Tese de Doutorado

ESTUDO DA RELAÇÃO ENTRE DOENÇA PERIODONTAL E FUNÇÃO ENDOTELIAL EM PACIENTES COM DOENÇA ARTERIAL CORONARIANA

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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE: CARDIOLOGIA E CIÊNCIAS CARDIOVASCULARES

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SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS EM PORTUGUÊS

CsE: Células endoteliais

CT: Colesterol total

CEP: Comitê de ética e pesquisa

DAC: Doença arterial coronariana

DCV: Doença cardiovascular

ECR: Ensaio clínico randomizado

GC: Grupo controle

GT: Grupo teste

HAS: Hipertensão arterial sistêmica

IAM: Infarto agudo do miocárdio

IC: Intervalo de confiança

IMC: Índice de massa corporal

MEV: Mudanças no estilo de vida

PI: Perda de inserção

PV: Placa visível

PS: Profundidade de sondagem

PCR: Proteína C reativa

RAP: Raspagem, alisamento e polimento supragengival

RASUB: Raspagem e alisamento radicular subgengival

RR: Risco relativo

SS: Sangramento subgengival

TCLE: Termo de Consentimento Livre e Esclarecido

TG: Triglicerídeos

VFM: Vasodilatação fluxo-mediada

LISTA DE ABREVIATURAS E SIGLAS EM INGLÊS

BOP: Bleeding on probing

CVD: Cardiovascular disease

CI: Confidence intervals

CG: Control group

CHD: Coronary heart disease

CRP: C-reactive protein

ECs: Endothelial cells

eNOS: endothelial Nitric Oxide synthase

FMD: Flow-mediated dilation

GR: Gingival recession

HDL-C: High density lipoprotein cholesterol

ICAM-1: intercellular adhesion molecule-1

IL-6: Interleukin-6

LDL-C: Low density lipoprotein cholesterol

NO: Nitric Oxide

PD: Periodontal disease

PD: Probing depth

SRP: subgingival scaling and root planing

TG: Test group

TG: Triglycerides

TNF-œ: Tumor necrosis factor-alpha

VCAM-1: Vascular cell adhesion molecule-1

VLDL-C: Very low density lipoprotein cholesterol

VP: Visible plaque

RESUMO

Objetivo: Testar o efeito do tratamento da periodontite na função endotelial, avaliada pela vasodilatação fluxo-mediada (VFM) em pacientes com doença arterial coronariana (DAC). **Métodos:** Ensaio clinico randomizado conduzido com pacientes com DAC e periodontite severa, atendidos em um hospital público universitário no sul do Brasil. O grupo teste (GT) recebeu tratamento periodontal intensivo com uma sessão de raspagem, alisamento e polimento supragengival (RAP) e orientação de higiene bucal, além de até quatro sessões de raspagem e alisamento radicular subgengival (RASUB) por quadrante, em um período máximo de 14 dias. O grupo controle (GC) recebeu uma única sessão de RAP, além de orientação de higiene bucal. A função endotelial foi avaliada através da VFM, antes e após três meses do tratamento periodontal. **Resultados:** Foram incluídos 69 pacientes nesta análise interina (amostra total 84); 31 no GT e 38 no GC. O GT apresentou condição periodontal significativamente melhor aos 3 meses no índice de placa visível (24,58%±23,36 vs. 48,77%±20,62), profundidade de sondagem (2,27±0,51 vs. 3,16±0,73), perda de inserção (4,31%±1,26 vs. 4,91%±1,35) e sangramento subgengival (34.08%±33.32 vs. 71.74%±21.39); após tratamento, houve melhora das medidas da VFM (hiperemia reativa) nos GT e GC (1,39% vs. 1,37%; p=0,84). **Conclusão:** Resultados preliminares indicam efeito semelhante na função endotelial, independente do tratamento periodontal em pacientes com DAC, durante o seguimento de 3 meses.

1. INTRODUÇÃO

Nos últimos anos, houve diversos ensaios clínicos randomizados desenhados para estudar o efeito do tratamento periodontal na doença cardiovascular. A maioria dos estudos usou diferentes biomarcadores inflamatórios e endoteliais em desfechos secundários, enquanto que os desfechos primários como morte, infarto, acidente vascular encefálico não foram avaliados [1-3]. Um destes estudos indicaram que o tratamento da periodontite reduziu as concentrações séricas de Proteína C-reativa (2,7±1,9 para 1,8±0,9 mg/L; $p<0,05$) e Interleucina-6 (2,6±3,4 para 1,6±2,6 mg/L; $p<0,05$) em pacientes com periodontite [1].

Na avaliação do endotélio vascular, além dos marcadores inflamatórios e moléculas de adesão, podemos utilizar a aferição da vasodilatação fluxo-mediada da artéria braquial (VFM) [4]. Um estudo publicado em 2005 avaliou a função endotelial em pacientes com diagnóstico de periodontite severa. Os principais resultados, após o tratamento periodontal, demonstraram uma melhora significativa na avaliação da VFM de 9,8% ± 5,7% ($p=0,003$) comparado com as medidas basais [3]. Outro estudo mostrou uma diferença de 2% na VFM, entre os grupos tratamento e controle, após 6 meses da terapia periodontal em indivíduos com periodontite severa, em uma amostra de 120 pacientes sem doença cardiovascular [2].

Alguns autores destacam que muitos desses estudos apresentam limitações metodológicas, que comprometem a extrapolação dos resultados. A maioria dos estudos avaliaram os efeitos do tratamento periodontal na VFM em pacientes sistemicamente saudáveis, sem doença arterial, o que limita a aplicabilidade de tal impacto terapêutico para pacientes cardíacos. Além disso, os efeitos do tratamento periodontal na função endotelial em diferentes grupos étnicos/sociais e outras co-morbidades (obesidade, diabetes, doença cardiovascular, síndrome metabólica) são desconhecidos [1,5].

Nessa perspectiva este estudo torna-se relevante, a medida que irá trazer informações sobre as respostas do endotélio vascular a um tratamento periodontal intensivo. Com isso

objetivamos testar o efeito do tratamento da periodontite na função endotelial, avaliada pela VFM em pacientes com doença arterial coronariana acompanhados durante um período de 3 meses.

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3. JUSTIFICATIVA

Na revisão da literatura poucos estudos randomizados têm avaliado os efeitos do tratamento periodontal na função endotelial por meio da vasodilatação fluxo-mediada. Especificamente, nenhum ensaio clínico randomizado foi conduzido até o momento para testar o efeito do tratamento da periodontite na função endotelial, avaliada pela vasodilatação fluxo-mediada, em pacientes com doença arterial coronariana. Nessa perspectiva este estudo torna-se relevante, a medida que irá trazer informações sobre as respostas do endotélio vascular a um tratamento intensivo em pacientes com periodontite grave comparado ao tratamento convencional comunitário.

4. OBJETIVO

Objetivo geral

Testar o efeito do tratamento da periodontite na função endotelial, avaliada pela vasodilatação fluxo-mediada em pacientes com doença arterial coronariana, acompanhados durante um período de 3 meses.

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Abstract

Inflammation and endothelial dysfunction are linked to the pathogenesis of atherosclerotic disease. Recent studies suggest that periodontal infection and the ensuing increase in the levels of inflammatory markers may be associated with myocardial infarction, peripheral vascular disease and cerebrovascular disease. The present article aimed at reviewing contemporary data on the pathophysiology of vascular endothelium and its association with periodontitis in the scenario of cardiovascular disease.

Keywords: endothelium, vascular; atherosclerosis; periodontal diseases; nitric oxide; cardiovascular diseases.

Core tip: Recent studies underscore the importance of endothelial dysfunction and inflammatory markers for the development of atherosclerotic disease. In addition, the literature suggests a direct association between periodontal and cardiovascular diseases. Nevertheless, more robust intervention studies are required to clarify specific gaps, especially in relation to the biological and clinical effects of periodontal disease on the genesis and progression of atherosclerotic disease.

Background

Cardiovascular disease is still the leading cause of morbidity and mortality worldwide. Nevertheless, as a result of new and effective strategies to prevent and treat atherosclerosis, the number of deaths associated with cardiovascular events has not increased, and seems to have stabilized in some countries[1].

Because it has regulatory, secretory, metabolic, immunological, and synthesizing properties, the vascular endothelium may be regarded as a heterogeneous and dynamic organ. An imbalance of these properties is linked to the onset of endothelial dysfunction and atherogenesis, and to increased risk of cardiovascular events[2]. Added to that, in the past years, the role of inflammation in the development of atherosclerosis has also been explored. Data from epidemiologic studies confirm the association between high levels of inflammatory markers and the progression of cardiovascular disease[3, 4].

Emerging evidence suggests that periodontal infection may be an independent risk factor for myocardial infarction, peripheral vascular disease, and cerebrovascular disease[5, 6]. A metanalysis has shown increased incidence of coronary heart disease (relative risk=1.14; 95%CI 1.07-1.21; P<0.001) in patients with periodontal disease even after adjustment for confounding factors such as smoking, diabetes, alcohol intake, obesity, and arterial hypertension, also suggesting a positive correlation between dental loss and coronary artery disease[6]. It should be noted that much of this evidence was generated by observational studies. In this sense, additional studies with more robust designs should be carried out to provide answers regarding the association between periodontal and cardiovascular diseases.

