

Effects of periodontal therapy on C-reactive protein and HDL in serum of subjects with periodontitis

Efeitos da terapia periodontal sobre proteína C-reativa e HDL no soro de indivíduos com periodontite

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Abstract

Objective: To investigate the effects of nonsurgical periodontal therapy on levels of high-sensitivity C-reactive protein in the sera and its association with body mass index and high density lipoprotein in subjects with severe periodontitis.

Methods: Sera from 28 subjects (mean age: 34.36±6.24; 32% men) with severe periodontitis and 27 healthy controls (mean age: 33.18±6.42; 33% men) were collected prior to periodontal therapy. Blood samples were obtained from 23 subjects who completed therapy (9-12 months). Oral and systemic parameters such as the number of blood cells, glucose examination, lipid profile, and high-sensitivity C-reactive protein levels accessed by high-sensitivity immunonephelometry assay, were included.

Results: Before therapy, in the periodontitis group, the ratio of subjects with high-sensitivity C-reactive protein <0.3 mg/dL was statistically lower than in the control group ($P<0.0216$).

After therapy, the ratio of subjects with high-sensitivity C-reactive protein <0.3 mg/dL was significantly higher (65.22%) ($P<0.0339$). The mean value for body mass index was statistically lower in subjects with high-sensitivity C-reactive protein <0.3 mg/dL (24.63±4.19), compared with those with high-sensitivity C-reactive protein ≥0.3 mg/dL (28.91±6.03) ($P<0.0411$). High density lipoprotein presented a mean value statistically higher after therapy ($P<0.0027$).

Conclusion: In systemically healthy subjects with periodontitis, periodontal therapy was associated with decreased levels of circulating high-sensitivity C-reactive protein and increase of high density lipoprotein in serum. The clinical trial was registered at <http://www.clinicaltrials.gov.br/>, No. RBR-24T799.

Descriptors: C-Reactive Protein. Periodontal Diseases. Cardiovascular Diseases.

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Abbreviations, acronyms & symbols	
Aa	Aggregatibacter actinomycetemcomitans
BMI	Body mass index
BOP	Bleeding on probing
CAL	Clinical attachment level
CG	Control group
CRP	C-reactive protein
CVD	Cardiovascular disease
HDL	High density lipoprotein
hs-CRP	High-sensitivity C-reactive protein
LDL	Low-density lipoprotein
NSPT	Nonsurgical periodontal therapy
NT	Number of teeth
PD	Probing depths
PG	Periodontitis group
Pg	Porphyromonas gingivalis
PI	Plaque index
Pi	Prevotella intermedia
PT	Periodontal therapy
SPT	Supportive periodontal therapy
Tf	Tannerella forsythia

Resumo

Objetivo: Investigar os efeitos da terapia periodontal não cirúrgica sobre níveis de proteína C-reativa ultrasensível no soro e associação dessa com o índice de massa corporal e lipoproteína de alta densidade em indivíduos com periodontite grave.

Métodos: O soro de 28 indivíduos (idade média: 34,36±6,24; 32% homens) com periodontite grave e 27 controles saudáveis

(idade média: 33,18±6,42; 33% homens) foi coletado antes da terapia periodontal. Novas amostras de sangue foram obtidas dos 23 indivíduos que completaram o tratamento periodontal (entre 9-12 meses após). Parâmetros bucais e sistêmicos, tais como contagem do número de células do sangue, exame complementar de glicose, lipidograma e níveis de proteína C-reativa ultrasensível, acessados pelo método de nefelometria (imunonefelometria ultrasensível) foram incluídos.

Resultados: Antes da terapia, a proporção de indivíduos com proteína C-reativa ultrasensível <0,3 mg/dL no grupo periodontite foi estatisticamente menor que a proporção de indivíduos com proteína C-reativa ultrasensível <0,3 mg/dL nos controles ($P<0,0216$). Após a terapia, a proporção de indivíduos com proteína C-reativa ultrasensível <0,3 mg/dL foi estatisticamente maior (65,22%) ($P<0,0339$). O valor médio para índice de massa corporal foi estatisticamente menor nos indivíduos com proteína C-reativa ultrasensível <0,3 mg/dL (24,63±4,19), comparados àqueles com proteína C-reativa ultrasensível ≥0,3 mg/dL (28,91±6,03) ($P<0,0411$). O colesterol lipoproteína de alta densidade pós-terapia apresentou valor médio estatisticamente maior ($P<0,0027$).

