Evaluation of periodontitis treatment effects on carotid intima-media thickness and expression of laboratory markers related to atherosclerosis

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The objective of this research was to evaluate the treatment of periodontal disease and its effects on carotid intima-media thickness (CIMT) and expression of laboratory markers related to atherosclerosis. Twenty-three healthy patients (group 1) and 21 patients with moderate to severe periodontitis (group 2) underwent evaluation of clinical periodontal parameters. The patients were submitted to CIMT measurements and laboratory evaluations at the start of the study (0 months), 6 months, and 12 months. All patients received oral hygiene instruction; patients in group 2 also underwent supragingival and subgingival scaling and root planing. A statistically significant improvement in clinical periodontal parameters occurred in both groups ($P < 0.05$). Improvements were more evident between the first and second evaluations and were greater in group 2. Both groups experienced a statistically significant decrease in CIMT in the first 6 months ($P < 0.05$). Treatments—oral hygiene instruction in group 1 and instruction plus mechanical periodontal instrumentation in group 2—were effective in improving clinical periodontal parameters of both groups and promoting reduction in CIMT at 6 months.

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Atherosclerosis is characterized by the development of atheromatous plaques along the arterial wall. The most accepted theory for atherogenesis argues that the lesions begin as a response to some type of injury to the arterial endothelium, which increases permeability to plasma components, including lipids, and allows monocytes and blood platelets to adhere to the endothelial wall, forming atherosclerotic plaques.[1,2] For several years, it has been the major cause of mortality in Brazil.[3] According to the World Health Organization, this disease accounted for 37% of deaths in the Brazilian population in 2008.[4]

Periodontal disease is an inflammatory condition caused by infection that promotes destruction of periodontal support and subsequent tooth loss.[5] Periodontitis is one of the most common chronic inflammatory diseases in the world. It has been reported that 44%-57% of the adult population has moderate periodontitis, whereas about 10%-30% has severe periodontitis.[5,6] Dental plaque is responsible for the onset and maintenance of periodontal disease. After the injury, host inflammatory mediators produced in the periodontium enter the circulation and cause multiple-organ systemic impairment.[7]

The biological relationship between periodontal diseases and cardiovascular diseases is manifested in elevation of cholesterol levels, aggravation of the atherosclerotic process by circulating oral bacteria, and higher production of acute stage proteins in patients with chronic periodontitis.[3,8] These relationships depend on the degree of development of periodontitis: The more intense the inflammatory process of the teeth, the greater the cardiovascular risk. Some individuals with periodontitis may have 2.6 times greater risk of severe forms of peripheral arterial disease.[9,10] The overall objective of the present study was to analyze the effect of treatment of periodontitis and its influence on the expression of markers related to atherosclerosis.

Materials and methods

The study was submitted to the Committee in Ethics Research of West Paraná State University (No. 097/2012-CEP, CAAE No. 04116012.4.0000.0107).

Ninety-eight patients were selected from among those who attended the dental clinic of West Paraná State University between June and November 2012. The following individuals were excluded from the study: patients who smoked more than 20 packs of cigarettes per year; patients who were pregnant or breastfeeding; patients who had undergone prolonged broad-spectrum antibiotic treatment in the last 6 months; patients who had taken high doses of anti-inflammatory steroids, anticoagulants, or immunosuppressants in the 3 months prior to the study; patients with a history of any type of severe systemic disease; and patients with a history of recent periodontal treatment. All patients included in the study had at least 20 aligned teeth in the dental arches. Of the 98 prospective subjects, 44 met the inclusion criteria. Data were collected at the following intervals: *stage 1* (0 months); *stage 2* (6 months); and *stage 3* (12 months). The total duration of the study was 12 months.

All patients had at least 20 aligned teeth in the dental arches; the buccal, lingual/palatal, mesial, and distal surfaces of the teeth were examined. A single, trained, calibrated examiner with extensive experience in periodontics performed the oral examinations with a Williams No. 23 periodontal probe, determining the plaque index (PI) of Silness & Löe, gingival index (GI) of Löe & Silness, probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP).[11,12]

The common carotid intima-media thickness (CIMT) was measured with a portable ultrasound featuring a 7- to 12-mHz linear transducer with

Table 1. Mean (SD) values for periodontal parameters in healthy patients (group 1) and patients with moderate to severe periodontitis (group 2).

	Group 1			Group 2		
Parameter	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
PI (%)	28.60 (19.72)	12.31 (14.69)[a]	10.45 (15.49)[c]	50.45 (27.40)	17.79 (17.10)[a]	13.38 (19.72)[c]
GI (%)	2.87 (4.23)	0.25 (0.66)[a]	0.19 (0.59)[c]	16.95 (17.58)	1.92 (2.96)[a]	3.11 (8.15)[c]
BOP (%)	7.71 (8.35)	0.91 (2.33)[a]	0.08 (0.20)[c]	35.26 (29.18)	11.61 (15.59)[a]	0.10 (0.19)[bc]
PD (mm)	1.71 (0.26)	1.60 (0.24)[a]	1.48 (0.17)[bc]	2.84 (0.65)	2.00 (0.28)[a]	1.97 (0.54)[c]
CAL (mm)	1.87 (0.33)	1.77 (0.30)	1.61 (0.25)[bc]	3.24 (0.96)	2.47 (0.69)[a]	2.39 (0.92)[c]

Abbreviations: BOP, bleeding on probing; CAL, clinical attachment level; GI, gingival index; PD, probing depth; PI, plaque index.

Stage 1, first assessment (0 months); stage 2, second assessment (6 months); stage 3, third assessment (12 months).

[a]Statistically significant difference between the mean values at stages 1 and 2 within each group ($P < 0.05$).

[b]Statistically significant difference between the mean values at stages 2 and 3 within each group ($P < 0.05$).

