Severe Chronic Periodontitis Is Associated With Endothelial and Microvascular Dysfunctions: A Pilot Study

Ronaldo Lira-Junior,* Carlos Marcelo Figueredo,* Eliete Bouskela,† and Ricardo Guimarães Fischer*

Background: Periodontitis is an inflammatory chronic disease that has been implicated as a risk factor for cardiovascular disease (CVD). Endothelium has a central role in CVD pathogenesis, and chronic inflammation can make it dysfunctional, contributing to CVD emergence. Thus, the aim of this study is to investigate the existence of an association between severe chronic periodontitis (CP) and nailfold microvascular, gingival microvascular, and endothelial functions.

Methods: Twenty-three patients were included, 13 with severe periodontitis (median age, 46 years; interquartile range, 9.5 years) and 10 healthy control patients (median age, 35.5 years; interquartile range, 12.5 years). Clinical and laboratorial variables were gathered, and patients were examined by the following: 1) nailfold videocapillaroscopy to assess functional capillary density (FCD), capillary diameters, red blood cell velocity at rest (RBCV) and after 1-minute arterial occlusion ($RBCV_{max}$), and time taken to reach $RBCV_{max}$ ($TRBCV_{max}$); 2) side-stream dark-field imaging to determine gingival capillary density (GCD); and 3) venous occlusion plethysmography to assess endothelium-dependent (% Hyper) and endothelium-independent vasodilatation (% Nitro).

Results: Patients with CP have smaller values for FCD, RBCV, $RBCV_{max}$, and % Hyper and higher values for $TRBCV_{max}$ and GCD compared with controls ($P<0.05$). There were significant correlations between periodontal parameters with FCD, RBCV, $RBCV_{max}$, $TRBCV_{max}$, GCD, and % Hyper. There was also a negative correlation between FCD and GCD ($r = -0.7$; $P < 0.01$). Associations between periodontitis and FCD, $RBCV_{max}$, $TRBCV_{max}$, GCD, and % Hyper remained significant after adjustments for age and systolic blood pressure.

Conclusion: Severe CP was directly associated with endothelial and microvascular dysfunctions. *J Periodontol 2014; 85:1648-1657.*

KEY WORDS

Capillaries; cardiovascular diseases; microcirculation; periodontitis; vasodilation.

* Department of Periodontology, Faculty of Odontology, Rio de Janeiro State University, Rio de Janeiro, Rio de Janeiro, Brazil.
† Clinical and Experimental Research Laboratory on Vascular Biology, Rio de Janeiro State University.

Periodontal disease is a chronic inflammatory disease that affects tooth-supporting tissues. It begins as gingivitis and, if left untreated, can evolve to periodontitis, in which connective tissue and bone breakdown can lead to tooth loss.[1] According to National Health and Nutrition Examination Survey data, >47% of the adult U.S. population presented periodontitis, with 8.5% having the severe form.[2] Atherosclerosis is a slow and progressive, chronic inflammatory disease, resulting from a group of specific cellular and molecular responses that leads to lipid and fibrous elements storage in large arteries.[3] It represents an important factor in the growing burden of cardiovascular diseases (CVDs).[3]

Epidemiologic data provided evidence that severe periodontitis is a risk factor to CVD,[4,5] and endothelial dysfunction is the initial marker in the development of atherosclerosis, leading to CVD.[6] Several studies showed an association between periodontitis and endothelial dysfunction, highlighting a possible causal relationship between atherosclerosis and periodontitis.[7-11] The effect of periodontal treatment on endothelial function was also evaluated, showing that its treatment reverses endothelial dysfunction frame associated with periodontitis.[8-12]

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Microcirculation comprises vessels <100 μm in mean internal diameter, including arterioles, capillaries, and venules.[13,14] Its main function is to optimize oxygen and nutrient supply within tissues in response to variations in demand, besides removing waste products. Another important function is to avoid large fluctuations in hydrostatic pressure at the capillary level, which cause disturbances in capillary exchange.[13-15] The microcirculation is the site at which the earliest manifestations of CVD occur, in particular the inflammatory processes, even before the onset of clinical symptoms of the disease.[13,16]