Conclusão: Em indivíduos com periodontite e saudáveis sistemicamente, a terapia periodontal foi associada com o decréscimo dos níveis de proteína C-reativa ultrasensível circulante no soro e aumento de lipoproteína de alta densidade. O ensaio clínico foi registrado no <http://www.clinicaltrials.gov.br/>, No. RBR-24T799.

Descritores: Proteína C-reativa. Doenças Periodontais. Doenças Cardiovasculares.

INTRODUCTION

Periodontal diseases are characterized as the pathological manifestation of the host immune response to the bacterial infection at the tooth/gingival interface. These are mainly caused by Gram-negative bacteria, including *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Aggregatibacter actinomycetemcomitans* (Aa), and *Tannerella forsythia* (Tf) [1]. Severe periodontitis affects up to 15% of most populations [2]. In Brazil, the most recent National Survey of Oral Health reported that the distribution of the most severe forms of periodontal disease are more significant in adults between 35-44 years of age, with a prevalence of 19.4% [3].

The term periodontitis comprises, generally, chronic forms of periodontal disease, which are the result of a polymicrobial infection and are characterized by the loss of collagen fibers and insertion in the cementum surface (mineralized tissue that covers the root surface), apical migration of junctional epithelium (epithelium continuous with the oral epithelium that promotes the insertion of the gum to the tooth), periodontal pocket formation (cementum surface devoid of periodontal fibers) and alveolar bone reabsorption.

Such damage compromises the function of the periodontal tissues and may result in tooth loss [1].

The chronic and cyclical nature of the periodontal disease provides an opportunity for continuous hematogenous dissemination of periodontal pathogens and, consequently, a direct exposure of blood vessels to these microorganisms and their endotoxins [4]. Hence, the invasion and proliferation of pathogens in specific sites of the host organism, as in periodontitis, may produce tissue damage and subsequent progression of other diseases through a variety of cellular mechanisms [1].

C-reactive protein (CRP), an acute phase protein, is a marker of systemic inflammation in response to infectious, inflammatory and/or traumatic stimulation [5]. Although produced primarily in the liver in response to proinflammatory cytokines (IL-1, IL-6, TNF- α), recently, extrahepatic synthesis of CRP has been reported in gingival biopsies [6]. Furthermore, it was also found in saliva and gingival crevicular fluid [7].

The potential role of CRP in cardiovascular pathogenesis is not fully understood. However, it has been suggested that it can directly damage blood vessels via activation of the complement

cascade, enhancing the formation of atherosclerotic lesions, and it is associated with endothelial dysfunction [8].

In healthy subjects, CRP levels are found in trace amounts with values <0.3 mg/L [9]. It is also known that levels below 1.0 mg/L indicate low risk for cardiovascular disease (CVD). Levels between 1.0 and 3.0 mg/L and above 3.0 mg/L indicate medium and high risk, respectively [10].

Chronic bacterial infections such as periodontitis, are one of the well-established risk factors for moderately elevated CRP level [7]. In otherwise, in healthy subjects with periodontitis, especially in severe forms, high systemic levels of IL-6 [11], dyslipidemia [12], and moderate leukocytosis [11] have been observed.

Experimental and clinical trials have been performed in order to investigate the biological mechanisms of association between periodontal disease and atherosclerosis, which are not yet completely understood [4,7,11]. The ability of the periodontal pathogen to induce platelet aggregation, formation of foam cells, and development of the atheroma has been demonstrated [4]. Evidence supports at least two mechanisms that are biologically plausible: increased levels of systemic inflammation in patients with periodontitis, and the frequent migration of Gram-negative bacteria from periodontal pockets into the bloodstream (bacteremia and endotoxemia) [4,13].

The treatment of periodontal disease, in both systemically healthy patients and those with history of cardiovascular events, has been shown to reduce systemic inflammation [14-16]. Recently, a systematic review and meta-analysis reported a reduction of 0.231 ($P=0.000$) in mean levels of CRP after nonsurgical periodontal therapy (NSPT) [15].

This study aimed to investigate the effect of severe periodontal disease on the systemic inflammatory response related to the elevation of CRP levels in serum and to determine the influence of NSPT on these levels. Furthermore, the correlation between systemic levels of hs-CRP and demographic characteristics of the population study (gender and age), body mass index (BMI), high-density lipoprotein (HDL), and clinical oral parameters before and after NSPT were assessed.