[c]Statistically significant difference between the mean values at stages 1 and 3 within each group (total variation; $P < 0.05$).

radiofrequency waves (Esaote North America). The apparatus was used by a single experienced physician, certified by the Brazilian Vascular Surgery Society. Right and left common CIMTs were measured 1.5 cm from the carotid bifurcation, with an orthogonal beam incidence to the axial course of the common carotid artery. Scanning was performed in 10 mm of the posterior artery wall, taking at least 6 measurements with a standard deviation less than 10 µm. The highest value of both was defined as *CIMTU* and the average of right and left values was defined as *CIMTM*. Data were compared with those established by Howard et al and the resulting values were defined as *CIMTEXP*.[13] The protocol for measuring the intima-media complex followed the parameters established by the Mannheim consensus.[14] Common carotid artery diameter and the presence of atherosclerotic plaques in the carotid arteries were also measured. Wave spectral analyses of the carotid and vertebral arteries were obtained, and the peak systolic velocity (PSV), end-diastolic velocity (EDV), pulsatility index, and resistivity index were analyzed.

Blood samples were obtained prior to treatment (stage 1) and at stages 2 and 3 to measure the following laboratory markers related to atherosclerosis:

- Total cholesterol
- Cholesterol fractions: high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL)
- Triglycerides
- C-reactive protein (CRP)
- Ultrasensitive C-reactive protein
- Fibrinogen
- Glycated hemoglobin (HbA_{1c})
- Fasting glucose
- Erythrocyte sedimentation rate
- Homocysteine
- Complete blood cell counts
- Creatinine

After initial evaluations (CIMT, clinical examination, and initial laboratory samples), patients were divided into 2 groups: healthy patients (n = 23) and patients with moderate to severe periodontitis (n = 21). In patients assigned to group 1, all tooth sites exhibited a probing depth of 3 mm or less and a gingival bleeding score of 5% or less. Patients assigned to group 2 had severe localized periodontitis or moderate or severe generalized chronic periodontitis, which was defined as at least 4 sites, not at the same tooth, exhibiting a probing depth of 5 mm or more, a clinical attachment level of 4 mm or more, BOP, and gingival inflammation.

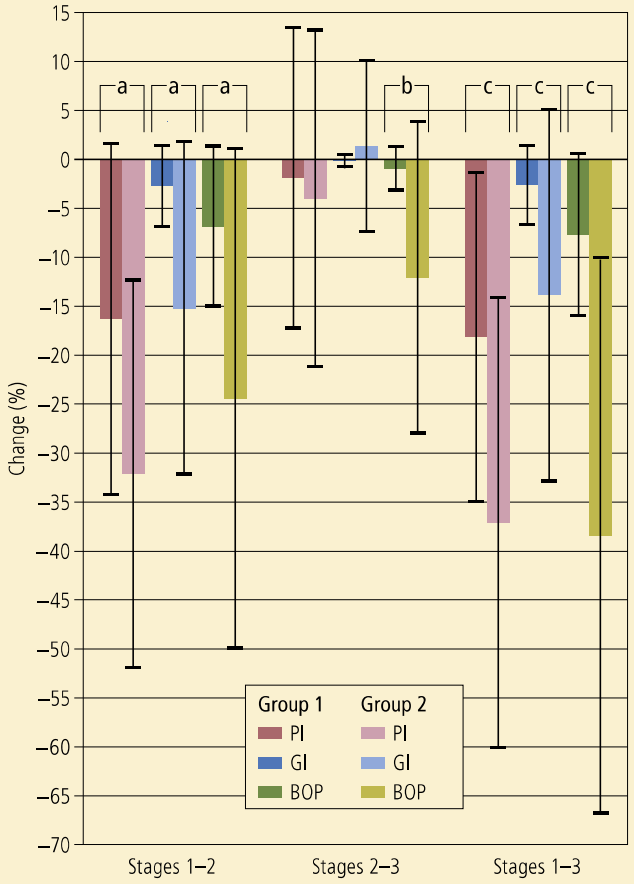
During regularly scheduled visits at the university dental clinic, both groups were provided with instruction on mechanical plaque control (modified Bass brushing technique and use of dental floss) and periodontal supportive therapy. In group 1, treatment at stage 1 consisted solely of oral hygiene instruction and motivation. Patients in group 2 also underwent supragingival and subgingival scaling and root planing at stage 1. Manual instrumentation with No. 5/6, 7/8, 11/12, and 13/14 Gracey curettes (Hu-Friedy Mfg Co, LLC) and ultrasonic instrumentation (Dabi Atlante) were performed under local anesthesia. Patients were evaluated for a total period of 12 months, and clinical examinations were performed with an interval of 6 months, at which time oral hygiene instructions were reinforced.

The means within each group were compared with a *t* test ($P < 0.05$) for statistical analysis of the data with normal distribution. A Mann-Whitney test ($P < 0.05$) was used for data that were not normally distributed. The Shapiro-Wilk test was used to evaluate the normal distribution of data. The changes between the 3 assessments—from stages 1 to 2 (0-6 months), from stages 2 to 3 (6-12 months), and from stages 1 to 3 (0-12 months)—were subjected to analysis of variance ($P < 0.05$) if data were normally distributed and the Kruskal-Wallis test ($P < 0.05$) if data were not normally distributed. Linear regression models were used to analyze the influence of periodontal treatment on CIMT and laboratory changes.

Results

Of the 44 patients included, 27.3% were men (30.4% in group 1 and 22.7% in group 2). The average age was 39.81(SD, 7.98) years (39.47 years in group 1 and 40.77 years in group 2).

Chart 1. Mean changes in periodontal indices in groups 1 and 2.



Abbreviations: BOP, bleeding on probing; GI, gingival index; PI, plaque index.

Stage 1, first assessment (0 months); stage 2, second assessment (6 months); stage 3, third assessment (12 months).