Microvascular dysfunction is a systemic process that occurs in a similar manner in multiple tissue beds,[17] and skin microcirculation offers the possibility to explore non-invasively the relationship among microvascular dysfunction, CVD, and its risk factors.[18,19] Changes in tissue perfusion attributable to microvascular system abnormalities are common among several cardiovascular risk factors.[20,21] Detecting impaired microvascular blood flow and vasoreactivity can lead to early identification of patients at risk for peripheral vascular and coronary artery disease.[16,19] To the best of the authors' knowledge, there are no data in the literature evaluating microvascular functions in patients with periodontitis, and therefore, the aim of the present study is to evaluate endothelial and microvascular functions in patients with severe chronic periodontitis (CP).

MATERIALS AND METHODS

Study Population

This is a cross-sectional study including patients with severe CP (test group, n = 13; six males and seven females, aged 34 to 56 years; mean age, 45.7 years; interquartile range, 9.5 years; seven females), as well as patients without periodontitis (control group, n = 10; three males and seven females, aged 30 to 48 years; mean age 37.1 years; interquartile range, 12.5 years; seven females) selected from the outpatient dental clinic at Rio de Janeiro State University. Patients were aged ≥30 years. Severe CP was defined when the patient presented at least two interproximal sites with clinical attachment loss (AL) ≥6 mm, not in the same tooth, and probing depth (PD) ≥5 mm in one or more interproximal sites, excluding third molars.[22] Control patients had no AL. Exclusion criteria were as follows: 1) the presence of CVD, diabetes mellitus, hypertension, obesity, smoking habit, hypercholesterolemia, or any other systemic disease that could alter the course of periodontal disease or cause microvascular disturbances; 2) pregnancy, lactation, or postmenopausal status; 3) use of anti-inflammatory, antibiotic, vasoactive, or hypolipidemic medications; or 4) use of antibiotics in the preceding 3 months or having received periodontal therapy in the preceding 6 months. The study was reviewed and approved by the Ethics Committee in Research of Pedro Ernesto University Hospital of the Rio de Janeiro State University, and all patients gave their written informed consent.

Medical History and Physical Examinations

Each patient underwent an interview and physical examination. The information gathered included the following: 1) sex; 2) age; 3) ethnicity; 4) socioeconomic status; 5) presence of systemic diseases; 6) family CVD history; 7) medications; 8) regular physical activities; 9) previous periodontal treatment; 10) smoking habit; 11) blood pressure (BP); and 12) anthropometric measurements. Body mass index (BMI), defined as the ratio of body weight (in kilograms) by the square of height (in meters), waist circumference (in centimeters), and waist-to-hip ratio were determined. Systolic and diastolic BP were checked after 5 minutes of acclimatization and resulted from the average of two measurements. Ethnicity was evaluated as white and non-white. Socioeconomic status was assessed using a combined measure of family economy, according to Brazilian economy criteria and the patient's education level as described by Susin et al.,[23] resulting in three statuses: 1) high; 2) medium; and 3) low.

Periodontal Examination

Patients underwent a comprehensive periodontal examination including the following: 1) PD; 2) AL; 3) bleeding on probing (BOP); and 4) visible plaque index (VPI). PD and AL were determined at six sites per tooth, excluding third molars, and BOP and VPI in four sites per tooth. A single calibrated examiner (RLJ) using a manual probe‡ performed all clinical examinations. Five patients were assessed twice, with at least a 1-hour interval between assessments to observe intraexaminer concordance, and 95% and 94% of concordance to PD and AL within 1 mm was obtained, respectively.