METHODS

Subjects and study groups

The total sample (convenience sampling) consisted of 55 systemically healthy subjects. The periodontitis group (PG) consisted of 19 women (68%) and nine men (age range 20-45; mean age: 34.36 ± 6.24 years old), with ≥ 18 teeth. The classification of periodontal disease was according to Armitage & Cullinan [17]. The control group (CG) consisted of 18 women (67%) and nine men (age range 21-44; mean age: 33.18 ± 6.42 years old), with clinical probing depths (PD) ≤ 3 mm and clinical attachment level (CAL) ≤ 3 mm, $\leq 10\%$ of sites with bleeding on probing (BOP), and no radiographic evidence of bone

loss. Exclusion criteria were history of smoking, pregnancy or lactation, periodontal therapy (PT), antimicrobial therapy for systemic conditions or use of topical oral antibiotics in the last twelve months, diabetes, autoimmune disease, acute infections, severe allergies, gastrointestinal and renal diseases, cancer, morbid obesity (BMI >40 kg/m²) or underweight (malnourished BMI <18.5 kg/m²) [18], and use of medications that alter the levels of inflammatory mediators. The study protocol was approved by the Ethics Committee of the Faculty of Health Sciences - University of Brasilia, Brazil (045/2008). All subjects were informed about the purpose of the study, both orally and written, and a written informed consent document prior to participation was also signed.

Clinical examination

The clinical examination performed at baseline and after six months of supportive periodontal therapy (SPT) included detection of visible plaque accumulation described as plaque index (PI), BOP, PD and CAL. Measurements were assessed at four sites around each tooth, buccal, lingual and both proximal sites using a manual probe (probe Michigan O with Williams markings), excluding third molars.

Treatment protocol/Retention and Laboratory analysis

PG subjects were treated in three stages: mechanical periodontal therapy, sites reinstrumentation, and SPT. The first stage was performed in ≤ 14 days. One month later, in stage 2, new mechanical instrumentation was performed in patients who persisted with deep pockets, BOP and calculus. At this stage, meticulous scaling and root planning were performed until reaching the following periodontal conditions: PD above 4 mm in at least three or fewer sites, PD above 5 mm in two places at most, PI $\leq 15\%$ and BOP $\leq 10\%$. In Stage 3, subjects were scheduled biweekly or monthly, according to the need to control biofilm formation. SPT was performed for six months.

Out of the 28 subjects in PG, five did not complete the treatment. Twenty-three completed the three stages of periodontal protocol. Among these, ten (43%) subjects completed the treatment in nine months, ten (43%) subjects in ten months, and three (14%) subjects in 12 months.

Blood samples were collected for biochemical analysis at baseline for all 28 PG subjects and 27 CG subjects. New blood samples were collected from 23 PG subjects who completed treatment. The fasting venous blood was collected in gel separator tubes. Each EDTA tube was assessed within three hours in order to perform blood counts with standard measurements for the number of neutrophils, lymphocytes, monocytes, basophils and eosinophils. Additionally, glucose examination was performed. Plasma and serum samples were immediately placed on ice and stored at -80°C .

Lipid profile included serum levels of triglycerides, total cholesterol, HDL cholesterol and low-density lipoprotein

(LDL) obtained by the esterase-oxidase method, homogeneous direct method, oxidase-peroxidase method, and Friedeald formula. The hs-CRP levels in serum were accessed by the nephelometry method (ultrasensitive immunonephelometry assay) with the lowest detection limit of 0.1 mg/dL. This method is characterized as highly sensitive [14].

Statistical analysis

The clinical oral parameters and the demographic and hematological characteristics (lipids, glucose, and blood cells) before and post-SPT for both groups (PG and CG) were presented as mean ± standard deviation. The clinical periodontal parameters PI, PD ≤3, 5 and 6 mm and BOP before and post-therapy in the PG were compared by paired Student’s t-test, as well as lymphocytes, monocytes, neutrophils, total white blood cell and blood glucose. In both periods evaluated for this group, a nonparametric Wilcoxon test was used for the following variables: number of teeth (NT), PD=4 and ≥7 mm, BMI, systolic and diastolic blood pressure (SBP and DBP, respectively), triglycerides, total cholesterol, HDL and LDL, eosinophils and basophils.

The measurements NT, BMI, SBP, DBP, PI, BOP and hematological characteristics, except hs-CRP, were compared between both groups before and after therapy by Student’s t-test for variables that showed the Gaussian distribution. In cases where normality was not observed for both groups, the nonparametric Mann-Whitney test was used. Results for hs-CRP were expressed as percentages. For the analysis of hs-CRP in the PG before and post-therapy McNemar’s test was used and, when compared to the CG, the chi-square test was used. Data

were assessed by SAS 9.2 for Windows. For purposes of analysis, a significance level of 5% was used ($P<0.05$).