With the aim of furthering the understanding of the relationship between vascular endothelium, periodontal disease, and the process of atherosclerosis, this article will review contemporary data about endothelial pathophysiology and its association with periodontitis in cardiovascular disease. For that, the MEDLINE-PubMed database was searched to retrieve articles published between 1980 and 2014, using the following DeCS terms: “endothelium, vascular”; “atherosclerosis”; “periodontal diseases”; “nitric oxide”; “cardiovascular diseases”.

Vascular endothelium and atherosclerosis

Endothelial cells (ECs) form an organ weighing approximately 1kg; they are distributed along the body (total estimated area: 7,000 m^2), and are characterized by heterogeneous structure and function, with phenotypic variation according to their location in different organs, tissues, or blood vessel type[7]. Located at the interface between blood and tissues, the vascular endothelium plays an important role in the cardiovascular system, including regulation of vascular tone (smooth muscle), synthesis and secretion of molecules, and control of homeostasis, coagulation, and inflammatory and atherogenic responses[8].

Atherosclerosis is a progressive disease, characterized by accumulation of lipid particles and fibrous elements on the arterial wall. A more recent concept has introduced the notion that, in addition to the thrombotic process, inflammation and endothelial dysfunction are also directly related to all stages of atherosclerosis. In the undamaged endothelium, ECs resist leukocyte adhesion and aggregation, in addition to promoting fibrinolysis. However, when associated with inflammatory factors, such as periodontal disease (PD), cardiovascular risk factors (smoking, obesity, sedentary lifestyle, dyslipidemia, diabetes) promote changes in endothelial permeability and hence endothelial function[9]. At this initial stage, ECs express adhesion molecules that selectively recruit various leukocyte classes into the tunica intima[10]. Monocytes mature into macrophages, forming foam cells that release cytokines and factors that affect ECs. This process induces migration of smooth muscle cells from the media to the intima and affects metabolism of the arterial extracellular matrix (metalloproteinase), synthesis and release of procoagulant factors, and the bioavailability of nitric oxide (NO)[9]. NO, initially defined by Furchgott and Zawadzki as an "endothelium-derived relaxing factor"[11], is synthesized by the action of an enzyme, endothelial nitric oxide synthase (eNOS), from the amino acid L-arginine. NO plays a fundamental part in endothelial function, promoting smooth muscle relaxation and consequently vasodilatation. In addition, NO supports inhibition of platelet aggregation, smooth muscle cell proliferation, and maintenance of anti-sclerotic effect[12].

The inflammatory process may also contribute to atherosclerotic plaque rupture and thrombosis. Inflammation regulates the fragility of the fibrous cap and the thrombogenicity of the atherosclerotic plaque, influencing collagen metabolism, which provides strength and stability to the cap[13]. Pro-inflammatory cytokines such as C-reactive protein (CRP), fibrinogen, tumor necrosis factor-alpha (TNF-œ), interleukin-1 (IL-1), and interleukin-6 (IL-6) reduce the endothelial expression of NOS[14], increasing endothelial synthesis of NADPH oxidases and promoting endothelial expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin and P-selectin[14, 15]. As a result, the absence of anti-atherogenic properties in the endothelium increases leukocyte migration and platelet activation to form the atherosclerotic plaque[4].

In this sense, endothelial function and inflammatory markers are important predictors of future cardiovascular events in individuals at risk for atherosclerotic disease.

Cell adhesion molecules

Cell adhesion molecules (CAMs) are glycoproteins expressed on the cellular surface. CAMs are involved in cell-cell and cell-extracellular matrix binding and encompass immunoglobulins such as ICAM-1 and VCAM-1, as well as the selectin family, including leukocyte-endothelial adhesion molecules (E-selectin), P-selectin and leukocyte-lymphocyte adhesion molecules (L-selectin), integrins, and cadherins[16].

Selectins are expressed on the surface of endothelial cells, leukocytes, and platelets, and their expression in the endothelium is induced by various inflammatory cytokines. In the first phase of leukocyte migration, selectins mediate the capture and transport of circulating leukocytes into the vascular endothelium. In the second phase, leukocytes adhere to the endothelium through the action of ICAM-1 and VCAM-1, migrating into the interstitial tissue space[17].

Soluble forms of CAMs are found in plasma and correlate to endothelial dysfunction[18]. Thus, these markers are associated with biological mechanisms that promote thrombus formation, plaque rupture, and subsequently acute coronary

events[19]. The summary of the involvement of pro-inflammatory cytokines and adhesion molecules in atherosclerosis is described in Table 1.

Shear stress

Even if the multifactorial pathophysiologic nature of atherosclerosis is recognized, special attention should be paid to a specific component in this scenario – shear stress. Shear stress is a biomechanical force determined by blood flow, vessel geometry, and fluid viscosity, aspects modulating the structure and function of the vascular endothelium. The presence of "disturbed" flow – that is, nonlaminar flow – favors atherosclerotic plaque formation. Atherosclerotic plaque development is favored by a combination of cardiovascular risk factors and altered arterial hemodynamics around curvatures, arterial branch ostia and bifurcations[20].

Studies have shown that different types of shear stress correlate with "resistant" or "susceptible" regions in the endothelium during atherogenesis[21]. Pulsatile blood flow triggers many types of hemodynamic, hydrostatic, and cyclic forces that have the ability to influence vascular endothelial physiology[22]. The most susceptible atherosclerotic lesions are associated with certain sites in the proximal branches, bifurcations, and in areas of greater curvature. However, regions with uniform laminar flow are typically more resistant to atherogenic plaque formation[23].

Periodontal disease, inflammatory markers, and endothelial dysfunction

Periodontal disease encompasses two large groups of gum diseases. Gingivitis, which is characterized by inflammation of the gingival margin, is easily reversed with adequate oral hygiene. Periodontitis entails a chronic infectious/inflammatory process involving the supporting tissues of the tooth, including periodontal ligament and alveolar bone. The main consequence of periodontitis is the loss of tooth support structures and tooth loss[24]. Data from different countries show a prevalence of periodontitis reaching up to 50%[25-27]; however, progression is usually slow[28].

Epidemiologic studies provide evidence of an association between periodontitis and cardiovascular disease (CVD)[6,29]. The biological plausibility for this association is based mainly on the fact that patients with periodontitis present increased levels of CRP, TNF-œ, interleukins, and other inflammatory markers, which are associated with endothelial dysfunction and cardiovascular events[30,31]. Most studies employ different inflammatory and endothelial biomarkers, with secondary outcomes, whereas primary outcomes such as death or brain stroke have not yet been evaluated[32-34].

A recent systematic review and metanalysis analyzed the effect of periodontal treatment on cardiovascular risk profile in patients with established periodontitis. The main findings show a significant reduction in CRP (-0.50 mg/dL), IL-6 (-0.48 ng/L), TNF-œ (-0.75 pg/mL), fibrinogen (-0.47 g/L) and total cholesterol (-0.11 mmol/L) in the intervention group. In addition, there was improvement of endothelial function and an additional benefit regarding inflammatory markers in patients with traditional cardiovascular risk factors[24]. Investigating the same outcome in a different scenario, another study compared patients with coronary heart disease with or without periodontitis. The results indicate that treatment of periodontal disease promoted a reduction in serum concentrations of CRP, from 2.7±1.9 mg/L to 1.8±0.9 mg/L ($P<0.05$), and of IL-6, from 2.6±3.4 mg/L to 1.6±2.6 mg/L ($P<0.05$) in patients with periodontitis[32].

In addition to inflammatory markers and adhesion molecules, the measurement of brachial artery flow-mediated dilation (FMD), a technique developed initially in 1992, is also useful to assess the endothelium[35]. This non-invasive technique evaluates the diameter of the brachial artery before and after induced forearm ischemia. A blood pressure cuff is inflated at the distal or proximal section of the arm, and FMD is expressed as the percent change in brachial artery diameter at the end of ischemia. This dilatation is mediated by endothelial release of NO in response to shear stress at the arterial wall[36].

FMD is decreased in individuals with cardiovascular risk factors (diabetes, hypertension, obesity, and smoking, among others) and established atherosclerosis[37]. A study published in 2005 evaluated endothelial function in

patients with a diagnosis of severe periodontitis. The main findings following periodontal treatment show significant improvement in FMD, of 9.8%±5.7% (P=0.003) as compared to baselines measures, accompanied by a reduction in the levels of CRP from 1.1±0.9 to 0.8±0.8 (P=0.026)[34]. In this sense, evaluation of FMD and cardiovascular disease biomarkers have recently been studied and associated with endothelial dysfunction and occurrence of cardiovascular events[38,39]. The summary of the effects of periodontal disease on pro-inflammatory cytokines and adhesion molecules is depicted in Table 2

Conclusion

The present literature review suggests that periodontal treatment reduces the risk of cardiovascular disease by improving plasma levels of inflammatory markers (CRP, TNF-œ, IL), thrombotic markers (fibrinogen) and adhesion molecules (VCAM-1, ICAM-1, P-selectin), in addition to improving endothelial function as assessed by FMD. Future intervention studies are required to further elucidate the association between periodontal and cardiovascular disease, especially in terms of the biological effects of periodontal disease on the atherogenic cascade affecting the vascular endothelium.