Nailfold Videocapillaroscopy

All patients were acclimatized for 10 minutes in a temperature-controlled room (22°C to 24°C) before the examination. Patients were seated in a fixed chair with the left upper arm raised at heart level. The fourth finger was supported on an acrylic base 2 cm above the palm level, mounted on the *x-y* stage of a three-eyepiece microscope,§ equipped with an epi-illumination system (100 W xenon lamp). The fingertip was fixed to the acrylic base by a metal loop to minimize movements. The skin temperature of the finger was monitored throughout the examination with a digital thermometer‖ with the thermistor probe taped within

‡ UNC-15, Hu-Friedy, Chicago, IL.
§ Leica DM LM microscope, Leica, Wetzlar, Germany.
‖ YSI Precision 4000A thermometer, YSI, Dayton, OH.

1 cm proximal to the nailfold. A pressure cuff (1 cm wide) was placed around the proximal phalanx and connected to a mercury manometer. A drop of mineral oil was spread over the observation site to improve image quality. Microcirculatory images were recorded and saved on digital video discs using a closed-circuit TV.

Functional capillary density (FCD), defined as the number of capillaries per square millimeter with flowing red blood cells (RBCs), was assessed using a ×250 magnification in an area of 3 mm of the distal row of capillaries into three different areas. Afferent (AF), apical (AP), and efferent (EF) capillary diameters and RBC velocity at rest (RBCV) were assessed at a magnification of ×680. After 1-minute ischemia, with cuff pressure above systolic BP, the maximal RBCV during the reactive hyperemia response ($RBCV_{max}$), and time taken to reach it ($TRBCV_{max}$) were measured, also at a final magnification of ×680. The relation of $RBCV_{max}$/RBCV was determined to observe precapillary dilatation from baseline condition.

Capillary diameters and RBCV were measured three times each. After ischemia, each variable was assessed once. The analysis of microvascular parameters was performed using a video image analysis system[24] by a single examiner (EB) who was not aware of the patients' data. Five patients were evaluated twice, and the interassay coefficient of variation (IACV) ranged from 3.2% to 10.5% for measured parameters.

Side-Stream Dark-Field Imaging

Gingival microcirculation was evaluated using a video microscope[¶] with side-stream dark-field technology. In the test group, the area most affected by the disease, i.e., greater PD, on the buccal aspect, attributable to visibility and accessibility, was evaluated. In the control group, the chosen area was set according to the evaluated area in the test group to seek balance between groups. Examinations were performed with patients comfortably seated, with care taken not to apply pressure on the mucosa and after saliva removal with gauze.

The analyzed area was set between the gingival margin and interdental papilla, because of the microvascular characteristics in this site and to standardize the evaluation. Three images per patient were captured and used to calculate gingival capillary density (GCD). A single examiner (RLJ) performed all examinations and analysis. Five patients were evaluated twice, and IACV was 3% to GCD.

Venous Occlusion Plethysmography

Patients were kept in a temperature-controlled room (20°C to 22°C). Forearm blood flow (FBF), in milliliters per minute per 100 mL tissue, was measured using a plethysmograph[#] in the non-dominant forearm, kept above the heart level, with a strain gauge placed on the upper third of the forearm at maximum circumference. Three electrodes were placed on the precordium region to evaluate heart rate. BP was measured in the dominant arm.

Venous collecting pressure was set to 50 mm Hg to avoid venous return, and wrist cuff occlusion, to avoid hand shunt, was set to 40 mm Hg above systolic BP. FBF was measured in four phases: 1) initial flow 1; 2) after reactive hyperemia; 3) the second flow; and 4) flow 5 minutes after 0.4 mg sublingual nitroglycerin.[**] The reactive hyperemia response was induced by inflating a cuff, 50 mm Hg above systolic BP, placed around the upper arm for 5 minutes. The percentage increase in blood flow during the reactive hyperemia response related to initial flow (% Hyper) and its increase after nitroglycerin related to second flow (% Nitro) were calculated. There was a 15-minute interval between the reactive hyperemia response and the second flow measurement. The mean of the first four measurements in each recording period was used for analysis. Furthermore, post-ischemic peak flow (first measurement) and its relation to initial flow 1 (peak flow) were determined. Five patients were evaluated twice, and IACV was <10% in all evaluated cycles.