Finally, hs-CRP levels were divided into two groups: hs-CRP <0.3 and ≥0.3 mg/dL. To compare means between these groups before and after treatment, and between the other variables mentioned above (gender, age, BMI, HDL, BOP, PD and CAL), the Student’s t-test was used for variables that showed a Gaussian distribution for both groups. When normality was not observed for both groups, the nonparametric Mann-Whitney test was used. To compare the ratio of clinical cases with hs-CRP <0.3 and ≥0.3 mg/dL, Fisher’s exact test was used. McNemar’s test was performed to verify whether the ratio of PG patients that exhibited hs-CRP between 0.3 and 3 mg/dL was different before and after the PT. The reduction of the mean levels of hs-CRP after SPT was calculated by paired Student’s t-test.

RESULTS

Demographic and hematological characteristics, BMI, and clinical oral parameters of the study population before and after NSPT

Initially, 28 patients with periodontal disease were involved in this study (32% men; mean age: 34.36±6.24 years old) and 27 healthy controls (33% men; mean age: 33.18±6.42 years old). There was no difference between PG and CG for age ($P=0.4955$) and gender ($P=0.9251$). Of all the patients in the PG, 23 were followed until remission of periodontal disease. Clinical oral parameters and characteristics before and post-SPT are shown in Table 1.

Table 1. Characteristics and clinical oral parameters before and after supportive periodontal therapy

Characteristics / Parameters*	Control (n=27)	Pre-therapy (n=28)	Post-therapy (n=23)	P value pre x post	P value control x pre	P value control x post
NT	28.78±2.01	27.25±4.84	24.70±5.79	0.0001 ¹	0.5317 ³	0.0294 ³
BMI (kg/m ²)	22.23±2.32	26.92±5.60	26.95±6.29	0.0923 ¹	0.0002 ⁴	0.0021 ⁴
SBP (mmHg)	120.85±4.75	120.74±13.60	117.33±10.87	0.1055 ¹	0.8584 ³	0.1689 ³
DBP (mmHg)	80.44±2.47	79.95±12.16	76.47±7.91	0.4230 ¹	0.0513 ³	0.0004 ³
PI (%)	4.74±2.30	63.61±33.64	4.83±6.73	< 0.0001 ²	< 0.0001 ³	0.0679 ³
BOP (%)	2.67±1.49	44.46±29.35	1.63±3.35	< 0.0001 ²	< 0.0001 ³	< 0.0001 ³
PD (mm)						
≤3 mm	100.00±0.00	68.71±14.38	98.32±1.79	< 0.0001 ²	NA	NA
4 mm	0	4.02±4.02	0.63±0.96	< 0.0001 ¹	NA	NA
5-6 mm	0	17.08±8.85	0.85±1.22	< 0.0001 ²	NA	NA
≥7 mm	0	10.45±8.89	0.17±0.61	< 0.0001 ¹	NA	NA
CAL (mm)						
≤3 mm	100.00±0.00	62.56±18.20	NA	NA	NA	NA
4 mm	0	4.97±4.77	NA	NA	NA	NA
5-6 mm	0	18.74±8.96	NA	NA	NA	NA
≥7 mm	0	13.73±11.49	NA	NA	NA	NA

*Results shown as mean ± standard deviation. 1 Wilcoxon test; 2 pair T test; 3 Mann-Whitney test; 4 T test; NA = not applicable. BMI = body mass index, PI = plaque index, NT = number of teeth, CAL = clinical attachment level, DBP = diastolic blood pressure, SBP = systolic blood pressure, PD = probing depth, BOP = bleeding on probing

In the PG, PT led to a significant decrease in all clinical periodontal parameters ($P<0.0001$). The mean values for BMI, SBP and DBP after SPT did not differ statistically from the mean values of the same measurements before therapy.

At baseline, the CG was significantly different from the PG for PI and BOP ($P<0.0001$). The mean values for PI and BOP in the PG were greatly reduced after the therapy.

As shown in Table 2, there were no statistical differences in hematological and biochemical parameters between the PG and the CG before and after therapy, except for hs-CRP levels. This reflects the systemic health of both groups and shows that PT did not modify other hematological and biochemical parameters.

For comparisons between the CG and the PG before therapy, the ratio of patients with hs-CRP <0.3 mg/dL was statistically lower in PG than in CG ($P<0.0216$). Therefore, among systemically healthy patients, those with severe periodontitis had higher levels of CRP. In addition, for comparisons between the PG and the CG after therapy, the ratio of patients in the PG with hs-CRP <0.3 mg/dL did not differ statistically from that observed in CG patients.