Competing interests

The authors declare they do not have any competing interest related to this study.

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Table 1. Summary of the involvement of pro-inflammatory cytokines and adhesion molecules in atherosclerosis.

Pro-inflammatory cytokines and adhesion molecules	**Cells involved**	**Atherogenic effect**
C-reactive protein (CRP)	Adhesion molecules and endothelial cells	Stimulates production of adhesion molecules and chemokines by endothelial cells [14]
Fibrinogen	Platelet, adhesion molecules and smooth muscle	Activates platelet aggregation and promotes the migration and proliferation of smooth muscle [14]
Tumor necrosis factor-alpha (TNF-œ)	Monocytes, neutrophils and endothelial cells	Activates monocytes, neutrophils and endothelial cells to express adhesion molecules [14]
Interleukin-6 (IL-6)	Epithelial cells, fibroblasts and macrophages/ monocytes	Is involved in promoting coagulation, which result in the development of atherosclerosis [14]
Interleukin-1ß (IL-1ß)	Macrophages/ monocytes	Impedes fibrinolysis, facilitates coagulation and thrombosis [14]
Vascular cell adhesion molecule-1 (VCAM-1)	Endothelial cells	Suggested as potential candidate markers of endothelial dysfunction [19]
Intercellular adhesion molecule-1 (ICAM-1)	Endothelial cells	Implicated in leukocyte recruitment and migration into the vessel wall [19]
Leukocyte-endothelial adhesion molecules (E-selectin)	Endothelial cells	migration of monocytes down into the subendothelial space [16]

Table 2. Summary of the effects of periodontal disease on pro-inflammatory cytokines and adhesion molecules.

Pro-inflammatory cytokines and adhesion molecules	**Effect of periodontal disease**
C-reactive protein (CRP)	Increased [24]
Fibrinogen	Increased [24]
Tumor necrosis factor-alpha (TNF-œ)	Increased [24]
Interleukin-6 (IL-6)	Increased [24]
Interleukin-1β (IL-1β)	Increased [24]
Vascular cell adhesion molecule-1 (VCAM-1)	Increased [30]
Intercellular adhesion molecule-1 (ICAM-1)	Increased [30]
Leukocyte-endothelial adhesion molecules (E-selectin)	Increased [30]

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ARTIGO 2: PROTOCOLO DO ENSAIO CLÍNICO RANDOMIZADO

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The effect of periodontal therapy on C reactive protein, endothelial function, lipids and pro-inflammatory biomarkers in patients with stable coronary artery disease: study protocol for a randomized controlled trial

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Abstract

Background: Scarce information exists regarding the preventive effect of periodontal treatment in the recurrence of cardiovascular events. Prevention may be achieved by targeting risk factors for recurrent coronary artery disease (CAD) in patients with previous history of cardiovascular events. The aim of the present trial is to compare the effect of two periodontal treatment approaches on levels of C reactive protein, lipids, flow-mediated dilation and serum concentrations of pro-inflammatory and endothelial markers in stable CAD patients with periodontitis over a period of 12 months.

Methods/Design: This is a randomized, parallel design, examiner blinded, controlled clinical trial. Individuals from both genders, 35 years of age and older, with concomitant diagnosis of CAD and periodontitis will be included. CAD will be defined as the occurrence of at least one of the following events 6 months prior to entering the trial: documented history of myocardial infarction; surgical or percutaneous myocardial revascularization and lesion >50% in at least one coronary artery assessed by angiography; presence of angina and positive noninvasive testing of ischemia. Diagnosis of periodontitis will be defined using the CDC-AAP case definition (≥2 interproximal sites with clinical attachment loss ≥6mm and ≥1 interproximal site with probing depth ≥5mm). Individuals will have to present at least 10 teeth present to be included. 100 individuals will be allocated to test (intensive periodontal treatment comprised by scaling and root planning) or control (community periodontal treatment consisting of one session of supragingival plaque removal only) treatment groups. Full-mouth six sites per tooth periodontal examinations and subgingival biofilm samples will be conducted at baseline, 3, 6 and 12 months after treatment. The primary outcome of this study will be C reactive protein changes over time. Secondary outcomes include total cholesterol, LDL-C, HDL-C, triglycerides, IL-1β, IL-6, TNFα, fibrinogen, ICAM-1, VCAM-

1 and E-Selectin. These outcomes will be assessed at all time-points over 12 months. Flow-mediated dilation will be assessed at baseline, 1, 3 and 6 months after periodontal therapy.

Discussion: This trial will provide new evidence regarding the effect of periodontal treatment on risk markers for recurrence of cardiovascular events in stable coronary artery disease patients.

Trial registration number: ClinicalTrials.gov Identifier: NCT01609725

Keywords: Periodontal diseases, cardiovascular diseases, C reactive protein, non-surgical periodontal therapy, coronary artery disease, randomized controlled trial, endothelial function, lipids

Background

Cardiovascular diseases (CVD) are still considered the main cause of mortality and morbidity all over the world [1]. In the last years, efforts have been made to define the inflammatory pathways that lead to cardiovascular events aiming to define more effective therapeutic and preventive strategies. In this regard, it has been demonstrated that many pro-inflammatory biomarkers play an important role in the cascade of events observed in CVD, mainly C reactive protein (CRP) and other molecules such interleukins, fibrinogen and adhesion molecules [2-4].

Periodontal diseases have been considered a probable risk factor for CVD with great amount of evidence from observational studies associating the two conditions [5-10]. The increase in the risk of coronary artery disease in periodontal patients is estimated to be 24% higher after adjusting for important confounding factors [11]. Despite the considerable amount of evidence associating the two conditions, there is still lack of interventional studies to better elucidate the effect of periodontal treatment on the prevention of CVD [12, 13].

The biological plausibility linking periodontal diseases to CVD is supported by the findings that periodontal inflammation and infection lead to an increase in blood levels of the abovementioned important biomarkers related to CVD. For instance, levels of CRP are higher in periodontitis compared to healthy patients, and reduction of this marker is observed after periodontal therapy in otherwise healthy individuals [14, 15]. Interleukin 1β, interleukin 6, fibrinogen and other pro-inflammatory biomarkers have also been found in elevated levels in healthy patients with periodontitis [16, 17]. It has also been demonstrated that periodontal disease may negatively influence endothelial function directly or indirectly [18-21].

Although there are some interventional studies evaluating the systemic effects of periodontal therapy [15], there is little information regarding the impact of periodontal treatment in the recurrence of cardiovascular events. Most of the studies have been conducted with otherwise healthy patients with periodontitis, limiting the applicability of the findings to patients suffering from CVD. To the best of our knowledge, there are no randomized controlled trials published to date assessing the preventive effect of periodontal therapy in true endpoints of cardiovascular disease, i.e. major cardiovascular events such as myocardial infarction and stroke. By reviewing the literature we were able to find two small interventional studies showing that periodontal treatment might reduce some CVD blood risk markers. One of the studies demonstrated that CRP and hemostatic factors improved after periodontal treatment in a small group of 18 non-smokers with a history of recent CVD event [22]. Another study evaluated hypertensive patients with periodontitis and showed that periodontal therapy might reduce levels of CRP and fibrinogen [23].

Recently, a study protocol of a short-term randomized controlled trial about the effect of periodontal therapy on endothelial function and some blood biomarkers for CVD was published [24]. The study is planned to be conducted with systemically healthy individuals with periodontitis and will provide data for primary prevention of CVD.

The aim of this article is to describe the protocol of a randomized controlled trial that was designed to compare the effect of intensive and community periodontal treatments on levels of C reactive protein, lipids, flow-mediated dilation and serum concentrations of pro-inflammatory and endothelial markers in stable coronary artery disease patients with periodontitis over a period of 12 months.

Methods/Design

Study design and centers

This is a randomized, parallel design, examiner blinded, controlled clinical trial. This study will be conducted at the School of Dentistry of the Federal University of Rio Grande do Sul and at the University Hospital of Porto Alegre, in Brazil. Cardiovascular patients will be recruited at the Ischemic Heart Disease Clinic at the University Hospital. Blood samples and laboratory analyses will be conducted at the Clinical Research Center at the University Hospital. Flow-mediated dilation will be performed at the sector of non-invasive methods of the University Hospital. Periodontal clinical examinations and treatments will be conducted at the Periodontal Department of the School of Dentistry.

Inclusion and exclusion criteria

Individuals from both genders, 35 years of age and older, with concomitant diagnosis of CAD and periodontitis will be selected for the study. The diagnosis of CAD will be recalled from the history of acute coronary syndrome episodes or percutaneous/surgical revascularization [25] recorded at the hospital files. Specifically, CAD will be defined as the occurrence of at least one of the following events 6 months prior to entering the trial: documented history of myocardial infarction; surgical or percutaneous myocardial

revascularization and lesion >50% in at least one coronary artery assessed by angiography; presence of angina and positive noninvasive testing of ischemia.

Diagnosis of periodontitis will be defined using the CDC-AAP case definition [26]. Individuals will be classified as having severe chronic periodontitis in the presence of ≥2 interproximal sites with clinical attachment loss ≥6mm and ≥1 interproximal site with probing depth ≥5mm in non-adjacent teeth. Moreover, included individuals have to present at least 12 teeth.

Individuals will be excluded from the study if they use antibiotics or anti-inflammatory drugs during the follow-up period of the study.