Laboratorial Analyses

Venous blood samples were collected in the morning after a 12-hour fasting period and evaluated using routine biochemical procedures. Total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol were measured by colorimetric method. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. Glucose and insulin levels were measured by hexokinase enzymatic and chemiluminescent methods, respectively. Glycated hemoglobin was obtained by high-performance liquid chromatography. Fibrinogen was measured by the automatized Clauss method. High-sensitivity C-reactive protein (hsCRP) determination was analyzed by turbidimetry.

Statistical Analyses

Data were analyzed using statistical software.[††] Data normality was verified with the Kolmogorov–Smirnov test. Continuous variables are presented as median (interquartile range), and categorical variables are presented as frequencies.

Comparisons between the two groups were performed using Mann–Whitney, χ^2, or Fisher exact tests

¶ Microscan, MicroVision Medical, Amsterdam, The Netherlands.
# AI6 Automated Strain Gauge Plethysmography System, Hokanson, Bellevue, WA.
** Nitrolingual, BurnsAdler Pharmaceuticals, Charlotte, NC.
†† SPSS v.19.0, IBM, Armonk, NY.

Table 1.

Medical and Demographic Characteristics of the Test and Control Groups

Variables	Test Group (n = 13)	Control Group (n = 10)	*P*
Age (years)	46.0 (9.5)	35.5 (12.5)	0.01*
Sex (male/female)	6/7	3/7	0.67†
Ethnicity (white/non-white)	7/6	5/5	0.86‡
Socioeconomic status (high/medium/low)	0/4/9	2/3/5	0.37†
Regular physical activity (yes/no)	1/12	2/8	0.56†
CVD family history (yes/no)	4/9	3/7	0.66†
BMI (kg/m^2)	24.2 (4.85)	25.4 (8.25)	0.71*
Systolic BP (mm Hg)	124 (17.00)	109 (6.75)	0.005*
Diastolic BP (mm Hg)	74.0 (10.50)	66.5 (8.25)	0.01*
Heart rate (bpm)	66.30 (10.45)	68.45 (9.58)	0.42*
Waist (cm)	85.0 (16.5)	87.0 (16.25)	0.73*
Waist-to-hip ratio	0.91 (0.08)	0.90 (0.04)	0.80*

bpm = beats per minute.
Continuous data are presented as median (interquartile range), and categorical data are presented as absolute frequency.
* Mann–Whitney test.
† Fisher exact test.
‡ χ^2 test.

whenever appropriate. Analysis of covariance (with log transformation when appropriate) was performed to evaluate the influence of possible confounding factors on associations between severe CP and endothelial and microvascular functions. Adjusted means and 95% confidence interval (95% CI) were calculated. Statistical significance was set at 0.05.

To detect a mean difference in FCD of 3.5 ± 2.0 capillaries/mm^2, with $\alpha = 0.05$, 10 patients per group should be included to achieve 80% of power.

RESULTS

Twenty-three patients participated in the study, 13 from the test group and 10 controls. The characteristics of the study population are described in Table 1. Patients in the test group were significantly older than controls ($P < 0.05$), and, although within the normal range, systolic and diastolic BP were significantly higher in the test group ($P < 0.05$). Clinical periodontal parameters are presented in Table 2, and, as expected, in the test group median percentages of VPI and BOP were higher, and the number of teeth, median percentages of PD ≤3 mm, and AL of 0 to 2 were lower ($P < 0.05$) compared with the control group.

Nailfold microcirculation analysis revealed that patients in the test group have lower FCD, RBCV, and $RBCV_{max}$ and higher $TRBCV_{max}$ when compared with controls ($P < 0.05$; Table 3). Median GCD was higher ($P < 0.05$; Table 3) and % Hyper, post-ischemic peak flow, and peak flow were lower ($P < 0.05$; Table 3) in the test group compared with the control group. There were no significant differences in laboratorial

Table 2.