In the PG, the mean values of triglycerides, total cholesterol, LDL cholesterol, glucose, and total leukocytes did not differ statistically before and after treatment. However, HDL cholesterol was statistically higher after treatment ($P<0.0027$). Moreover, the ratio of patients in the PG with hs-CRP <0.3 mg/dL was statistically higher after than before treatment ($P<0.0339$). This result suggests that NSPT led to reduce levels of hs-CRP in the PG and reduced levels below 0.3 mg/dL in 65.22% of patients with severe periodontitis.

Relationship between high-sensitivity C-reactive protein (hs-CRP) and demographic/hematological characteristics, BMI, and clinical oral parameters

At baseline, the ratio of patients with hs-CRP <0.3 and

≥ 0.3 mg/dL did not differ between males and females, among PG 28 subjects ($P<0.6891$). The mean BMI was statistically lower in subjects with hs-CRP <0.3 mg/dL than in those with ≥ 0.3 mg/dL ($P<0.0411$). As for HDL cholesterol, patients with hs-CRP <0.3 mg/dL had statistically higher mean value than those with ≥ 0.3 mg/dL ($P<0.0171$). The mean value for all the clinical oral parameters suggested that patients with lower levels of CRP (<0.3 mg/dl) have less severe periodontal disease, even though, for this sample, the mean value for the measurements BOP, PD ≥ 7 mm and CAL ≥ 7 mm contradicted this suggestion (Table 3).

After treatment, the mean value of all variables did not differ statistically among 23 PG subjects with hs-CRP <0.3 and ≥ 0.3 mg/dL. However, the mean value for HDL cholesterol was slightly higher in patients with hs-CRP <0.3 mg/dL (Table 4).

NSPT effect on levels of hs-CRP

In this study, the PG was found to be within the range of low or medium risk for CVD (CRP levels <1 mg/L and between 1-3 mg/L, respectively). No subject with periodontitis exhibited CRP levels ≥ 3 mg/L (high risk for CVD). The frequency of the levels of hs-CRP in the PG before and after therapy is given in Table 5 and Figure 1. The ratio of subjects in the PG with high levels of hs-CRP (≥ 0.3 to 3 mg/dL) and low levels as considered for healthy subjects (<0.3 mg/dL) was different before and after SPT. The percentage of subjects with hs-CRP <0.3 mg/dL was statistically lower before (39.13%) than after SPT (65.22%, $P<0.0339$). Thus, hs-CRP levels from ≥ 0.3 -3 mg/dL were observed in a higher percentage of subjects before treatment (60.87%) than after SPT (34.78%).

Regarding two subgroups of levels of hs-CRP (<0.3 and from ≥ 0.3 -3 mg/dL), it was observed that, out of nine patients with hs-CRP level <0.3 mg/dL before treatment, eight

Table 2. Hematologic and biochemical parameters before and after supportive periodontal therapy.

Characteristics / Parameters*	Control (n=27)	Pre-therapy (n=28)	Post-therapy (n=23)	P value pre x post	P value control x pre	P value control x post
Triglycerides (mg/dL)	87.22±35.24	101.32±46.86	106.26±43.34	0.7012 ¹	0.2254 ³	0.0848 ³
Total Cholesterol (mg/dL)	172.70±30.93	176.21±30.07	182.78±38.15	0.0975 ¹	0.6713 ⁴	0.3074 ⁴
HDL Cholesterol (mg/dL)	47.56±12.28	42.82±12.60	49.17±20.07	0.0027 ¹	0.1641 ⁴	0.6194 ³
LDL Cholesterol (mg/dL)	107.17±24.59	112.94±27.09	112.36±31.14	0.9765 ¹	0.4128 ⁴	0.5138 ⁴
Glucose (mg/dL)	85.07±6.63	90.96±14.51	88.83±11.21	0.7528 ²	0.0589 ⁴	0.1679 ⁴
Eosinophils	143.74±102.00	227.96±179.80	178.26±95.26	0.1666 ¹	0.0549 ³	0.2548 ³
Basophils	14.19±31.35	8.71±21.97	13.30±21.30	0.2188 ¹	0.5765 ³	0.6033 ³
Lymphocytes	2238.52±520.45	2136.75±508.12	2075.22±550.65	0.3018 ²	0.4663 ⁴	0.2870 ⁴
Monocytes	428.22±141.26	368.75±128.59	366.65±138.50	0.5187 ²	0.1082 ⁴	0.1277 ⁴
Neutrophils	3208.56±865.86	3548.64±1279.78	3104.59±1496.55	0.0871 ²	0.3279 ⁴	0.8049 ⁴
Total Leukocytes	6171.48±1260.51	6297.50±1515.88	5970.00±1734.72	0.1808 ²	0.7393 ⁴	0.6373 ⁴
hs-CRP ($<0,3$ mg/dL) †	76.92	39.13	65.22	0.0339 ⁵	0.0216 ⁶	0.3654 ⁶

* Results shown as mean ± standard deviation. 1 Wilcoxon test; 2 pair T test; 3 Mann-Whitney test; 4 T test; 5 Mc Nemar test; 6 X-square test. HDL = high-density lipoprotein, LDL = low-density lipoprotein, hs-CRP = high-sensitivity C-reactive protein

Table 3. Comparison between levels of hs-CRP and demographic/hematological characteristics, BMI, and clinical oral parameters at baseline.