Ethical considerations

All participants will read and sign an informed consent before entering the study. The study protocol was approved by the Institutional Review Boards of the University Hospital (protocol number 12-265) and the Federal University of Rio Grande do Sul (protocol number 18341). The study will be conducted according to the principles of the Declaration of Helsinki for human studies and to the Brazilian legislation for human studies of the Ministry of Health.

Cardiovascular care

This study will be conducted with stable coronary artery disease patients who will be receiving cardiovascular care for at least 6 months at the Ischemic Heart Disease Clinic, a tertiary care cardiovascular clinic, at the University Hospital of Porto Alegre. Cardiovascular care provided in this tertiary clinic includes medication and counseling. The staff of the clinic comprises cardiologists, nurses, nutritionists and physiotherapists. The protocol of cardiovascular care in this clinic includes statins for the majority of the patients. When

appropriate, oral hypoglycemic, insulin, acetylsalicylic acid and anti hypertensive drugs are also prescribed. Counseling includes mainly health-related life style behavioral modifications such as daily exercise, smoking cessation and dietary therapy. The mean follow-up time of the patients that attend the clinic is 5 years.

Sample size

The sample size of the present trial was estimated using a statistical software (G*Power 3 for Macintosh). The change in serum levels of C reactive protein was considered the main outcome for sample size estimation. It was estimated that 42 individuals in each treatment arm will be necessary to find reductions in C reactive protein of 1.5 mg/L (standard deviation = 1.5) and 0.5 mg/L (standard deviation = 0.5) in test and control groups, respectively. Alpha and beta errors of 5% and 20%, respectively, were used in the estimation. An attrition rate of 20% is expected during the study; consequently, 50 patients in each group will be included.

Interventions

The test group (TG) will consist of intensive periodontal treatment comprising one session of supragingival scaling and personalized oral hygiene instructions, followed by one to four sessions of subgingival scaling and root planing (SRP) by quadrant, under local anesthesia, in a period of 14 days. Individuals will be followed monthly during the first six months and at each 3 months until the end of the 12 months study period. In the follow-up sessions, professional plaque removal, oral hygiene instructions and reinforcement will be provided.

The control group (CG) will receive community periodontal treatment similar to that provided by the Brazilian public health system, consisting of only one session of supragingival scaling followed by standard oral hygiene instructions.

Treatments will be performed by two experienced periodontists (I.W.J.R. and C.K.). Before the beginning of the study, the two clinicians will undergo a period of training and discussion of the therapeutic approaches with the aim of standardization of the test and control interventions.

Study protocol

Eligible individuals will be invited to follow the protocol of visits described bellow (Figure 1):

- Visit I: application of a structured questionnaire, general clinical evaluation, baseline periodontal examination and subgingival microbiological samples.
- Visit II: baseline flow-mediated dilation, anthropometric assessment and blood samples.
- Visit III: Start of periodontal treatment according to randomization. After this visit, periodontal therapy will be conducted according to randomization.
- Visit IV (30 days after the last session of periodontal treatment): first follow-up flow-mediated dilation assessment.
- Visit V (3 months after the last session of periodontal treatment): second follow-up flow-mediated dilation assessment, first follow-up blood samples, periodontal clinical examination and subgingival microbiological samples.
- Visit VI (6 months after the last session of periodontal treatment): third follow-up flow-mediated dilation assessment, second follow-up blood samples and periodontal clinical examination and subgingival microbiological samples.

- Visit VII (12 months after the last session of periodontal treatment): final follow-up blood samples, periodontal clinical examination and subgingival microbiological samples.

Randomization

Stratified randomization will be conducted. Individuals will be stratified into localized or generalized periodontitis using a cut-off point of 30% of teeth with clinical attachment loss ≥6mm since the extent of disease may be a prognostic factor for the response to non-surgical periodontal treatment.

A specific program (randomization.com) for allocation in test and control groups will be used by generating a random numbers sequence. Randomization will be conducted in blocks of 20 individuals after attributing codes to each participant. An external assistant not involved in the study will conduct randomization to warrant allocation concealment. Codes will be kept until the end of analyses to maintain blindness of the examiner and statistician.

Blood markers

Each individual will provide two samples of 10ml of blood collected by a trained nurse from the anticubital fossa. Fasting blood samples will be obtained between 7:00am and 12:00pm to control for possible diurnal variations. Blood samples for traditional cardiovascular blood markers will be immediately centrifuged and analyzed. Blood samples used for pro-inflammatory and endothelial markers will be centrifuged at 4^{o}C and 4000rpm for 10 minutes (ALC PK 120 R, ALC International, Milan, Italy). The serum will be sampled and stored in Eppendorf tubes at -80^{o}C until analysis.

Traditional cardiovascular blood risk markers to be assessed will be glucose, glycated hemoglobin, triglycerides (TG), total cholesterol, high- and low-density lipoprotein

cholesterol (HDL-C and LDL-C). The following pro-inflammatory markers will be assessed: C reactive protein, interleukin 1β (IL-1β), IL-6, tumor necrosis factor α (TNFα) and fibrinogen. ICAM-1, VCAM-1 and E-Selectin will be the measured to assess the endothelial function. These markers will be measured at baseline, 3, 6 and 12 months after treatment.

High-sensitive CRP, glucose, TG, total cholesterol and HDL-C will be measured by automated enzymatic colorimetric methods (ADVIA 1800, Siemens, Germany) following the manufacturer instructions. CRP will be measured using the intensified immunoturbidimetry by latex (CRP_2). Glucose will be obtained using the glucose-hexokinase method II (GLUH). Total cholesterol will be dosed by the colorimetric enzymatic method (CHOL_2) with cholesterol-esterase, cholesterol oxidase followed by an end point Trinder type. Triglycerides will be measured by the Trinder GPO method. HDL-C will be assessed by the HDL-*Directo* (HDL-D) using the principles of elimination/catalase. LDL-C will be calculated using the Friedwald formula [LDL-C=total cholesterol – (HDL-C + TG/5)]. Non-HDL-C will be calculated by the subtraction of HDL-C from total cholesterol. Very low density lipoprotein cholesterol (VLDL-C) will be calculated dividing TG by 5. Glycated hemoglobin will be obtained by high precision chromatography (Merck-Hitachi L-9100, Merck, Germany).

Pro-inflammatory and endothelial function blood markers will be measured using specific kits for Luminex (Milliplex map human panels, EMD Millipore Corporation, Billerica, USA).

Periodontal clinical examination

The periodontal clinical examinations will be conducted by one single calibrated examiner to assess the periodontal status of each participant. Clinical examinations will be performed using a round tip manual Williams periodontal probe of 10mm. All permanent teeth, excluding third molars, will be examined in six sites per tooth (disto-buccal, mid-

buccal, mesio-buccal, disto-palatal, mid-palatal, mesio-palatal). Visible plaque (VP), gingival recession (GR), periodontal probing depth (PD) and bleeding on probing (BOP) will be recorded. Clinical attachment loss will be obtained by the sum of GR and PD.

Subgingival biofilm

The supragingival biofilm will be removed using sterile cotton and curettes. Relative isolation will be conducted in the area to be sampled. The subgingival biofilm samples will be obtained using sterile paper points number 30 inserted for 1 minute into the pocket. Paper points will be stored in one Eppendorf tube at -20^{o}C until analyzed. The four deepest sites of each individual will be sampled.

The presence of periodontopathogenic bacteria in the subgingival biofilm will be assessed by real-time polymerase chai reaction (RT-PCR). Three species will be evaluated (*T. forsythia*, *P. gingivalis* and *T. denticola*).

Endothelial function

Non-invasive measurements of endothelial function will be conducted by flow-mediated dilation (FMD) of the brachial artery using bidimensional ultrasound equipment. Individuals will have to be rested and will lay in a controlled temperature room. All vasodilation medication will have to be interrupted for at least 4 hours before the examination, if possible. Individuals will also be advised to refrain from exercising, drinking caffeine and smoking for at least 4 hours before the examination. The examination will start after 15 minutes of rest in supine position with the arm in a comfortable position. An image of the brachial artery will be obtained above the anticubital fossa in a longitudinal plane. The flow will be monitored with the Doppler positioned in a 65^{o} angle, and images will be obtained between the lumen and the wall of the vein. A sphygmomanometer will be inflated

at the right forearm for 5 minutes in at least 50 mmHg above the systolic pressure for measurement of reactive hyperemia. Images will be established 30 seconds before and until 2 minutes after sphygmomanometer deflation, synchronized with "R" wave of the electrocardiogram. FMD will be expresses as relative variation of the brachial diameter in the hyperemic phase and defined as [(post-hyperemic diameter – baseline diameter) / baseline diameter] x 100.

After 15 minutes of the reactive hyperemic measurement and artery diameter normalization, the baseline artery diameter will be measured again. In the sequence, a dose (spray) of sublingual NTG will be administered to evaluate dependent endothelial vasodilation with images acquired after 5 minutes [27].

Demographics, behavioral and clinical variables

A structured questionnaire will be applied to all participants to obtain demographic and behavioral data. Age, socioeconomic level, education, medication use, smoking habits and frequency of physical activities will be recorded. Individuals will be examined clinically to measure height, weight and blood pressure. Body mass index (BMI) will be calculated dividing the weight by the square of the height.