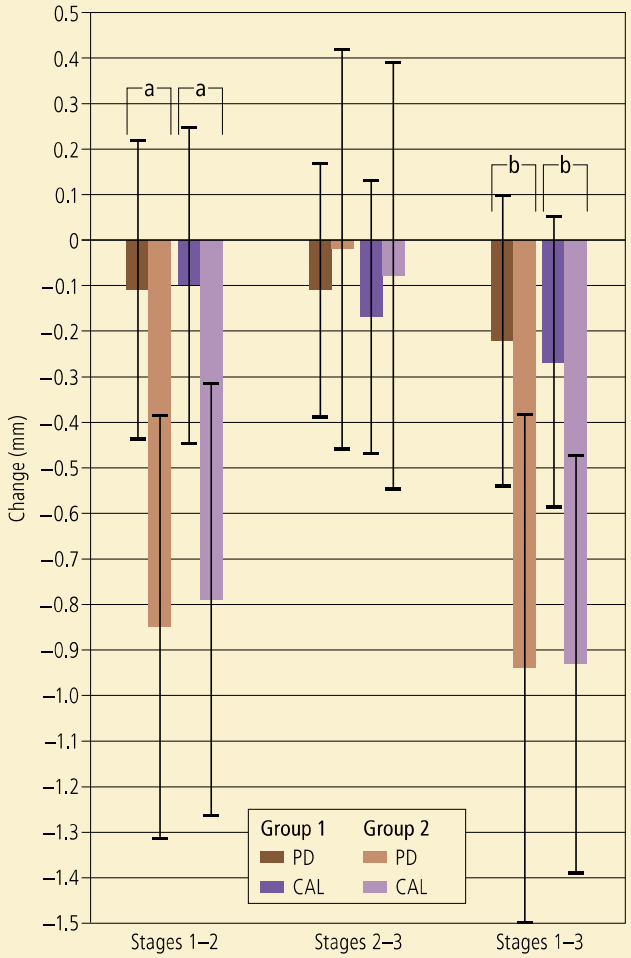
Error bars represent standard deviations.

[a]Statistically significant difference between groups 1 and 2 in changes from stages 1 to 2 ($P < 0.05$).

[b]Statistically significant difference between groups 1 and 2 in changes from stages 2 to 3 ($P < 0.05$).

[c]Statistically significant difference between groups 1 and 2 in changes from stages 1 to 3 ($P < 0.05$).

Chart 2. Mean changes in periodontal attachment in groups 1 and 2.



Abbreviations: CAL, clinical attachment level; PD, probing depth.

Stage 1, first assessment (0 months); stage 2, second assessment (6 months); stage 3, third assessment (12 months).

Error bars represent standard deviations.

[a]Statistically significant difference between groups 1 and 2 in changes from stages 1 to 2 ($P < 0.05$).

[b]Statistically significant difference between groups 1 and 2 in changes from stages 1 to 3 ($P < 0.05$).

There were statistically significant ($P < 0.05$) improvements in the clinical parameters PI, GI, BOP, and PD in both groups, and for CAL only in group 2, from stages 1 to 2 (Table 1). There were significant improvements from stages 2 to 3 only in PD and CAL in group 1 and in BOP in group 2. All periodontal parameters improved significantly from stages 1 to 3 in both groups (Charts 1 and 2). The changes in mean values from stages 1 to 2 and stages 1 to 3 were significantly greater in group 2 for all parameters studied ($P < 0.05$). From stages 2 to 3, only the change in BOP was significantly greater in group 2.

The inflammatory activity tests and blood samples revealed a statistically significant difference between the changes in mean levels of glycated hemoglobin from stages 2 to 3 and stages 1 to 3 in both groups ($P < 0.05$); the amount of HbA_{1c} value decreased over the course of the study (Table 2). There were no statistically significant changes between assessments in either group in the lipid profile: triglycerides, total cholesterol, LDL, HDL, and VLDL. However, there were significant differences between groups in the observed changes in triglycerides and VLDL; the changes were greater in group 1, and the amounts of change increased from stages 1 to 2 to stages 2 to 3 ($P < 0.05$).

Table 2. Mean (SD) laboratory values and inflammatory biomarkers in healthy patients (group 1) and patients with moderate to severe periodontitis (group 2).

	Group 1			Group 2		
Parameter	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
CRP (mg/dL)	0.82 (0.69)	1.02 (1.25)	0.88 (0.62)	0.94 (1.05)	0.70 (0.39)	0.75 (0.45)
CRP ultra (mg/L)	1.47 (1.00)	4.49 (6.61)	6.67 (9.41)	3.43 (4.51)	3.18 (2.84)	3.03 (1.84)
ESR (mm/h)	10.74 (11.39)	10.00 (13.85)	12.71 (12.86)	8.00 (4.98)	9.40 (5.60)	9.86 (7.55)
Fibrinogen (g/L)	2.71 (0.76)	3.09 (1.82)	3.75 (1.18)	2.73 (0.59)	2.84 (0.71)	3.24 (0.64)
Creatinine (mg/dL)	0.92 (0.16)	1.01 (0.19)	0.90 (0.24)[b]	0.93 (0.23)	0.93 (0.18)	0.83 (0.18)
Glucose (mg/dL)	90.26 (6.84)	92.50 (23.05)	90.86 (10.04)	91.45 (6.26)	92.10 (14.80)	97.57 (19.84)
HbA_{1c} (%)	5.45 (0.23)	5.46 (0.34)	4.75 (0.53)[bc]	5.47 (0.17)	5.44 (0.41)	4.77 (0.44)[bc]
Homocysteine (µmol/L)	8.39 (1.92)	7.71 (1.64)	8.75 (2.41)	8.98 (3.34)	7.85 (2.05)	9.61 (3.95)
Triglycerides (mg/dL)	175.64 (197.97)	163.30 (182.44)	122.23 (76.47)	121.21 (65.51)	141.20 (96.08)	127.76 (68.25)
TC (mg/dL)	221.91 (57.08)	206.61 (65.66)	201.05 (39.30)	190.37 (27.76)	196.50 (31.66)	186.90 (32.49)
HDL (mg/dL)	55.36 (18.86)	56.52 (16.05)	51.27 (17.97)	51.84 (12.48)	50.95 (12.86)	48.33 (8.26)
LDL (mg/dL)	139.05 (50.92)	127.43 (54.15)	127.18 (33.44)	114.32 (28.14)	117.35 (23.22)	112.95 (26.97)
VLDL (mg/dL)	27.50 (14.73)	26.57 (10.69)	22.59 (9.45)	24.21 (13.15)	28.20 (19.26)	25.62 (13.64)
Erythrocytes ($\times 10^6$/µL)	4.85 (0.43)	4.72 (0.39)	4.61 (0.36)[bc]	4.90 (0.51)	4.74 (0.44)[a]	4.68 (0.46)[c]
Platelets ($\times 10^3$/µL)	282.95 (40.64)	238.15 (41.37)[a]	268.71 (45.01)[c]	258.68 (54.91)	244.71 (44.87)[a]	233.21 (55.57)[c]
Leukocytes ($\times 10^3$/µL)	7.39 (1.70)	7.46 (2.47)	7.04 (2.73)	6.70 (1.45)	6.20 (2.06)	6.87 (1.46)