Variable*	hs-CRP <0.3 mg/dL	hs-CRP ≥0.3 mg/dL	P-value#
Gender†			0.6891§
Female	8 (42.11)	11 (57.89)	
Male	5 (55.56)	4 (44.44)	
Age (years)	34.62 ± 6.19	34.13 ± 6.50	0.8430
BMI (kg/m ²)	24.63 ± 4.19	28.91 ± 6.03	0.0411
HDL Cholesterol (mg/dL)	49.08 ± 14.19	37.40 ± 8.10	0.0171
BOP (%)	49.08 ± 33.23	40.47 ± 26.04	0.4492
PD <3 mm	69.63 ± 12.92	67.91 ± 15.95	0.7587
PD 4 mm	2.63 ± 1.92	5.22 ± 4.97	0.0779
PD 5-6 mm	15.90 ± 5.79	18.11 ± 10.95	0.5025
PD ≥7 mm‡	11.83 ± 9.12	9.25 ± 8.82	0.4067
CAL <3 mm	64.68 ± 19.81	60.72 ± 17.17	0.5765
CAL 4 mm	3.03 ± 2.25	6.65 ± 5.75	0.0369
CAL 5-6 mm	17.12 ± 6.99	20.15 ± 10.41	0.3825
CAL ≥7 mm	15.18 ± 13.58	12.48 ± 9.64	0.5462

* Values expressed as mean ± standard deviation. † Values expressed as frequency (percentage). # P-values are results of Student's t-test. ‡ P-values are results of Mann-Whitney test. § P-value is result of Fisher's test. HDL = high-density lipoprotein, BMI = body mass index, CAL = clinical attachment level, hs-CRP = high-sensitive C-reactive protein, PD = probing depth, BOP = bleeding on probing

Table 4. Comparison between levels of hs-CRP and demographic/hematological characteristics, BMI, and clinical oral parameters after supportive periodontal therapy.

Variable*	hs-CRP <0.3 mg/dL	hs-CRP ≥0.3 mg/dL	P-value#
Gender†			0.3452§
Female	9 (56.25)	7 (43.75)	
Male	6 (85.71)	1 (14.29)	
Age (years)	34.47 ± 6.40	33.88 ± 6.29	0.8339
BMI (kg/m ²)	26.20 ± 5.86	28.35 ± 7.23	0.4472
HDL Cholesterol (mg/dL) ‡	50.53 ± 22.06	46.63 ± 16.74	0.7712
BOP (%)‡	1.47 ± 3.48	1.94 ± 3.30	0.0659
PD <3 mm	98.09 ± 1.97	98.75 ± 1.42	0.4135
PD 4 mm‡	0.83 ± 1.02	0.26 ± 0.74	0.1490
PD 5-6 mm‡	0.86 ± 1.34	0.84 ± 1.06	0.6867
PD ≥7 mm‡	0.18 ± 0.68	0.17 ± 0.49	0.7414

* Values expressed as mean ± standard deviation. † Values expressed as frequency (percentage). # P-values are results of Student's t-test. ‡ P-values are results of Mann-Whitney test. § P-value is result of Fisher's test. HDL = high-density lipoprotein, BMI = body mass index, CAL = clinical attachment level, hs-CRP = high-sensitive C-reactive protein, PD = probing depth, BOP = bleeding on probing

Table 5. Frequency of hs-CRP in periodontitis group before and after supportive periodontal therapy.

hs-CRP (before therapy)	hs-CRP (after therapy)		Total
	<0.3 mg/dL	≥0.3 to 3 mg/dL	
<0.3 mg/dL	8 (88.89)	1 (11.11)	9 (39.13)
≥0.3 to 3 mg/dL	7 (50.00)	7 (50.00)	14 (60.87)
Total	15 (65.22)	8 (34.78)	23 (100.00)

Values expressed as frequency (percentage); McNemar test. hs-CRP = high-sensitive C-reactive protein

(88.89%) maintained this level, and only one (11.11%) had a hs-CRP level ≥0.3-3 mg/dL post-therapy. Out of 14 patients with hs-CRP levels from ≥0.3-3 mg/dL before therapy, seven (50%) maintained this level, and seven had hs-CRP level <0.3 mg/dL after treatment. Therefore, 26.09% of 23 PG patients showed a decrease in hs-CRP levels: between ≥0.3 and 3 mg/dL to levels <0.3 mg/dL after therapy. The reduction of hs-CRP levels was 0.1487±0.6290 after SPT, which was considered not statistically significant (P=0.2691).