Primary outcome

The primary outcome of the present trial will be changes in serum concentrations of C reactive protein.

Secondary outcomes

Secondary outcomes will include:

- Non-invasive measurements of endothelial function (FMD);

- Traditional cardiovascular risk markers (total cholesterol, LDL-C, HDL-C and triglycerides);
- Pro-inflammatory biomarkers (IL-1β, IL-6, TNFα and fibrinogen);
- Endothelial function biomarkers (ICAM-1, VCAM-1 and E-Selectin).

Independent/exposure variables and confounding

The main exposure variable will be composed by test and control periodontal treatments. The impact of each treatment on the primary and secondary outcomes will be evaluated controlling for the following confounding factors: age, gender, BMI, physical activity, glycated hemoglobin, use of oral hypoglycemic drugs, smoking exposure and subgingival microbiota.

Statistical analyses

Continuous variables will be described using means and standard deviations or median and range in case of asymmetric distribution of data. Categorical variables will be presented using frequency distribution. An intention-to-treat approach will be used in the analysis of this trial taking into consideration all dropouts during the follow-up period.

Univariable analyses will be conducted using chi-square and t tests for independent samples. Multivariable models will be fitted using generalized estimating equations to compare changes in each outcome according to test and control periodontal treatment groups controlling for confounding factors. P values <0.05 will be considered statistically significant. A statistical package (Stata 12 for Macintosh, STATA Corp. College Station, USA) will be used. The individual will be considered the unit of analysis.

Trial status

This is an ongoing trial. One first block of randomized patients is receiving the interventions and more participants are being recruited.

Competing interests

The authors declare they do not have any competing interest related to this study.

Authors' contributions

MALS will conduct the flow-mediated dilation measurements and drafted this manuscript.

MVF conceived the study and drafted this manuscript.

MMM will conduct all the periodontal examinations and drafted this manuscript.

IWJR will conduct the microbiological analyses and drafted this manuscript.

CK will conduct periodontal treatments and drafted this manuscript.

ERRS conceived the study and drafted this manuscript.

CAP is Head of the Cardiovascular Clinic, conceived the study and drafted this manuscript.

CKR is Head of Periodontology, conceived the study and drafted this manuscript.

ANH will conduct all statistical analyses, conceived the study and drafted this manuscript.

All authors read and approved the final manuscript.

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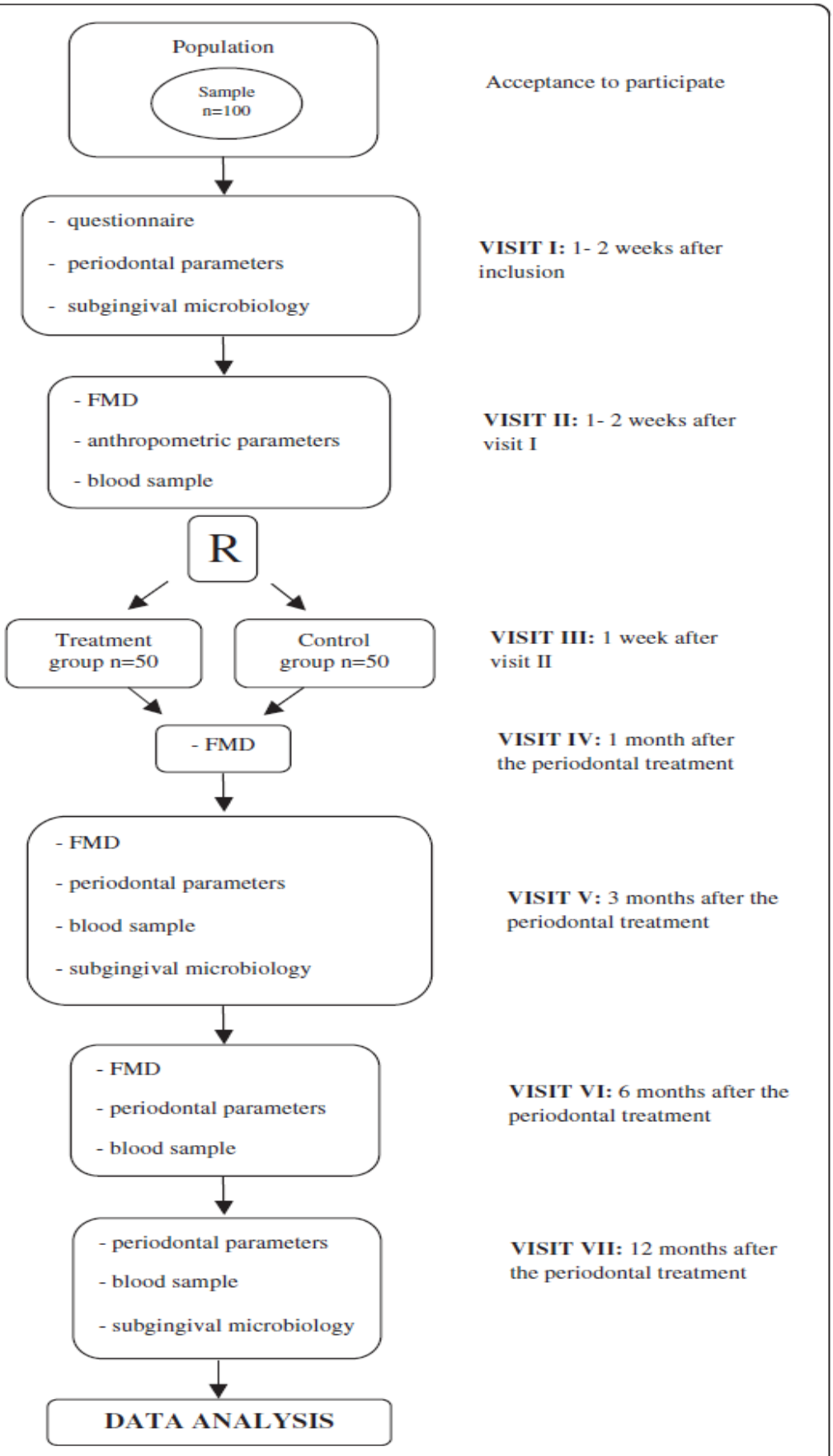

Figure 1 Flowchart of the study participation and examinations.

ARTIGO 3: ARTIGO ORIGINAL-VERSÃO EM INGLÊS

Artigo a ser submetido: *Atherosclerosis - Journal*

EFFECT OF PERIODONTAL THERAPY ON ENDOTHELIAL FUNCTION IN PATIENTS WITH CORONARY ARTERY DISEASE: A RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Objective: to assess the effects of periodontitis treatment on endothelial function, as assessed by flow-mediated dilation (FMD), in patients with coronary artery disease (CAD). **Methods:** Randomized clinical trial of adult patients of both sexes, with chronic heart disease and severe periodontitis, treated at a public teaching hospital in Southern Brazil. Subjects in the test group underwent intensive periodontal therapy, consisting of one session of supragingival scaling and polishing (S&P), followed by oral hygiene instructions and up to four sessions of subgingival scaling and root planing (SRP) per quadrant, over a maximum of 14 days. Control subjects received a single session of S&P followed by standard oral hygiene instructions. Endothelial function was assessed by measurement of brachial artery FMD before and 3 months after periodontal therapy. **Results:** This interim analysis (total sample 84) included 69 patients (31 test, 38 control). At 3 months, patients in the test group exhibited significant improvement in periodontal health as assessed by the visible plaque index (24.58±23.36% vs. 48.77±20.62%), probing depth (2.27±0.51 vs. 3.16±0.73), attachment loss (4.31±1.26% vs. 4.91±1.35%) and bleeding on probing (34.08±33.32% vs. 71.74±21.39%). After treatment, FMD (reactive hyperemia) improved in both the test and control groups (1.39% vs. 1.37%; p=0.84).

Conclusion: Preliminary results indicate a similar effect on endothelial function, regardless of periodontal treatment in patients with CAD during follow-up of 3-months.

Clinical trial registry: ClinicalTrials.gov (NCT01609725)

Keywords: vascular endothelium; atherosclerosis; periodontitis.

INTRODUCTION

Periodontitis is defined as a chronic infectious and inflammatory disease of the supporting structures of the teeth, including the periodontal ligament and alveolar bone[1]. The vascular endothelium has regulatory, secretory, metabolic, immune, and synthesizing properties. Imbalances of these properties are associated with endothelial dysfunction, increased levels of markers of inflammation, atherogenesis, and risk of cardiovascular events[2]. Epidemiological studies provide evidence of an association between periodontitis and cardiovascular disease[1,3]. A meta-analysis of prospective cohort studies assessing approximately 86000 patients over a 6-year period found that individuals with periodontal disease have a 1.14 times higher risk of developing coronary artery disease (CAD) than controls (95%CI 1.07-1.21; $p<0.001$)[4]. The mechanisms implicated in this process involve direct or indirect interactions between periodontal pathogens, systemic inflammation, and the vascular endothelium in the progression of atherosclerosis[5].