Abbreviations: CRP, C-reactive protein; CRP ultra, ultrasensitive C-reactive protein; ESR, erythrocyte sedimentation rate; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; VLDL, very low-density lipoprotein.

Stage 1, first assessment (0 months); stage 2, second assessment (6 months); stage 3, third assessment (12 months).

[a]Statistically significant difference between the mean values at stages 1 and 2 within each group ($P < 0.05$).

[b]Statistically significant difference between the mean values at stages 2 and 3 within each group ($P < 0.05$).

[c]Statistically significant difference between the mean values at stages 1 and 3 within each group (total variation; $P < 0.05$).

There was a significant decrease in creatinine concentrations at stage 3 in group 1 ($P < 0.05$). There was a statistically significant difference between groups in the changes of creatinine concentration from stages 1 to 2; the value was higher in group 2 ($P < 0.05$). There was also a statistically significant difference between groups 1 and 2 in the change in fibrinogen levels from stages 1 to 3; a greater change occurred in group 1 ($P < 0.05$). There were no statistically significant differences between groups 1 and 2 in the mean changes for inflammatory markers over time.

The red blood cell counts showed statistically significant differences in the change in amount of erythrocytes from stages 2 to 3 in group 1, stages 1 to 2 in group 2, and stages 1 to 3 in both groups ($P < 0.05$). There was a decrease in the platelet counts when comparisons were made from stages 1 to 2 and 1 to 3 in both groups ($P < 0.05$). There were no statistically significant differences between the amounts of white blood cells at the different stages of the study. There were no statistically significant differences between groups 1 and 2 in the changes in red and white blood cells over time.

The ultrasound evaluation showed a reduction in intima-media thickness in both groups from stages 1 to 2; these changes were statistically significant ($P < 0.05$) for both CIMTM and CIMTU (Table 3). There were no statistically significant differences between groups 1 and 2 in changes of CIMTM and CIMTU at any stage of the study (Table 4). When CIMTM measurements at the initial evaluation (stage 1) were compared with the threshold defined by Howard et al, it was shown that 65.22%, and 71.43% of subjects in groups 1 and 2, respectively, had CIMTs greater than the threshold value.[13] At 6 months (stage 2), there was significant decrease in the proportion of subjects with CIMT values above the threshold, to 39.13% and 47.62% for groups 1 and 2, respectively.[13] There was no statistically significant difference in either group in changes from stages 1 to 3, demonstrating that the CIMT measurements increased from stages 2 to 3, returning to values close to the original.

There were statistically significant differences in both groups in the measurements of velocity parameters at stages 2 and 3, represented by falling PSV, EDV, pulsatility index, and resistivity index ($P < 0.05$), but no there was no difference between groups 1 and 2 when the changes in these parameters were compared (Table 5). There was a significant decrease in EDV from stages 1 to 3 in group 2. There was no significant change in the average diameter of the carotid arteries at any stage of the study.

Table 3. Mean (SD) values for carotid artery parameters in healthy patients (group 1) and patients with moderate to severe periodontitis (group 2).

	Group 1			Group 2		
Parameter	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
CIMTM[a] (µm)	532.52 (93.23)	484.96 (104.92)[c]	520.78 (114.19)	561.05 (100.38)	513.42 (87.75)[c]	542.00 (105.19)
CIMTU (µm)	561.70 (99.89)	520.61 (122.78)[c]	557.74 (122.99)	599.38 (107.08)	543.79 (107.77)[c]	584.76 (105.41)
CIMTEXP (µm)	510.13 (89.17)	512.30 (89.22)	516.17 (89.69)	512.57 (79.31)	523.26 (80.14)	528.36 (79.17)
Howard (%)	65.22	39.13[c]	60.87	71.43	47.62[c]	57.14
Diameter[a] (cm)	6.59 (0.54)	6.51 (0.56)	6.52 (0.75)	6.69 (0.57)	6.70 (0.48)	6.86 (0.70)
Pulsatility index[b]	1.70 (0.32)	1.96 (0.37)	1.65 (0.32)[d]	1.59 (0.42)	1.80 (0.37)	1.45 (0.22)[d]
Resistivity index[b]	0.69 (0.04)	0.80 (0.07)	0.71 (0.05)[d]	0.70 (0.17)	0.76 (0.07)	0.68 (0.04)[d]
PSV[b](cm/s)	71.58 (10.19)	82.21 (14.56)	71.79 (13.85)[d]	68.96 (9.56)	76.87 (9.94)	68.46 (10.43)[d]
EDV[b] (cm/s)	21.70 (2.75)	29.63 (15.90)	20.16 (3.33)[d]	23.65 (4.66)	25.44 (5.32)	21.09 (2.73)[de]

Abbreviations: CIMTM, measured average intima-media thickness of right and left common carotid arteries; CIMTU, automated measurement of the common carotid intima-media thickness by portable ultrasound (greater value of the left and right measurements); CIMTEXP, reference value adjusted for age using the data reported by Howard et al[13]; EDV, end-diastolic velocity; PSV, peak systolic velocity.