DISCUSSION

This study included a homogeneous group of patients with severe periodontitis. The ratio of patients in the PG with hs-CRP levels >0.3 mg/dL was higher than in the CG (60.87 versus 23.08, respectively; $P=0.0216$). These results are consistent with previous reports [11,12], in that higher levels of hs-CRP were observed in the serum of patients with severe periodontitis.

The levels of serum IL-6 and CRP have been reported to increase with age [14]. However, in this study, there was no difference in the ratio of patients with levels of hs-CRP <0.3 and ≥ 0.3 mg/dL, with a mean age in the PG of 34.36 ± 6.24 . Also, increased levels of CRP have been reported in women due to hormonal changes [19]. Interestingly, the ratio of patients in the PG with hs-CRP <0.3 and ≥ 0.3 mg/dL did not differ between female and male subjects ($P < 0.6891$), although, before NSPT, the ratio of subjects with hs-CRP ≥ 0.3 mg/dL was higher for women than men.

Race/ethnicity has been described to affect CRP levels [20]. Asian populations have a lower range of systemic levels of CRP than black populations [21]. Since the Brazilian population presents miscegenation of browns, whites, blacks and Indians, race/ethnicity may have influenced the levels of CRP in this population study. Here, subjects with severe periodontitis were at “low risk” (<1 mg/L) or “medium risk” (1 to 3 mg/L) for CVD, as shown in previous studies [20,21].

There is a strong link between CRP levels in the blood and future development of high blood pressure [19]. This association is stronger for SBP than for DBP [22]. In this study, no difference was found between the mean values of SBP and DBP in the PG before and after therapy.

White blood cell count also has been associated with significant prediction of future cardiovascular events and glucose intolerance in different populations [22]. In this study, the non-observation of leucocytosis indicated that patients with severe periodontitis exhibited less pronounced systemic inflammation due to periodontal disease.

A positive relationship between the severity of periodontitis and initial systemic levels of hs-CRP has been observed [11]. Also, in this study, the clinical periodontal parameters before and after SPT suggested that patients with lower levels of CRP (<0.3 mg/dl) had less severe periodontal disease.

NSPT led to a significant decrease of all clinical periodontal parameters ($P < 0.0001$). It should be noted that there was a significant reduction of PI from 63.61 ± 33.64 to 4.83 ± 6.73 and of BOP from 44.46 ± 29.35 to 1.63 ± 3.35 . After therapy, the NT was statistically fewer due to the extraction of periodontally condemned teeth ($P = 0.0001$). Regarding the levels of hs-CRP, the ratio of PG subjects with hs-CRP <0.3 mg/dL was statistically higher after therapy ($P < 0.0339$). This result indicates that NSPT led to reduced levels of hs-CRP in the PG and demonstrated levels <0.3 mg/dL in most subjects

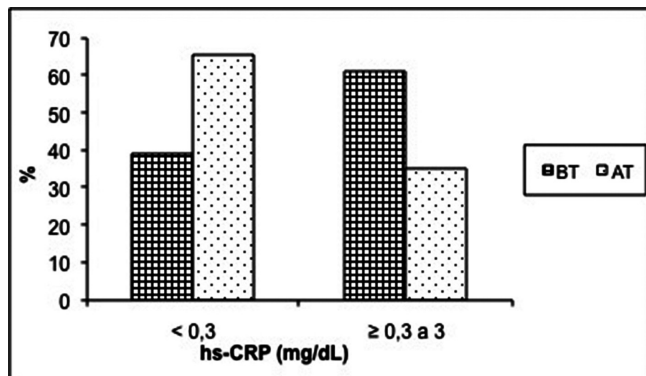


Fig. 1 – Frequency of the hs-CRP in the periodontitis group before and after periodontal therapy. hs-CRP - high-sensitivity C-reactive protein; BT - before therapy; AR - after therapy

with severe periodontitis (65.22%). It should be emphasized that for 26.09% of the 23 subjects that completed treatment, changes in the risk stratification for CVD was detected.