One marker of endothelial function is flow-mediated vasodilation (FMD) of the brachial artery[6]. Consistent data have demonstrated a significant inverse association between baseline FMD measurements and cardiovascular risk[7,8]. A recent meta-analysis sought to evaluate the effect of periodontal therapy on vascular function as assessed by FMD. In subjects with periodontitis, the difference in mean baseline FMD was 5.1% (95%CI 2.08-8.11; $p<0.001$) as compared with controls. After periodontal therapy, the difference in mean FMD between the test and control groups was 6.64% (95%CI 2.83-10.44). However, analysis of the included studies showed a high level of heterogeneity in outcomes, with I^2=80.1% and I^2=78% respectively[9]. The same research group had previously published a randomized trial that showed a 2% increase in mean FMD (95%CI 1.2-2.8; $p<0.001$) in the treatment group 6 months after periodontal therapy[10].

To the best of our knowledge, no studies have assessed the effect of periodontal therapy on endothelial function (as measured by FMD) among patients with CAD and periodontitis. Therefore, the present study sought to assess the effects of periodontitis treatment on endothelial function, as assessed by flow-mediated dilation (FMD), in patients with coronary artery disease (CAD).

METHODS

Study design

This was a randomized, parallel-design, examiner-blinded, controlled trial of patients with chronic heart disease treated at a public university hospital in Southern Brazil from August 2012 through August 2014. The study was approved by the local Research Ethics Committee and all patients provided written informed consent for participation. The trial protocol was registered on ClinicalTrials.gov (identifier NCT01609725) and published (*Trials* 2013;14:283) [11].

Participants

Individuals of both sexes, 18 years of age and older, with a diagnosis of stable CAD (last cardiovascular event >6 months prior to trial inclusion) and severe chronic periodontitis. Subjects were selected from a specialty outpatient clinic. The diagnosis of CAD was defined as a clinical history of: at least one documented episode of acute coronary syndrome; percutaneous/surgical revascularization; coronary angiography showing ≥50% lesion in at least one artery; or positive noninvasive testing of ischemia[12].

The diagnosis of periodontitis was defined in accordance with the Centers for Disease Control – American Academy of Periodontology (CDC-AAP) criteria[13], i.e., as the presence of at least two teeth with probing depth (PD) ≥5 mm and attachment loss (AL) ≥6 mm. Furthermore, subjects were required to have at least 12 teeth. Patients were excluded from the

study if they had received periodontal therapy in the last 6 months or used antibiotics or anti-inflammatory drugs in the 3 months prior to trial inclusion.

Interventions

Periodontal therapy was performed by two experienced periodontists, who were not involved in outcome assessment. The test group received intensive periodontal therapy. This consisted of one session of supragingival scaling, planing and polishing (S&P) and personalized oral hygiene instructions, followed by up to four sessions of subgingival scaling and root planing (SRP) per quadrant, under local anesthesia, over a maximum period of 14 days. Patients were followed by means of individual periodontal maintenance or recall visits (professional plaque biofilm removal and reinforcement of oral hygiene instructions) once monthly during 3-month follow-up.

The control group received community periodontal treatment similar to that provided by the Brazilian public health system, consisting of a single session of S&P followed by standard oral hygiene instructions.

Primary outcome

Endothelial function

Noninvasive assessment of endothelial function was carried out by a trained nurse blinded to patient allocation and treatment. Brachial artery FMD was measured using a two-dimensional (2D) Philips EnVisor ultrasound system, with an electrocardiogram module and a high-frequency (7-12 MHz) vascular transducer. Examination of endothelial function was carried out in a temperature-controlled environment with the patient at rest. All vasoactive medications were discontinued at least 4 h before examination. Patients were also instructed to refrain from physical activity, caffeine-containing foods, and smoking for at least 4 h before examination. After a 15-minute rest in the supine position with the arm placed comfortably, the examination was started. The brachial artery was identified above the

antecubital fossa on the longitudinal plane. Flow was monitored with a Doppler angle of 65° and images obtained between the vessel lumen and vessel wall (anterior and posterior intimal interface). A sphygmomanometer was inflated on the forearm for 5 minutes, at least 50 mmHg above systolic blood pressure, for measurement of reactive hyperemia. Images were obtained 30 seconds before (baseline) and 60 seconds after deflation of the sphygmomanometer cuff (reactive hyperemia) and gated to the R-wave of the electrocardiogram. FMD was expressed as the relative change in brachial artery diameter during the hyperemic phase, and defined as: [(post-hyperemic diameter – baseline diameter) / baseline diameter] × 100.

After 10 minutes since measurement of reactive hyperemia and return of arterial diameter to normal, the baseline arterial diameter was measured again. Endothelium-independent dilation was then measured 5 minutes after administration of a single 0.4-mg dose (pump) of sublingual nitroglycerin (Nitrolingual Pumpspray). All images were R-wave gated. The relative change in arterial diameter was calculated as: [(post-NTG diameter – baseline diameter) / baseline diameter] × 100[6]. Still images were used to calculate the mean of six measurements of arterial diameter obtained at different sites along the vessel.

Blood markers

At the start of the study, each participant provided 15 mL of blood from an antecubital vein, after an 8-h fast, for measurement of cardiovascular risk markers in blood. Part of each blood sample was taken immediately to the HCPA laboratory for analysis of lipid profile and blood sugar, whereas part was centrifuged at 4^{o}C and 4,000 rpm for 10 minutes (ALC PK 120 R, ALC International, Milan, Italy). Serum was frozen at -80^{o}C in labeled Eppendorf tubes for quantitation of C-reactive protein (CRP).

The cardiovascular risk markers of interest for measurement in blood were CRP, glucose, glycated hemoglobin (HbA1c), triglycerides (TG), total cholesterol (TC), high-

density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). The valued measured for these markers were recorded and classified as per Brazilian Cardiology Society guidelines[14,15].

Glucose, TG, TC, and HDL-C were measured by automated enzymatic colorimetric methods (ADVIA 1800, Siemens, Germany). CRP was quantitated using the latex-enhanced immunoturbidimetry method (CRP_2). LDL-C was calculated using the Friedewald formula [LDL-C = TC – HDL-C – (TG/5)]. Glycated hemoglobin was measured by chromatography (Merck-Hitachi L-9100, Merck, Germany).

Sample size calculation

The sample size was calculated using FMD as the primary outcome of the present study. It was estimated that 42 patients per group (n=84) would be necessary, considering a 1% reduction in mean FMD between groups, a standard deviation of 1.5, alpha and beta errors of 5% and 20% respectively, and a 15% attrition rate during follow-up. At this interim analysis, 69 patients were included, 31 in the test group and 38 in the control group.

Randomization

Patients were allocated into the test and control groups by randomization in blocks of 10 individuals. A specific program *(randomization.com)* was used for random allocation into the test and control groups.

Blinding and allocation concealment

The randomization procedure was performed entirely by an external assistant using numeric codes and opaque sealed envelopes, thus ensuring allocation concealment. FMD was measured by a blinded examiner, i.e., one unaware of patient allocation.

Periodontal examination

Periodontal clinical examination was conducted by a calibrated examiner to assess the periodontal health status of each patient, using a mouth mirror and a manual periodontal

probe with Williams markings. All erupted permanent teeth, excluding the permanent molars, were examined at six sites per tooth. The visible plaque index (VPI), gingival recession (GR), probing depth (PD), and bleeding on probing (BOP) were measured. Clinical attachment loss (AL) was calculated as the sum of PD and GR.

Clinical and demographic data

A structured questionnaire was administered to all participants to collect clinical and demographic data and thus characterize the profile of the study sample. Clinical parameters included age, weight, height, history of present illness and past medical history, comorbidities, and current medication use. The demographic parameters were socioeconomic status and educational attainment.

Patients were classified as diabetic if they were receiving medication for diabetes, reported having diabetes, and/or had a diagnosis of diabetes in their medical records. Blood pressure was measured using a sphygmomanometer placed on the left arm, with the patient seated, after a 15-minute rest and before FMD measurement. Patients were classified as smokers if they smoked regularly (at least one cigarette per day) or had smoked within 6 months of inclusion in the trial; nonsmokers if they had never smoked or had quit smoking at least 10 years prior to inclusion in the trial; and former smokers if they had not smoked for at least 6 months prior to inclusion in the trial. Finally, patients were classified as physically active if they reported engaging in moderate physical activity for at least 30 minutes, at least five times a week. Body mass index (BMI) was calculated by dividing the patient's weight (in kg) by the height (in m) squared [weight (kg) / height (m^2)].

Reproducibility of the raters

Before the start of the trial, the periodontal examiner was calibrated by conducting repeated measurements of PD and GR, at 1-hour intervals, in 10 periodontal patients who did

not take part in the study. The weighted kappa coefficients (considering a ±1mm error) for PD and AL were 0.91 and 0.88 respectively.

All FMD measurements were obtained by a single trained examiner. Interobserver correlation coefficients for measurements (% change) of FMD in reactive hyperemia and endothelium-independent dilation were 0.89 and 0.93 respectively. Considering a significance level of $\alpha = 0.05$, a statistical power of 80%, a 3% mean difference in FMD and a standard deviation of 5%, 11 individuals in the sample were assessed randomly.

Statistical analysis

The primary outcome of this trial was FMD after reactive hyperemia and assessment of endothelium-independent dilation. These two variables were asymmetrically distributed and were thus analyzed by means of nonparametric tests (Mann–Whitney *U* and Wilcoxon test). Categorical variables were compared between groups at baseline using the chi-square or Fisher's exact tests as appropriate.