Stage 1, first assessment (0 months); stage 2, second assessment (6 months); stage 3, third assessment (12 months); Howard, percentage of patients with intima-media thickness greater than expected for age, based on findings of Howard et al[13]; diameter, average diameter of the common carotid artery; pulsatility index, (PSV – EDV)/timed mean velocity; resistivity index, (PSV – EDV)/PSV.

[a]Average of right and left common carotid arteries.

[b]Average of right and left carotid (common, internal, and external) and vertebral arteries.

[c]Statistically significant difference between the mean values at stages 1 and 2 within each group ($P < 0.05$).

[d]Statistically significant difference between the mean values at stages 2 and 3 within each group ($P < 0.05$).

[e]Statistically significant difference between the mean values at stages 1 and 3 within each group (total variation; $P < 0.05$).

Discussion

The prevalence of mild and moderate forms of periodontal disease ranges from 20% to 50% of the world population, reaching 85% in older people.[15] Serious forms affect 30% of adults older than 50 years of age.[16,17] Effective actions to reduce the number of individuals exposed to periodontal disease are necessary to avoid negative influences on the rest of the body.

In the present study population, there was low prevalence of associated systemic diseases because the sample was chosen according to criteria that excluded patients who had moderate to severe forms of hypertension, diabetes, or other serious diseases. Patients who smoked more than 20 packs of cigarettes per year were also excluded because such patients have been shown to have more evident clinical problems, and the objective in the present study was to minimize confounding factors.[18]

The periodontal evaluation quickly and efficiently promoted the identification of patients with different degrees of periodontitis. Relatively healthy patients were defined by the criteria of PD of 3 mm or less, GI of 5% or less, and CAL of 2 mm or less. The presence of at least 4 sites with PD of 5 mm or more and CAL of 4 mm or more have been cited as relevant parameters for defining the severity of periodontal disease.[19]

In the present study, the 44 patients who were evaluated showed initial mean values of 2.52 (SD, 0.97) mm for CAL; 2.25 (SD, 0.74) mm for PD; and 39.00% (SD, 25.80%) for PI. When the sample was divided into healthy patients and patients with moderate to severe periodontitis, the differences in these parameters were statistically significant ($P < 0.05$; Table 1). It was not possible to identify the reasons why the patients in group 2 were most affected by periodontal disease since many social factors, and perhaps some genetic factors, may have contributed to the onset of periodontitis; these issues were not evaluated in this study. Periodontal disease is a complex chronic inflammatory disease in which dental plaque is the main etiologic factor. Plaque accumulation as a result of poor oral hygiene contributes to an increase in the number of periodontopathogenic microorganisms, since efficient oral hygiene is the only means of preventing periodontal disease.[15,16]

Treatment of periodontal disease plays an important role not only in the improvement of oral health but also in the prevention of systemic diseases. The basic principles of clinical management have been described for more than 100 years, and the efficiency of simplified models of treatment in the control of periodontal disease has been exhaustively proven.[20,21] In the present study, simple toothbrushing (modified Bass brushing technique associated with flossing) promoted improvements in all clinical periodontal parameters. There were additional effects in individuals with severe periodontal disease, in whom the combination of clinical management and mechanical periodontal instrumentation mitigated the inflammatory process. In this study, the changes were more evident from

Table 4. Mean (SD) changes (in µm) in carotid intima-media thickness in groups 1 and 2.

	Stages 1 to 2		Stages 2 to 3		Stages 1 to 3	
Measurement	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
CIMTM	–47.57 (64.31)	–53.26 (49.37)	35.83 (74.60)	35.84 (74.82)	–11.74 (74.29)	–19.05 (61.95)
CIMTU	–41.09 (87.14)	–62.32 (56.91)	37.13 (82.97)	49.16 (73.99)	–3.96 (79.14)	–14.62 (58.25)
CIMTEXP	2.17 (4.23)	3.05 (4.64)	3.87 (18.37)	5.11 (10.04)	6.04 (19.57)	8.33 (8.99)

Abbreviations: CIMTM, measured average intima-media thickness of right and left common carotid arteries; CIMTU, automated measurement of the common carotid intima-media thickness by portable ultrasound (greater value of the left and right measurements); CIMTEXP, reference value adjusted for age using the data reported by Howard et al.[13]

Stage 1, first assessment (0 months); stage 2, second assessment (6 months); stage 3, third assessment (12 months).

There are no statistically significant differences between groups 1 and 2 in changes at any stage ($P > 0.05$).

Table 5. Mean (SD) changes in carotid artery parameters in groups 1 and 2.

	Stages 1 to 2		Stages 2 to 3		Stages 1 to 3	
Parameter	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Diameter (cm)	–0.08 (0.34)	0.06 (0.42)	0.01 (0.48)	0.15 (0.49)	–0.07 (0.59)	0.17 (0.56)
Pulsatility index	0.26 (0.31)	0.23 (0.49)	–0.31 (0.29)	–0.37 (0.33)	–0.05 (0.33)	–0.15 (0.37)
Resistivity index	0.10 (0.07)	0.09 (0.07)	–0.09 (0.06)	–0.07 (0.07)	0.02 (0.05)	–0.02 (0.16)
PSV (cm/s)	10.63 (9.46)	8.61 (9.19)	–10.42 (15.58)	–8.54 (13.67)	0.21 (13.29)	–0.51 (9.43)
EDV (cm/s)	7.93 (6.12)	2.14 (0.12)	–9.46 (16.80)	–4.24 (5.42)	–1.53 (4.70)	–2.56 (4.80)

Abbreviations: EDV, end-diastolic velocity; PSV, peak systolic velocity.