These patients, prior to therapy, had hs-CRP levels from ≥ 0.3 to 3 mg/dL and, after therapy, levels were <0.3 mg/dL, i.e., considered as normal levels for subjects. Although the reduction in levels of hs-CRP was 0.1487 ± 0.6290 , i.e., moderate and not statistically significant ($P = 0.2691$), the ratio of subjects in PG with hs-CRP <0.3mg/dL did not differ statistically from that observed in subjects in CG after SPT. There are reports of significant reductions in CRP levels, especially for subjects with high levels of CRP at baseline [23]. However, it is known that among systemically healthy patients, NSPT may decrease levels of CRP that were initially below 3 mg/dL [23].

It is known that anti-inflammatory and non-steroidal drugs (aspirin and ibuprofen), oral drugs of synergistic combinations of cardiovascular agents, and low doses of steroids can potentially decrease the serum CRP levels [19]. In this study, the use of any medication was prohibited because it could affect the immunoinflammatory response and directly interfere with clinical outcomes. Since smoking has been reported as an independent risk factor for periodontitis and elevated serum CRP levels, patients with history of smoking and obesity were excluded in order to avoid adjustments to their potential effects on CPR response to the periodontal treatment [23].

NSPT employed with the reinstrumentation of residual periodontal pockets with BOP and calculus, reassessment, and SPT for 6 months indicate that maintaining a healthy periodontium without signs of periodontal inflammation had a positive impact on the reduction of serum CRP levels. This supports previous results showing that a greater reduction in CRP levels occurs among those with better clinical responses to PT [23]. In contrast, other studies found no significant reduction in serum CRP levels after NSPT, despite

improvement in clinical periodontal parameters [21]. A systematic review and meta-analysis justified that these differences are due to the fact that the authors have not studied the same type or severity of periodontal disease [24]. Another explanation refers to the medical follow-up and the methodology proposed by each study (for example, the assay used to detect CRP).

Furthermore, our results showed no association between periodontitis and BMI. However, subjects with BMI >27 kg/m² were not included in this study. There is evidence that increase of BMI also increases risk for periodontitis [12,23]. However, our data showed a significant increase in HDL ($P=0.0027$) and no significant change in other serum lipids in the PG after therapy. The concentration of CRP has also been correlated with the levels of lipids (e.g., triglycerides and HDL) and an inverse relationship between CRP and HDL levels has been observed [19]. In this study, PG subjects with hs-CRP <0.3 mg/dL presented a mean value of HDL statistically higher than those with hs-CRP ≥ 0.3 mg/dL ($P<0.0171$) before treatment. Furthermore, the mean value for BMI was lower in patients with hs-CRP <0.3 mg/dL compared with those with hs-CRP ≥ 0.3 mg/dL ($P<0.0411$).

However, between patients with hs-CRP <0.3 and ≥ 0.3 mg/dL the mean values of HDL and BMI were not statistically different after therapy. Therefore, these results demonstrate the significant association between periodontitis and decrease in HDL cholesterol. This association has been related to local production of inflammatory cytokines (IL-1, TNF- α) due to periodontal infection and its effect on other systemic mediators (IL-6) that can induce changes in lipid metabolism [25], such as increased LDL and triglycerides. This is due to increased hepatic lipogenesis, lipolysis of adipose tissue or reduced clearance of blood. Bacterial toxins (LPS) may also induce changes in cholesterol levels (reduced HDL and increase LDL), or to target the metabolism of glucose and produce a state of insulin resistance [22].

Hence, our results showed that periodontitis significantly increases the levels of serum CRP, and therefore might be related with moderate risk of atherosclerosis and its consequences. Nevertheless, to better understand the association between periodontal disease and atherosclerosis, additional studies with larger samples are required in order to statistically compensate the other various covariates such as age, adiposity, smoking, and insulin resistance. A higher number of subjects certainly contains a sample with a broader variation of systemic CRP levels, that would help to clarify whether PT has a significant impact on CVD, i.e., if the control of periodontal infections leads to decreased risk of future cardiovascular events. Additionally, a longitudinal follow-up of patients with periodontal risk would evaluate the duration of the therapeutic effects of the SPT on systemic biomarkers.

CONCLUSION

This study showed that severe periodontitis is associated with increased levels of hs-CRP in serum, and NSPT is able to reduce these levels to values close to that of healthy subjects, and therefore reduce the category of inflammatory risk for CVD. Moreover, a significant increase in HDL cholesterol also was shown after the periodontal treatment. The results emphasize the importance of NSPT and the maintenance of periodontal health to avoid high levels of hs-CRP, which are also increased in other systemic inflammatory diseases such as arthritis, diabetes mellitus, and obesity.

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Authors' roles & responsibilities	
ACEL	Main author
VMAC	Coauthor
MCMG	Coauthor

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