Mean VPI, PD, AL, and BOP values were calculated for each participant. These periodontal variables were then compared between groups using Wald tests.

All analyses were conducted in the STATA v.10 software environment. Two-tailed p-values <0.05 were deemed statistically significant.

RESULTS

Of the 428 eligible patients, 262 were excluded for failure to meet the inclusion criteria, 60 refused to take part in the study, 19 missed their first periodontal clinical examination visit, and 11 had a new CV event or died before randomization. Therefore, a total of 76 patients were randomized: 36 to the test group and 40 to the control group. Of these, 5 patients in the test group and 2 in the control group did not receive the allocated

intervention because of a new CV event, death, or failure to attend the first visit. At 3-month follow-up, 31 patients in the test group and 38 in the control group were assessed (Figure 1).

Baseline profile and cardiovascular risk factors

The baseline profile of the sample is descried in Table 1. There were no significant between-group differences for any of the variables of interest. Mean age was 61.7±8.3 years in the control group and 58.6±8.5 years in the test group. Most patients in both groups were male and had well-controlled TG, TC, LDL-C, and HbA1c levels. Appropriate HDL-C levels were observed in approximately one-third of patients in each group. The baseline brachial artery diameter was 0.41 cm in the control group and 0.43 cm in the test group (p=0.07).

Periodontal response

At 3-month follow-up, the test group exhibited significantly better periodontal status as compared with the control group (Table 2). At baseline, there were no significant between-group differences in VPI, PD, AL, or BOP. At 3 months, there was a significant reduction in visible plaque in both groups, but this reduction was significantly greater in the test group than in the control group. At 3 months, there were no significant differences from baseline in PD or AL in controls; conversely, both PD and AL were significantly reduced in the test group. Furthermore, at 3 months, both PD and AL were significantly lower in tests than in controls. Both groups exhibited reductions in BOP at 3 months, but this reduction was greater in the test group. The final BOP index was significantly lower in tests than in controls.

Endothelial function

Figure 2 shows the FMD results obtained over time. There were no significant between-group differences in baseline assessment of reactive hyperemia (control, 7.10±6.09; test, 7.05±5.6). After periodontal therapy, nonsignificant improvements were observed in the control (1.37%) and test (1.39%) groups, with no significant between-group difference (p=0.84). A similar pattern was observed on measurement of endothelium-independent

dilation. There were no significant between-group difference in baseline measurements (control, 12.3±6.9; test, 13.9±7.6) and final (control, 11.9±8.4; test, 13.3±6.8; p=0.68). When the sample was analyzed as a whole without considering group allocation, there were no significant improvements in FMD at 3-month follow-up (1.38% reduction, p=0.15).

DISCUSSION

The present analysis sought to ascertain the effects of intensive periodontal therapy on endothelial function in patients with CAD. Intensive periodontal therapy was significantly superior to a single session of S&P in reducing periodontal inflammation and recovering clinical attachment. However, intensive periodontal therapy was not associated with a positive effect on FMD superior to that observed in the control intervention group.

The response to periodontal therapy observed in the present trial was similar or superior to that observed in prior studies[10,16]. In interventional studies in the field of periodontology, recovery of periodontal health is of critical methodological importance, as the absence of systemic impact after periodontal therapy cannot be explained by an absence of periodontal response. Within this context, it bears stressing that the absence of significant between-group differences in FMD observed in this trial should not be attributed to failure of the periodontal therapy provided to test group participants, but rather to other aspects that warrant discussion.

The literature has consistently demonstrated that periodontitis correlates positively – both directly and indirectly – with CAD. A recent meta-analysis reported that individuals with periodontal disease were at 14% higher risk of developing CAD than controls[4]. Periodontal infection contributes to systemic inflammation, as demonstrated by elevated levels of inflammatory markers such as CRP, fibrinogen, and interleukins, all of which are determining factors in the development of atherosclerosis[17]. A cross-sectional study

previously published by our group found a significant association between severe periodontitis and elevated CRP levels in patients with stable CAD[18]. The cardiovascular risk profile of patients treated for periodontitis was assessed in another recent study. Notably, patients with periodontitis and other comorbidities (cardiovascular disease, metabolic syndrome) derived greater benefit from periodontal therapy in terms of reductions in CRP, interleukin-6, TG, TC, HDL-C, and HbA1c[1].

The vascular endothelium as a selectively permeable barrier between the extravascular and intravascular compartments. Furthermore, it has anti-inflammatory, antithrombotic, and anticoagulant properties[19]. In epidemiological studies, endothelial dysfunction is an independent predictor of cardiovascular events[20,21]. Within this context, studies have shown that periodontitis can have an impact on endothelial function as assessed by several outcome measures, including FMD. In a systematic review of the literature, Orlandi et al.[9] found three cross-sectional observational studies with FMD as outcome. After meta-analysis, they found a significant reduction in FMD (5.1%) in patients with periodontitis as compared with periodontally healthy subjects. This systematic review also found six controlled trials of the effects of periodontal therapy on FMD; three were included in the meta-analysis, which demonstrated a 6.64% increase in FMD, although there was a high degree of heterogeneity.

The findings of the present randomized clinical trial do not corroborate these results; neither the absence of difference between the test and control groups nor the effect size of periodontal therapy on FMD are consistent with the aforementioned literature. On the other hand, one must bear in mind that the present study was unique in its assessment of a population of patients with chronic heart disease. All other studies published to date were carried out exclusively on systemically healthy patients with no cardiovascular disease. Moreover, the patients in the present sample were already receiving cardiovascular care

(including medication and nonpharmacological interventions) at the time of the study, which may explain the improvement in FMD observed in the control group as well.

From another standpoint, the improvements in FMD observed in both periodontal therapy groups in the present trial may be discussed from the perspective of the cardiovascular care provided to these patients. This included a lifestyle modification approach with proven impact on cardiovascular risk reduction, as demonstrated in previous studies by our group[22]. A systematic review of prospective studies[7] showed that FMD was significantly and inversely associated with cardiovascular risk in several populations, with an overall estimated risk of 0.90 per 1% higher FMD (95%CI 0.86–0.94; $p<0.01$). Furthermore, this review noted that a 1% increase in FMD may be relatively more important in terms of cardiovascular risk in diseased populations than in populations with healthy vascular endothelium. Comparatively, in the present study of CAD patients, FMD values improved 1.37% in the control group and 1.39% in the test group. Therefore, from a clinical standpoint, the entire sample derived benefit in terms of cardiovascular risk reduction.

The results of the various studies and scenarios analyzed to date cannot prove that FMD is a causal risk factor for cardiovascular disease, but one can hypothesize that worsening endothelial function would contribute to vascular deterioration and hasten the atherosclerotic process. In short, the impact of periodontal therapy on cardiovascular outcomes in patients with DAC remains inconsistent. Further studies are necessary to ascertain whether treatment of periodontitis can contribute to the prevention of atherosclerosis and cardiovascular events. Recruitment by our group is ongoing. Our objective is to follow these patients for 1 year and assess other indicators of cardiovascular risk, such as markers of inflammation and adhesion molecules.

LIMITATIONS

The findings of the present study should be interpreted in light of potential methodological limitations. The first such issue concerns the sample size, which, at interim analysis, was smaller than calculated *a priori*. Nevertheless, the effect size of periodontal therapy appears to have been small in this sample, and increasing the sample size would be unlikely to alter the results obtained. Although the systemic impacts of periodontal therapy occur within 3 months, this trial will continue to follow patients for up to 1 year, as systemic inflammatory changes with the potential to contribute to improvements in endothelial function may still occur during this period.

CONCLUSION

Preliminary results indicate a similar effect on endothelial function, regardless of periodontal treatment in patients with CAD during follow-up of 3-months.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

