate. Despite this reduction in numbers at the follow-up, no significant differences were observed in demographic, dental and clinical parameters.

Group 1 (10 periodontitis subjects) had: a baseline periodontal examination; the collection of a blood sample for evaluation of eritrosedimentation rate (ESR) and CRP; special oral hygiene instructions named hygienic therapy (see Box **1**); gross scaling (full mouth); 4 sessions of scaling and root planing (one for each quadrant); periodontal revaluation and collection of a second blood sample one month after the completion of treatment.

Box 1:   Special Oral Hygiene Instructions Named Hygienic Therapy

Each subject was instructed in proper oral hygiene practices:

Bass technique for brushing lingual and buccal surfaces

Tip for cleaning crevices and to create space (when needed) for the interdental brush

Interdental brush in interproximal areas

Plaque disclosing agent at least once a week.

Group 2 (8 periodontal patients) had: a baseline periodontal examination; the collection of a blood sample for evaluation of ESR and CRP; special oral hygiene instructions (hygienic therapy) as with group 1;

periodontal revaluation and collection of a second blood sample one month after the first. The aim of choosing different treatments was to verify if supragingival plaque control alone, obtained through hygienic therapy, could influence CRP levels.

Group 3 (14 periodontally healthy subjects) received a periodontal visit to confirm the periodontal health and the collection of a blood sample for evaluation of ESR and CRP.

Subjects of groups 1 and 2 underwent a radiological examination to obtain a radiographical status. Clinical and periodontal parameters were collected by an expert calibrated operator at baseline and at revaluation, without any blinding. The principles outlined in the Declaration Of Helsinki on clinical research involving human subjects were adhered to. All the research was conducted in accordance with Italian law.

Dental Indices

Clinical and radiographic examinations were used for each subject to obtain three dental indices that, singularly or partially associated, had already been used in previous epidemiological studies [9, 15, 16]. The indices were used to evaluate severity of periodontal disease and changes after treatment.

Panoramic Tomography Score (PTS). This was obtained by the sum of radiolucent periapical lesions, third-degree carious lesions, vertical bone pockets, furcation lesions and pericoronitis.

Clinical Periodontal Sum Score (CPSS). This represents the sum of the number of sites with probing pocket depth of 4 mm or greater, the number of gingival sites with bleeding after probing, the number of sites with visible suppuration after probing and the number of furcation lesions exceeding grade 1.

Clinical and Radiographic Sum Score (CRSS). This was calculated by the sum of the number of radiographic vertical defects, the number of furcation lesions and the CPSS.

Serum CRP Analysis

Blood samples of each patient were analyzed at the Central Laboratory of Sant'Orsola Hospital, Bologna. Serum was separated using standard laboratory techniques and C-reactive Protein was quantified by latex-enhanced nephelometry using a fully automated Behring Nephelometer Analyzer System (Dade Behring). Samples of serum were mixed with latex particles coated with anti-C-reactive protein antibodies, forming antigen-antibody complexes. Light scattering, proportional to the concentration of the analyte in the sample, was measured by nephelometric procedure. C-reactive protein concentrations in mg/dl were calculated with reference to a calibration curve. With this method the minimum detectable concentration of CRP was 0,3 mg/dl.

STATISTICAL ANALYSIS

Statistical analysis was performed using a computer statistical package (SPSS 11.0, SPSS Inc. 2001, Chicago IL). Levene statistic was used to verify the homoschedasticity of variance. The significance of differences of CRP and ESR between Groups 1, 2 (periodontal subjects) and 3 (controls) was calculated using analysis of covariance, by taking age as a covariate because of the different mean age between periodontal and healthy subjects, or Kruskal – Wallis analysis. The significance of differences between periodontal subjects (Groups 1+2) and controls (Group 3) was calculated with the Mann Whitney test or with the Student t-test. Subgroup analyses were carried out in order to verify internal comparability of each subgroup (pre-specified analyses). Finally, 95% confidence intervals were calculated.

RESULTS

Four patients of Group 1 and 6 patients of Group 2 did not come back for periodontal revaluation, so the number of periodontal patients for our analyses was reduced to 10 for Group 1 and 8 for Group 2.