Stage 1, first assessment (0 months); stage 2, second assessment (6 months); stage 3, third assessment (12 months); diameter, average diameter of the common carotid artery; pulsatility index, (PSV – EDV)/timed mean velocity; resistivity index, (PSV – EDV)/PSV.

There are no statistically significant differences between groups 1 and 2 in changes at any stage ($P > 0.05$).

stages 1 to 2, when patients received more intensive treatment and thereby obtained greater clinical effect. After 6 months, most patients received only maintenance treatment with toothbrushing and dental floss.

In the present study, there was no evident correlation of periodontal disease with results of laboratory tests. Assessment of the lipid profiles of patients with periodontitis did not demonstrate a direct correlation between regression of periodontal inflammation and positive changes in the lipid profiles, which corroborated results reported by Sridhar et al, who found no influence of periodontal status on lipid levels and that lipid levels did not correlate with loss of attachment.[22] Some authors, however, have shown elevated concentrations of LDL and VLDL and decreased HDL in patients with severe periodontitis.[23] The oxidized forms of LDL, which are more atherogenic, are also influenced by the intensity of periodontal disease.[24] Oz et al and Bresolin et al reported that the regression of periodontal inflammation promoted by mechanical treatment was beneficial to lipid metabolism, including a decrease in LDLs and an increase in HDLs.[25,26] After a systematic review, Tonetti et al concluded that, while in vitro, animal, and clinical studies support the interaction of the biological mechanisms between periodontal disease and changes in lipid metabolism, the results of interventional studies have been insufficient to allow further conclusions.[27]

C-reactive protein, produced in the liver in response to circulating cytokines, stimulates the systemic inflammatory process.[28,29] In the present study, the mean CRP, fibrinogen, and homocysteine values of groups 1 and 2 showed no statistically significant differences at baseline (Table 2). There was also no significant change over 12 months or correlation with the degree of periodontal inflammation. According to some authors, the higher the level of periodontal inflammation, the greater are the concentrations of CRP, fibrinogen, homocysteine, and white blood cells; when periodontitis is treated, the concentration of these markers decreases.[28,30-32] Patients with high levels of inflammatory markers and with high levels of type A serum amyloid, intercellular adhesion molecule 1, E-selectin, and vascular cell adhesion molecule 1 have greater chances of developing atherosclerotic disease.[17] Other authors, however, have identified individuals in whom the inflammatory response of periodontal disease was not as evident, suggesting that only some individuals respond systemically to periodontal inflammation.[32,33]

When groups 1 and 2 were analyzed—because the greatest changes in clinical periodontal data occurred from stages 1 to 2—the expected results would be a decrease in CRP, fibrinogen, and erythrocyte sedimentation rate. This did not happen in these patients. Several factors may have influenced these results. The most plausible are the existence of other inflammatory processes not identified in the clinical evaluation, the difficulty in evaluating each patient for systemic susceptibility to periodontal inflammation, and even genetic predisposition to periodontal inflammation.

Patients with periodontal disease have greater concentrations of glucose and glycated hemoglobin than healthy individuals.[34] The relationship between periodontal disease and diabetes is related to insulin resistance, which can be explained in several ways: modification of the insulin receptor, changes in adipocyte function that result in increased production of free fatty acids, decreased production of endothelial nitric oxide, and direct damage to the beta cells of the pancreas.[35] Patients in groups 1 and 2 showed similar glycemic profiles, which therefore were unrelated to the severity of periodontal disease. The fall in the proportion of glycated hemoglobin that occurred from stages 1 to 2, although statistically significant when analyzed within each group ($P < 0.05$), showed no relation to the clinical periodontal parameters by means of the linear regression model ($P > 0.05$).

Although high plasma homocysteine concentrations have been correlated with some cardiovascular diseases, it may be difficult to establish correlation with periodontal disease. The lack of correlation may be due to the large number of mutations in the enzymes that carry out metabolism and the large dietary variability in vitamin B_{12} and folic acid.[36] In the present study, plasma homocysteine levels did not differ significantly between the groups.

Patients in both groups had a significant reduction in erythrocyte and platelet counts from stages 1 to 3 of the study. The inflammatory response to periodontal disease, as already mentioned, promotes systemic changes that are dependent on the susceptibility of each individual. Agarwal et al concluded that treatment of periodontal disease increases the red blood cell count, thus reducing the anemia that may be related to chronic inflammation.[37] In contrast, the results of the present study were corroborated by Aljohani, who failed to establish a positive relationship between the severity of periodontal disease and changes in hematologic parameters.[38] In the present study both groups 1 and 2 had the same decrease in red blood cell counts from stages 1 to 3, indicating the influence of toothbrushing and flossing on periodontal status in both groups and, possibly, on hematologic parameters.

White blood cell counts are described in the literature as a marker of inflammation; however, they are nonspecific and may be influenced by factors such as stress and diet.[39] There were no statistically significant differences between the white cell counts of groups 1 and 2, nor was it possible to establish a correlation in the present study between the severity of the inflammatory process and the number of leukocytes.

Evaluation of subclinical atherosclerosis by measurement of CIMT has been performed routinely.[14,40] According to the Mannheim consensus, the greatest concern in the assessment of CIMT is the need to perform a standardized examination, reducing biases that may occur in the collection of images.[14] For this reason, the protocols for patients in groups 1 and 2 strictly followed the same routine evaluation, and, at each examination, the position of the transducer was recorded to facilitate consistent placement with minimal variation at the next review.