Clinical Periodontal Parameters in the Test and Control Groups

Variables	Test Group (n = 13)	Control Group (n = 10)	*P**
Number of teeth	23 (8.5)	27 (2.0)	0.01
VPI	83.0 (28.9)	15.7 (8.2)	0.001
BOP	83.0 (30.8)	13.7 (2.5)	0.001
PD			
≤3 mm	77.6 (14.5)	100	0.001
4 to 5 mm	13.6 (5.5)	—	—
≥6 mm	8.8 (9.2)	—	—
AL			
0 to 2 mm	77.6 (12.7)	100	0.001
3 to 4 mm	12.9 (6.3)	—	—
≥5 mm	9.5 (8.8)	—	—

Data are presented as median (interquartile range).
* Mann–Whitney test.

Table 3.

Nailfold and Gingival Microcirculation and Endothelial Function in the Test and Control Groups

Variables	Test Group (n = 13)	Control Group (n = 10)	*P**
Nailfold microcirculation			
FCD (n/mm^2)	6.01 (0.95)	8.86 (2.09)	0.001
AF (μm)	9.7 (2.7)	10.9 (3.6)	0.25
EF (μm)	12.5 (3.1)	13.0 (2.8)	>0.99
AP (μm)	14.2 (3.0)	15.1 (5.2)	0.84
RBCV (mm/second)	0.599 (0.216)	0.747 (0.085)	0.04
$RBCV_{max}$ (mm/second)	0.811 (0.237)	0.976 (0.062)	0.001
$TRBCV_{max}$ (seconds)	8.6 (2.4)	5.2 (0.4)	0.001
$RBCV_{max}$/RBCV	1.14 (0.4)	1.25 (0.1)	0.39
Gingival microcirculation			
GCD (n)	70.1 (12.1)	56.6 (10.1)	0.001
Endothelial function			
% Hyper	280.89 (156.45)	328.10 (118.04)	0.047
% Nitro	102.07 (29.17)	111.79 (11.28)	0.11
Post-ischemic peak flow	8.46 (3.87)	11.27 (3.47)	0.048
Peak flow	4.0 (3.1)	6.4 (3.1)	0.02

Data are presented as median (interquartile range).
* Mann–Whitney test.

Table 4.

Laboratorial Parameters in Patients With Periodontitis and Healthy Controls

Variables	Test Group (n = 13)	Control Group (n = 10)	*P**
Glucose (mg/dL)	93 (5.3)	89 (12.0)	0.39
Insulin (μUI/mL)	8.6 (3.1)	7.0 (2.4)	0.07
Glycated hemoglobin (%)	5.6 (0.5)	5.6 (1.0)	0.94
Total cholesterol (mg/dL)	191 (18.0)	173 (58.0)	0.11
HDL (mg/dL)	56 (15.0)	57 (19.0)	0.68
LDL (mg/dL)	123 (33.0)	139 (61.5)	0.71
Triglycerides (mg/dL)	84 (34.2)	104 (39.0)	0.84
Fibrinogen (mg/dL)	298.0 (68.0)	256.5 (92.5)	0.56
hsCRP (mg/dL)	1.85 (3.92)	1.28 (1.89)	0.73

Data are presented as median (interquartile range).
* Mann–Whitney test.

parameters between the test and control groups ($P > 0.05$; Table 4).

Correlational analysis revealed significant correlations between clinical periodontal parameters and FCD, RBCV, $RBCV_{max}$, $TRBCV_{max}$, and GCD (Table 5). VPI, BOP, and PD showed significant negative correlations with % Hyper (Table 5). There was a strong negative correlation between FCD and GCD ($r = -0.7$; $P < 0.01$). Age reached significant correlations only with $TRBCV_{max}$ ($r = 0.461$; $P < 0.05$). There were significant correlations between systolic BP and FCD ($r = -0.465$; $P < 0.05$), RBCV ($r = -0.572$; $P < 0.05$), $RBCV_{max}$ ($r = -0.649$; $P < 0.01$), and $TRBCV_{max}$ ($r = 0.705$; $P < 0.01$). Diastolic BP showed significant correlations with RBCV ($r = -0.625$; $P < 0.01$), $RBCV_{max}$ ($r = -0.571$; $P < 0.05$), and $TRBCV_{max}$ ($r = 0.504$; $P < 0.05$). Insulin reached significant correlation

Table 5.