Thirty-two subjects (53% males) completed the study. Participants were mostly non-smokers (75%). The two groups of periodontal subjects (groups 1 and 2) displayed a similar median age of 44-48 years. Control subjects had a median age of 33±15 years (Table **2**). No significant differences were observed in demographic, dental and clinical parameters after the loss of subjects at follow-up.

Table **3** describes the subject population considering dental and clinical parameters. A reduction of clinical index CPSS at revaluation for periodontal patients was observed: the reduction was 34% for group 1 patients and 38% for group 2 patients. Non statistically significant differences between Groups 1 and 2 (periodontal patients) were observed for clinical

Table 2:   Description of the Subject Population using Age (mean ± SD), Gender and Smoking Habits

		Group 1 (n=10)	Group 2 (n=8)	Group 3 (n=14)
Age		44±12	48±8	33±15
Gender	males	4 (40%)	5 (63%)	8 (57%)
	females	6 (60%)	3 (38%)	6 (43%)
Smoker	No	7 (70%)	7 (87%)	10 (71%)
	≤10/day	3 (30%)	-	2 (14%)
	>10/day	-	1 (13%)	2 (14%)

Table 3:   Description of Subject Population Considering Dental and Clinical Parameters (mean value ± SD)

		Group 1 (n=10)	Group 2 (n=8)	Group 3 (n=14)
Number of natural teeth		25±5	28±2	30±2
Number of sites with probing pocket depth ≥ 4 mm		62±34	84±27	-
Panoramic Tomography Score		10±6	9±2	-
Clinical Periodontal Sum Score	baseline	70±36 (47-93)	97±53 (59-135)	-
	revaluation	46±33 (25-67)	60±33 (36-84)	-
Clinical and Radiographic Sum Score		77±35	100±52	-

(95% confidence interval).

index CPSS, radiographic index PTS, clinical and radiographic sum score CRSS and number of sites with probing pocket depth ≥ 4 mm.

Table **4** shows the mean values of CRP and ESR among the three groups of subjects examined at baseline and at second examination. At baseline p was equal to 0.007 for ESR with a significant interaction group-age. All the other differences were not statistically significant. Even by grouping periodontal subjects (group1 + group2), no statistically significant differences were observed with the healthy control group (group3). No significant difference of CRP values between smokers and non-smokers was observed.

No important side effects or adverse events were observed.

DISCUSSION

Several studies in the last years have shown a possible association between periodontal disease and ischaemic heart disease (IHD) [3, 10, 11, 16, 18-20]. This association may partially be explained considering common risk factors like high levels of serum lipids, inflammatory and haemostatic factors. However, this hypothesis has not been supported by all authors. Hujoel *et al.* (2001) [21] argued that while periodontitis may coexist with IHD, it does not necessarily imply an

Table 4:   C-reactive Protein (mg/dl) and Eritro-Sedimentation Rate (mm/h) Values Among the Subject Population (mean ± SD)

		Group 1 (n=10)	Group 2 (n=8)	Group 3 (n=14)
CRP	baseline	0,34±0,13 (0,25-0,42)	0,32±0,01 (0,30-0,34)	0,32±0,01 (0,12-0,52)
	revaluation	0,42±0,28 (0,24-0,60)	0,32±0,01 (0,30-0,34)	-
ESR	baseline	13,0±9,5 (7,0-19,0)	9,4±7,0 (4,4-14,4)	6,0±3,4 (4,2-7,8)
	revaluation	10,9±10,0 (4,7-17,1)	5,9±3,9 (3,1-8,7)	

CRP, C-reactive protein; ESR, eritrosedimentation rate (95% confidence interval).

increased risk of myocardial infarction or stroke. In this case, periodontitis treatment would remove the risk of inducing coronary disease and completely edentulous subjects would have a reduced risk of IHD. Hujoel et al. found edentulous patients to have no lesser risk of developing coronary heart disease than periodontal patients [21].