Beck et al correlated a CIMT greater than 1 mm to severe periodontitis with an odds ratio of 2.09 and moderate periodontitis to atherosclerotic disease with an odds ratio of 1.5.[41] Patients with higher CIMTs have a 1.2-3.2 times increase in relative risk for cardiovascular disease.[42,43] Desvarieux et al investigated the effect of the number of bacterial colonies on the CIMT of the carotid artery and found that individuals with greater periodontal bacterial load had higher CIMTs, indicating an important role of periodontal bacteria in the pathogenesis of periodontal disease and the possible relation to systemic effects.[44] In the present sample, there was significant variation in CIMTM and CIMTU from stages 1 to 2 in both groups, and a clear reduction was found in the proportion of individuals who exceeded the average values reported by Howard et al.[13] Most of the studied parameters underwent significant changes from stages 1 to 2; however, in contrast to the results reported by Beck et al, it was not possible to establish a statistically significant relationship between changes in clinical periodontal indices and values of CIMTM and CIMTU by means of a linear regression model.[41]

Apart from the initial CIMT regression in both groups from stages 1 to 2, the subjects aged 12 months over the study, and, as patients age, CIMT increases. Therefore, the results of CIMT measured after stage 2, when patients had aged, become more relevant. Because there was less variation in the periodontal parameters after stage 2, when only maintenance of periodontal status occurred without any additional interventions, aging may have had a negative influence on CIMT; this is probably why results were significant only from stages 1 to 2 and not from stages 2 to 3 or stages 1 to 3. According to Rundek et al, the most relevant traditional cardiovascular risk factor for variance in CIMT is age.[45]

Conclusion

All treatments were effective in improving clinical periodontal parameters in both groups, promoting a reduction in CIMT at 6 months, but no significant effectiveness was demonstrated with regard to lipid and blood parameters at 6 or 12 months. Data suggest that the reduction in periodontal inflammation can positively influence the measurement of common CIMT for up to 6 months of treatment.