Correlational Analysis Among Periodontal, Laboratorial, Microvascular, and Endothelial Variables

	VPI (%)	BOP (%)	PD (mm)	AL (mm)	hsCRP
FCD	–0.795†	–0.772†	–0.737†	–0.789†	0.158
RBCV	–0.595†	–0.642†	–0.533*	–0.486*	–0.262
$RBCV_{max}$	–0.733†	–0.742†	–0.634†	–0.582†	0.141
$TRBCV_{max}$	0.763†	0.743†	0.734†	0.662*	–0.334
GCD	0.583†	0.508†	0.625†	0.712†	–0.133
% Hyper	–0.524*	–0.416†	–0.543†	–0.341	–0.138
Post-ischemic peak flow	–0.389	–0.390	–0.437*	–0.373	0.452
Peak flow	–0.558†	–0.570†	–0.555†	–0.326	0.054

Data are presented as Spearman correlation coefficient.
* $P < 0.05$.
† $P < 0.01$.

Table 6.

Comparisons of Microvascular and Endothelial Parameters Between the Test and Control Groups After Adjustments for Potential Confounding Factors

Models*	Test Group (n = 13)	Control Group (n = 10)	P*
FCD adjusted	5.89 (5.08 to 6.69)	10.04 (9.12 to 10.96)	0.001
RBCV adjusted	0.678 (0.583 to 0.772)	0.712 (0.593 to 0.830)	0.69
$RBCV_{max}$ adjusted	0.791 (0.712 to 0.870)	0.983 (0.884 to 1.08)	0.02
$TRBCV_{max}$ adjusted	7.95 (7.06 to 8.83)	5.50 (4.39 to 6.61)	0.007
GCD adjusted	72.9 (68.5 to 77.4)	59.3 (54.1 to 64.6)	0.001
% Hyper adjusted	237.83 (179.8 to 295.7)	344.78 (276.5 to 413.0)	0.04
Post-ischemic peak flow adjusted	7.70 (6.08 to 9.32)	12.16 (10.35 to 13.98)	0.003
Peak flow adjusted	3.98 (2.57 to 5.38)	7.12 (5.54 to 8.69)	0.01

Data are presented as adjusted mean (95% CI).
* Adjusted for age and systolic BP.

with $TRBCV_{max}$ ($r = 0.669$; $P < 0.01$) and GCD ($r = 0.669$; $P < 0.01$).

Except for RBCV, associations between periodontitis and endothelial (% Hyper, post-ischemic peak flow, and peak flow) and microvascular (FCD, $RBCV_{max}$, $TRBCV_{max}$, and GCD) variables remained essentially unchanged after controlling for possible confounding factors, age, and systolic BP (Table 6).

DISCUSSION

This study is designed to investigate the existence of an association between severe CP and nailfold and gingival microvascular and endothelial functions, and the present results indicate that periodontitis was associated with endothelial and microvascular dysfunctions. These associations remained significant even after adjustments to age and systolic BP. Impairments of cutaneous microvascular function were shown in hypertension,[25] diabetes,[26] obesity,[27] coronary artery disease,[28] and others.

Nailfold videocapillaroscopy is a non-invasive method used to assess patients' microcirculation in vivo, and its results were already associated with cardiovascular risk.[19] To the best of the authors'

knowledge, this is the first time an impairment of cutaneous microvascular function, evaluated by nailfold videocapillaroscopy, in patients with severe CP is reported.