Chong et al. (2000) [22] and Armitage (2000) [23], in a review of studies published in the last years, concluded that the association between periodontitis and IHD is not evident and that they could simply be parallel phenomena [24-28]. Seymour [29] and Beck et al. [27] concluded that, while many studies indicate a relationship between this two multifactorial disorders, a cause-effect relationship has not been demonstrated in any of them. However, several studies in the last years have shown that increased serum C-reactive Protein levels seem to be a risk predictor for coronary heart disease [1, 2, 5, 6, 9].

As shown in Table 1, in the National Health and Nutrition Estimates Survey III (NHANES III) reported by Slade et al. [30] and Beck et al. [31] the median value of CRP was 0,41 mg/dl for patients with more than 10% of sites with probing pocket depth ≥ 4mm, and 0,28 mg/dl for healthy patients. Ebersole et al. [17] reported an even more evident difference between periodontal patients and controls: 0,91 mg/dl vs. 0,22 mg/dl. Instead, Loos et al. [21], Mattila et al. [9] and D'Aiuto et al. [32] observed much smaller values for both periodontal patients and controls.

In the present study, at the baseline, median CRP values among periodontal patients were not as high as those reported by Ebersole et al. [17], but similar to those reported by Ide et al. [33] and to those of the NHANES III reported by Slade et al. [30] and Beck et al. [31] , where the same nephelometric method was used. With this method, the minimum detectable concentration of CRP was 0,3 mg/dl. The same nephelometric method was used in the NHANES III. The nephelometric method was also used in the studies of Ide et al. [33] and Loos et al. [21], but with a different ratio of reagent to serum in the samples, obtaining smaller minimum detectable concentrations of CRP: 0,02 mg/dl and 0,03 mg/dl respectively. Different methods were used in the studies of Montebugnoli et al. [16] and D'Aiuto et al. [32], with CRP minimum detectable values of 0,02 mg/dl. At the second examination group 1 showed an increase in the mean CRP value, in spite of a statistically significant reduction of clinical periodontal sum score. Even if this result is not statistically significant, it seems to indicate a trend completely opposite to the expected one. This could be explained by the presence of a single subject with a CRP value much bigger than the others (1,21 mg/dl). The cause of this higher value is not clear, but it was probably due to non-periodontal unrecognized reasons. The absence of statistically significant differences among periodontal and healthy groups before and after the treatment could be explained by the use of the standard nephelometric method that does not appear sensitive enough for periodontal purposes.

Smokers were included in our study, like in the studies of Ebersole et al. [17], Beck et al. [31] and Mattila et al. [9] Smoke is considered a factor able to alter CRP levels [10, 34, 35]. However in our study, as in the studies of Friedrikkson et al. [36] and Loos et al. [3], there was no statistical difference of CRP values between smokers and non-smokers.

In a recent study of D'Aiuto et al. (2004) [32] on 94 sistemically healthy subjects with severe generalized periodontitis, there was a median decrease of 0,05 mg/dl of CRP 6 months after non-surgical therapy. This decrease was observed in subjects that presented less than 30% of residual pockets and less than 30% of residual bleeding sites.

Mattila et al. [9] obtained a median decrease of 0,03 mg/dl after mechanical therapy and, when indicated, the use of metronidazole (Table 4). On the contrary no statistically significant difference for CRP values is reported before and after mechanical treatment in the study of Ebersole et al. [17].

One month after completion of mechanical treatment we observed a reduction of sites with pd ≥4 mm, bleeding after probing and visible suppuration in both group 1 and 2, with a consequent decrease of CPSS clinical index of 34% for patients of group 1 and 38% for patients of group 2. The greater reduction in Group 2 patients, that were not treated with scaling and root planning, was unexpected but it could be explained by the small number of patients.

Even though it is necessary to take into consideration the limited number of the subjects examined, the present study shows that (i) non-surgical therapy induces an improvement of clinical periodontal indices, even in subjects that were only instructed to correct plaque control. Moreover it appears evident that (ii) a standard nephelometric method is not sensitive

enough to detect difference in CRP values between periodontal and non-periodontal subjects.

ACKNOWLEDGEMENTS

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