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References

1. Jorge PA. Endothelium, lipids and atherosclerosis [in Portuguese]. *Arq Bras Cardiol.* 1997;68(2):129-134.
2. Dumitrescu AL. Influence of periodontal disease on cardiovascular diseases. *Rom J Intern Med.* 2005; 43(1-2):9-21.
3. Fischer RG. Doença periodontal e doenças cardiovasculares. In: Paiva JS, Almeida RV, eds. *Periodontia: A Atuação Clínica Baseada em Evidências Científicas.* Vol 1. São Paulo: Artes Médicas; 2005: 285-289.
4. World Health Organization. *World Health Statistics 2013.* Geneva: World Health Organization; 2013. http://www.who.int/gho/publications/world_health_statistics/EN_WHS2013_Full.pdf. Accessed November 25, 2015.
5. Piconi S, Trabattoni D, Luraghi C, et al. Treatment of periodontal disease results in improvements in endothelial dysfunction and reduction of the carotid intima-media thickness. *FASEB J.* 2009;23(4):1196-1204.
6. Beck JD, Offenbacher S. The association between periodontal diseases and cardiovascular diseases: a state-of-the-science review. *Ann Periodontol.* 2001; 6(1):9-15.
7. Genco R, Offenbacher S, Beck J. Periodontal disease and cardiovascular disease: epidemiology and possible mechanisms. *J Am Dent Assoc.* 2002;133(suppl):14S-22S.
8. Katz J, Flugelman MY, Goldberg A, Heft M. Association between periodontal pockets and elevated cholesterol and low density lipoprotein cholesterol levels. *J Periodontol.* 2002;73(5):494-500.
9. Hung HC, Willett W, Merchant A, Rosner BA, Ascherio A, Joshipura KJ. Oral health and peripheral arterial disease. *Circulation.* 2003;107(8):1152-1157.
10. Lu B, Parker D, Eaton CB. Relationship of periodontal attachment loss to peripheral vascular disease: an analysis of NHANES 1999-2002 data. *Atherosclerosis.* 2008;200(1):199-205.
11. Silness J, Löe H. Periodontal disease in pregnancy, 2: correlation between oral hygiene and periodontal condition. *Acta Odontol Scand.* 1964;22:121-135.
12. Löe H, Silness J. Periodontal disease in pregnancy, 1: prevalence and severity. *Acta Odontol Scand.* 1963;21: 533-551.
13. Howard G, Sharrett AR, Heiss G, et al. Carotid artery intimal-medial thickness distribution in general populations as evaluated by B-mode ultrasound. ARIC Investigators. *Stroke.* 1993;24(9):1297-1304.
14. Touboul PJ, Hennerici MG, Meairs S, et al. Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. *Cerebrovasc Dis.* 2007;23(1):75-80.
15. Balan H. Do cardio-vascular and periodontal diseases have a close, causal relationship? *Rom J Intern Med.* 2010;48(2):121-129.
16. Paizan ML, Martin JFV. Association between periodontal disease and hypertension [in Portuguese]. *Rev Bras Hipertens.* 2009;16(3):183-185.
17. Ramírez JH, Arce RM, Contreras A. Periodontal treatment effects on endothelial function and cardiovascular disease biomarkers in subjects with chronic periodontitis: protocol for a randomized clinical trial. *Trials.* 2011;12:46.
18. Kumpel C, Porto EF, Castro AAM, Leite JRO, Margarida U, Oliveira EFS. Clinical characteristics of smoking related to assist elderly persons by PSF [in Portuguese]. *Rev Bras Med.* 2009;67(6):208-213.
19. Hefti AF. Periodontal probing. *Crit Rev Oral Biol Med.* 1997;8(3):336-356.
20. Nassar PO, Bombardelli CG, Walker CS, et al. Periodontal evaluation of different toothbrushing techniques in patients with fixed orthodontic appliances. *Dental Press J Orthod.* 2013;18(1):76-80.
21. Zingale J, Harpenau L, Bruce G, Chambers D, Lundergan W. The effectiveness of scaling and root planing with adjunctive time-release minocycline using an open and closed approach for the treatment of periodontitis. *Gen Dent.* 2012;60(4):300-305.
22. Sridhar R, Byakod G, Pudakalkatti P, Patil R. A study to evaluate the relationship between periodontitis, cardiovascular disease and serum lipid levels. *Int J Dent Hyg.* 2009;7(2):144-150.
23. Wu T, Trevisan M, Genco RJ, Falkner KL, Dorn JP, Sempos CT. Examination of the relation between periodontal health status and cardiovascular risk factors: serum total and high density lipoprotein cholesterol, C-reactive protein, and plasma fibrinogen. *Am J Epidemiol.* 2000;151(3):273-282.
24. Tilakaratne A, Soory M. Osteoblastic responses to LPS, glucose-oxidised LDL and minocycline: therapeutic targets for periodontal and cardiometabolic diseases. *Recent Pat Endocr Metab Immune Drug Discov.* 2012;6(1):73-84.
25. Oz SG, Fentoglu O, Kilicarslan A, et al. Beneficial effects of periodontal treatment on metabolic control of hypercholesterolemia. *South Med J.* 2007;100(7):686-691.
26. Bresolin AC, Pronsatti MM, Pasqualotto LN, et al. Lipid profiles and inflammatory markers after periodontal treatment in children with congenital heart disease and at risk for atherosclerosis. *Vasc Health Risk Manag.* 2013;9:703-709.
27. Tonetti MS, Van Dyke TE; Working Group 1 of the Joint EFP/AAP workshop. Periodontitis and atherosclerotic cardiovascular disease: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *J Clin Periodontol.* 2013; 40(Suppl 14):S24-S29.
28. de Oliveira C, Watt R, Hamer M. Toothbrushing, inflammation, and risk of cardiovascular disease: results from Scottish Health Survey. *BMJ.* 2010;340:c2451.
29. Volp AC, Alfenas Rde C, Costa NM, Minim VP, Stringueta PC, Bressan J. Inflammation biomarkers capacity in predicting the metabolic syndrome [in Portuguese]. *Arq Bras Endocrinol Metabol.* 2008;52(3):537-549.
30. Vidal F, Figueredo CM, Cordovil I, Fischer RG. Periodontal therapy reduces plasma levels of interleukin-6, C-reactive protein, and fibrinogen in patients with severe periodontitis and refractory arterial hypertension. *J Periodontol.* 2009;80(5):786-791.
31. D'Aiuto F, Parkar M, Andreou G, Brett PM, Ready D, Tonetti MS. Periodontitis and atherogenesis: causal association or simple coincidence? *J Clin Periodontol.* 2004;31(5):402-411.
32. Mattila K, Vesanen M, Valtonen V, et al. Effect of treating periodontitis on C-reactive protein levels: a pilot study. *BMC Infect Dis.* 2002;2:30.
33. Bezerra CFR, Amaral BA, Camelo FP, Aquino ARL, Seabra EG, Carreiro AFP. The C-reactive protein role in the relationship between periodontal and cardiovascular diseases [in Portuguese]. *R Periodontia.* 2008;18(1):7-13.
34. Rajan P, Nera M, Pavalura AK, Medandrao N, Kumar SC. Comparison of glycosylated hemoglobin (HbA_{1C}) levels in patients with chronic periodontitis and healthy controls. *Dent Res J (Isfahan).* 2013;10(3): 389-393.
35. Southerland JH, Taylor GW, Offenbacher S. Diabetes and periodontal infection: making the connection. *Clin Diabetes.* 2005;23(4):171-178.
36. Helfand M, Buckley DI, Freeman M, et al. Emerging risk factors for coronary heart disease: a summary of systematic reviews conducted for the U.S. Preventive Services Task Force. *Ann Intern Med.* 2009;151(7): 496-507.
37. Agarwal N, Kumar VS, Gujjari SA. Effect of periodontal therapy on hemoglobin and erythrocyte levels in chronic generalized periodontitis patients: an interventional study. *J Indian Soc Periodontol.* 2009; 13(1):6-11.
38. Aljohani HA. Association between hemoglobin level and severity of chronic periodontitis. *JKAU Med Sci.* 2010;17(1):53-64.
39. Al-Rasheed A. Elevation of white blood cells and platelet counts in patients having chronic periodontitis. *Saudi Dent J.* 2012;24(1):17-21.
40. Stein JH, Korcarz CE, Hurst RT, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *J Am Soc Echocardiogr.* 2008;21(2):93-111.
41. Beck JD, Elter JR, Heiss G, Couper D, Mauriello SM, Offenbacher S. Relationship of periodontal disease to carotid artery intima-media wall thickness: the Atherosclerosis Risk in Communities (ARIC) Study. *Arterioscler Thromb Vasc Biol.* 2001;21(11):1816-1822.
42. Den Ruijter HM, Peters SA, Anderson TJ, et al. Common carotid intima-media thickness measurements in cardiovascular risk prediction: a meta-analysis. *JAMA.* 2012;308(8):796-803.
43. Hodis HN, Mack WJ, LaBree L, et al. The role of carotid arterial intima-media thickness in predicting clinical coronary events. *Ann Intern Med.* 1998;128(4):262-269.
44. Desvarieux M, Demmer RT, Rundek T, et al. Periodontal microbiota and carotid intima-media thickness: the Oral Infections and Vascular Disease Epidemiology Study (INVEST). *Circulation.* 2005;111(5):576-582.
45. Rundek T, Blanton SH, Bartels S, et al. Traditional risk factors are not major contributors to the variance in carotid intima-media thickness. *Stroke.* 2013;44(8): 2101-2108.