Patients with periodontitis did not present changes in capillary morphology. However, they showed reduced FCD and $RBCV_{max}$ and longer $TRBCV_{max}$ when compared with healthy control patients, pointing to microvascular function impairment at rest and after physiologic stimuli. These microcirculatory impairments affect capillary perfusion and blood-flow patterns.[27] According to the present results, there are strong negative correlations between periodontal parameters and FCD. Lower capillary density reduces the available surface area to oxygen delivery, increases diffusion distance between microvessel and target cell and thus diffusion time, and contributes to the increase in the total resistance of the capillary bed.[13] Capillary rarefaction may result from oxidative stress and nitric oxide inactivation[20] and may impair muscle metabolism and perfusion.[29] D'Aiuto et al.[30] described an increased oxidative stress and decreased antioxidant capacity in patients with severe periodontitis.

Microcirculatory hemodynamic behavior was observed through measurements of RBCV before and after 1-minute arterial occlusion and reperfusion time. The post-occlusive reactive hyperemia response is thought to happen at the level of small arterioles[31] and to be independent of the autonomic nervous system.[32] This response depends on endothelial and smooth-muscle cell reactivity and the amount of reactive oxygen species and metabolites produced. After arterial occlusion release, followed by an increase in perfusion pressure to an ischemic area, strong constriction at the arteriolar level may occur for a short period, the so-called myogenic response, followed by a sharp increase of blood flow to the tissue that gradually returns to resting level. Therefore, the degree of dilatation, which influences $RBCV_{max}$ and $TRBCV_{max}$, depends on the interplay between vasoconstrictor (myogenic response and reactive oxygen species) and vasodilator (nitric oxide and others dilator agents) stimuli.[33]

The present data show an association between periodontitis and impaired $RBCV_{max}$ and $TRBCV_{max}$, indicating that microvascular autoregulatory responses are altered in patients with periodontitis. There was also a significant correlation between clinical periodontal parameters and the aforementioned variables, showing that increases in VPI, BOP, PD, and AL resulted in $RBCV_{max}$ reduction and $TRBCV_{max}$ increase. A defective microvascular myogenic response to an intraluminal pressure increase suddenly after arterial occlusion could lead to decreased maximum erythrocyte velocity and longer $TRBCV_{max}$.[33] Furthermore, changes in mediators (nitric oxide, adenosine, endothelium-derived hyperpolarizing factor, and others) that affect arterial occlusion response may contribute to the observed findings. Periodontitis was associated with a decreased nitric oxide bioavailability and increased amount of reactive oxygen species.[10,11,30]

Other factors may contribute to microvascular alterations observed in the present study. RBCs are used to visualize the capillary wall, and thus an increase in blood viscosity may affect RBCV.[34] RBC deformability and hematocrit are also factors that may compromise microvascular function.[35] Although these parameters are evaluated in this study, results from other investigations are conflicting, showing similar values[36] or a decrease[37] in hematocrit values in patients with periodontitis. Blood rheologic properties should be further investigated in periodontitis.

Although the functional consequences of these microvascular abnormalities in patients with severe CP are unknown, it may be speculated that they might contribute to changes in BP and glucose metabolism frequently observed in these patients. Tsakos et al.[38] showed higher systolic BP in patients with periodontitis. Significant associations were found between periodontitis and hypertension.[38,39] Antonios et al.[40] and Serné et al.[25] demonstrated that hypertension was associated with capillary rarefaction. Penna et al.[41] found a reduction in FCD and RBCV in hypertensive patients compared with normotensive patients. Moreover, significant correlations were demonstrated between functional microvascular parameters and systolic BP.[33,42,43] In agreement with these results, correlations were also found between functional microvascular parameters and systolic BP.

Evidence has also pointed to an association between increased plasmatic glucose levels,[44] abnormal glucose metabolism,[45,46] poor glycemic control in patients with diabetes,[47] and periodontitis. Irving et al.[42] and Czernichow et al.[48] presented a negative correlation between plasmatic glucose level and capillary density. Correlations were also found between functional microvascular parameters and insulin resistance.[27,43] In this study, a significant correlation was found between insulin and reperfusion time. Pazos-Moura et al.[26] found a reduction in $RBCV_{max}$ and an increase in reperfusion time in patients with diabetes. Accordingly, tissue perfusion impairment, which is dependent on FCD and blood velocity and attributable to microvascular abnormalities, is common among cardiovascular risk factors, and microvascular changes resulting from one risk factor may predispose to the emergence of another one.[20] Thereby, it is attractive to infer

that microvascular dysfunction might be part of the pathophysiologic basis for the associations between periodontitis and the mentioned pathologic conditions, increasing cardiovascular risk in these patients.

Regarding capillary gingival density, patients with periodontitis presented a significantly higher number of capillaries when compared with control patients with no periodontitis. Furthermore, there were significant correlations between VPI ($r = 0.583$; $P<0.01$), BOP ($r = 0.508$; $P<0.05$), PD ($r = 0.625$; $P<0.01$), and AL ($r = 0.712$; $P<0.01$) with GCD. Vascular remodeling that occurs in periodontitis was documented, indicating vascular tissue expansion with disease development. Bonakdar et al.[49] and Chapple et al.[50] showed a significant increase in the number of blood vessels in the gingival tissue of patients with CP. These results are in agreement with the present findings. Mechanisms responsible for that increase in the number of vessels are still not clarified, and it was speculated that it may be caused by an increase in the number of open vessels, in length of blood vessels, or formation of new vessels.[49] Altered angiogenic activity was described in diseased periodontal tissue.[50]

An interesting finding of the present study was the negative correlation between GCD and FCD ($r = –0.7$; $P<0.01$). Because GCD seems to be a reflection of the severe chronic inflammatory process in the periodontal environment and, thus, of periodontitis, this correlation endorses the observed association between periodontitis and FCD.

This study also revealed a significant decrease in endothelium-dependent vascular reactivity in resistance vessels in patients with periodontitis, confirming results of videocapillaroscopy. The existence of an association between periodontitis and endothelial dysfunction was reported, but, in general, this association was assessed in the brachial artery, i.e., a conduit vessel.[7-9] Other studies found similar results in resistance vessels evaluating FBF in response to intra-arterial infusion of acetylcholine and sodium nitroprusside. Endothelial dysfunction could result from reduced nitric oxide bioavailability.[10,11] Similar results were found using a non-invasive method showing impairment of post-ischemic peak flow and peak flow in patients with periodontitis.

The cross-section design of this study does not allow for inferring causality about evaluated variables. In addition, the small sample size limits the drawing of definitive conclusions about observed associations. Patients with periodontitis were significantly older and presented a significant increase, although within normal range, in BP. However, associations remained significant after adjustments to the aforementioned variables, but some residual confounding factors may have remained. Hypotheses generated in this study deserve additional investigations.

Skin microcirculation has its own peculiarities and does not express precisely what happens on other vascular beds.[33] Nevertheless, there is a correlation between skin and coronary microcirculations,[18] and results derived from the former were consistently shown among cardiovascular risk factors.[19-21] Thus, results of this study add evidence to the relationship between periodontitis and CVD and include endothelial and microvascular dysfunctions as possible mechanistic explanations for the associations between both diseases.

CONCLUSION

Severe CP was associated with nailfold and gingival microvascular and endothelial dysfunctions, as highlighted by decreases in FCD, $RBCV_{max}$, % Hyper, post-ischemic peak flow, and peak flow; increases in GCD; and longer $TRBCV_{max}$ in patients with the disease.

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Correspondence: Mr. Ricardo Guimarães Fischer, Rio de Janeiro State University, Faculdade de Odontologia, Boulevard 28 de Setembro 157, 2o Andar, Vila Isabel, Rio de Janeiro, RJ, CEP 20551-030, Brazil. E-mail: ricfischer@globo.com.

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