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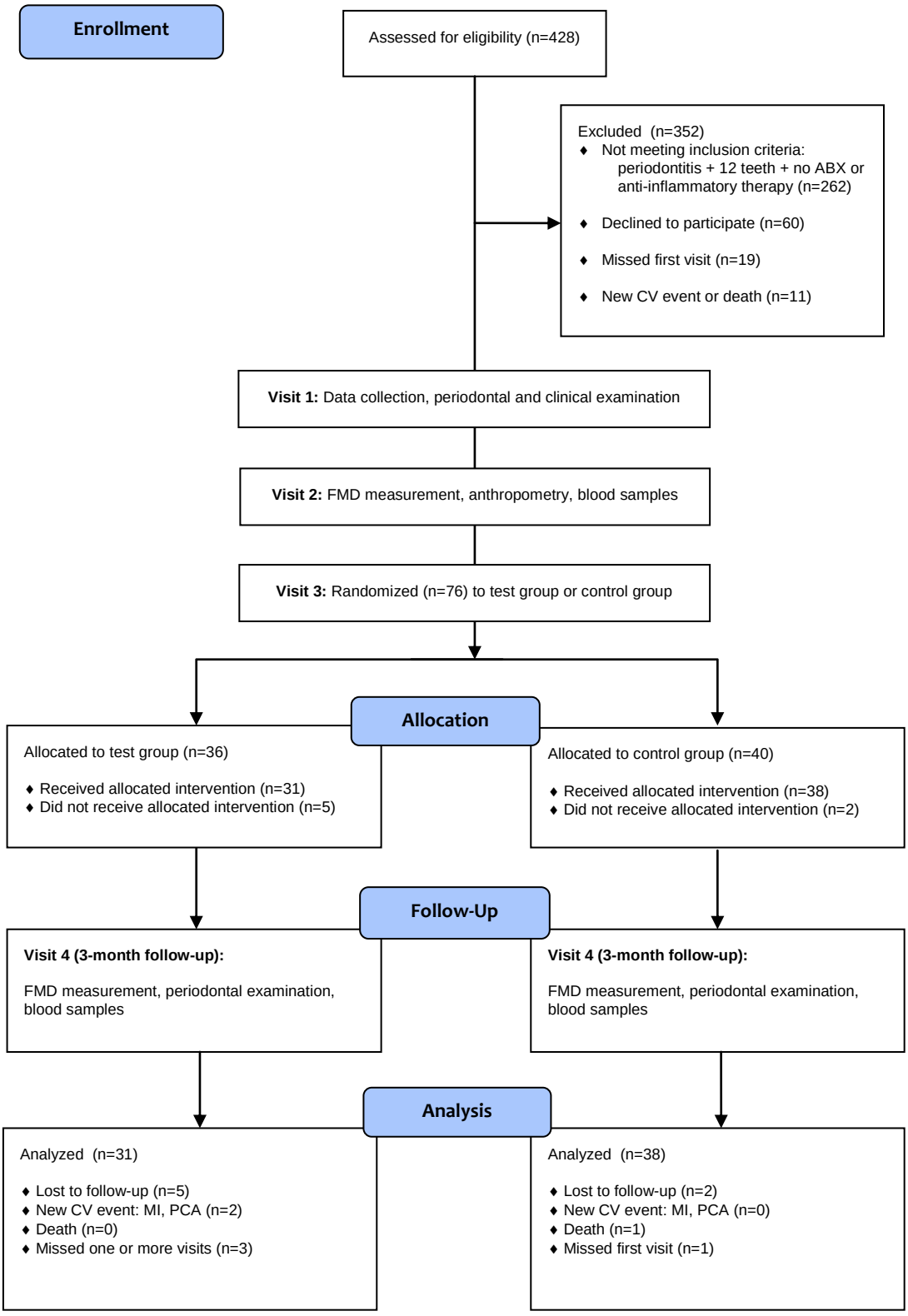

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) flow diagram.

Table 1. Sample profile at baseline.*

Variable	Control group (n=38)	Test group (n=31)	p†
Age (mean ± SD)	61.7±8.3	58.6±8.5	0.14
Male sex (n/%)	28 (74)	24 (77)	0.78
Smoking status			
Nonsmoker	10 (26)	7 (23)	
Former smoker	24 (63)	20 (64)	
Current smoker	4 (11)	4 (13)	0.18
White race	33 (87)	24 (77)	0.35
Occupation (inactive)	28 (74)	20 (64)	0.44
Educational attainment			
≤8 years formal schooling	25 (66)	16 (52)	
≥9 years formal schooling	13 (34)	15 (48)	0.33
Diabetes	16 (42)	13 (42)	1.00
Sedentary lifestyle	25 (66)	19 (61)	0.80
Total cholesterol (mg/dL)	163±42	181±49	0.10
Triglycerides (mg/dL)	172±117	217±140	0.14
LDL-C (mg/dL)	92±40	98±39	0.57
HDL-C (mg/dL)	39±10	40±11	0.80
HbA1c (%)	6.7±1.8	6.6±1.7	0.70
Glucose (mg/dL)	115±41	130±61	0.23
SBP (mmHg)	126±18	123±19	0.39
DBP (mmHg)	77±8	77±9	0.67
BMI (Kg/m^2)	28.2±4.1	27±3.6	0.20
Artery diameter (cm)	0.41±0.06	0.43±0.06	0.07
Treatment goals			
CRP <3 mg/dL	16 (51.6)	15 (48.4)	1.00
Triglycerides <150 mg/dL	23 (60)	15 (48)	0.34
Total cholesterol <200 mg/dL	29 (76)	22 (71)	0.78
LDL-C <100 mg/dL	30 (79)	22 (71)	0.57
HDL-C >40 mg/dL (males) or >50 mg/dL (females)	12 (32)	11 (35)	0.80
HbA1c <7%	30 (79)	24 (77)	1.00
BMI 18.5-25 Kg/m^2	17 (45)	17 (55)	0.50

BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure. *Variables expressed as mean ± standard deviation (SD) or n (%). † Student's *t*-test for independent samples.

Table 2. Markers of periodontal health in the control and test groups over time.

	Baseline	**3 months**	**p***
Visible plaque (%)			
Control	75.39±15.54	48.77±20.62	<0.001
Test	78.20±21.60	24.58±23.36	<0.001
p**	0.54	<0.001	
Probing depth			
Control	3.07±0.54	3.16±0.73	0.25
Test	3.22±0.54	2.27±0.51	<0.001
p**	0.25	<0.001	
Attachment loss (%)			
Control	4.89±1.33	4.91±1.35	0.79
Test	5.12±1.46	4.31±1.26	<0.001
p**	0.49	<0.001	
Bleeding on probing (%)			
Control	88.21±11.56	71.74±21.39	<0.001
Test	92.49±10.10	34.08±33.32	<0.001
p**	0.10	<0.001	

*Within-group comparison; **Between-group comparison. P-values from Wald test.

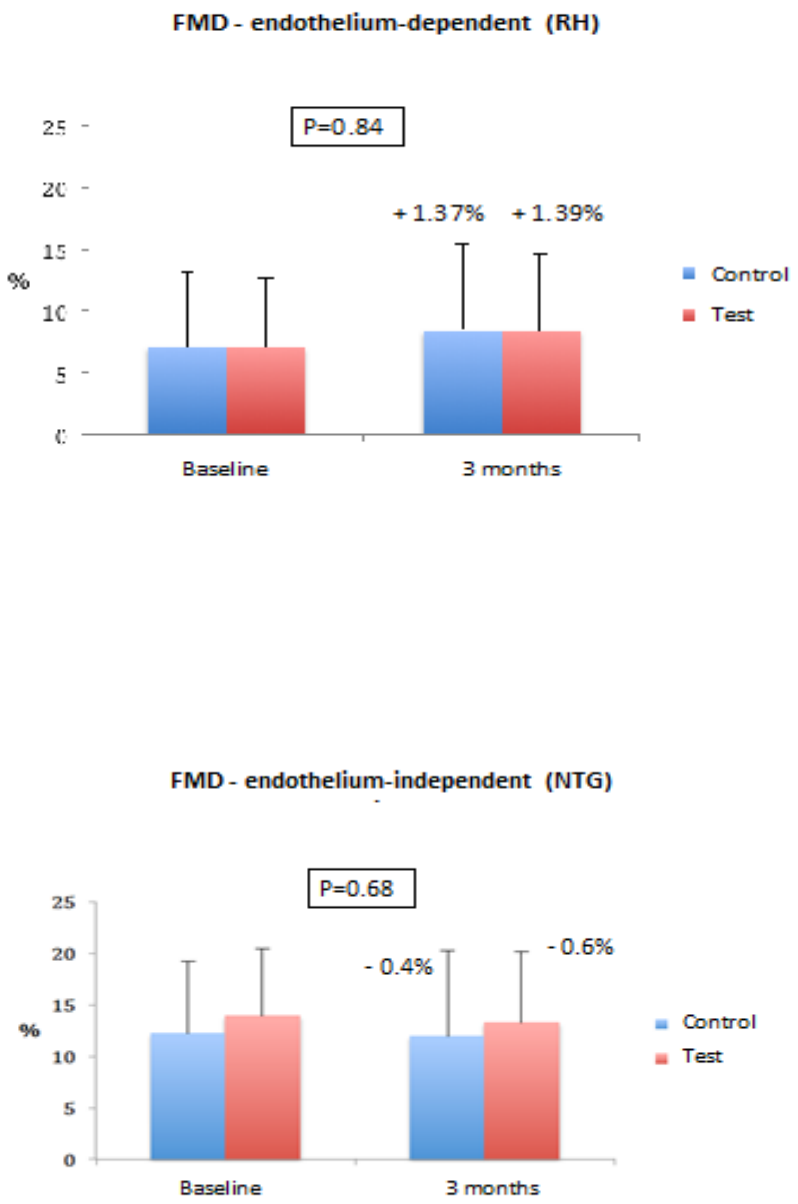

Figure 2. Flow-mediated dilation and nitroglycerin-mediated dilation in the control and test groups at baseline and at 3-month follow-up.

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ARTIGO 3: ARTIGO ORIGINAL-VERSÃO EM PORTUGUÊS

EFEITO DO TRATAMENTO PERIODONTAL NA FUNÇÃO ENDOTELIAL DE PACIENTES COM DOENÇA ARTERIAL CORONARIANA: ENSAIO CLÍNICO RANDOMIZADO

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RESUMO

Objetivo: Testar o efeito do tratamento da periodontite na função endotelial, avaliada pela vasodilatação fluxo-mediada (VFM), em pacientes com doença arterial coronariana (DAC). **Métodos:** Ensaio clinico randomizado em pacientes com DAC e periodontite severa, maiores de 18 anos, ambos os sexos, atendidos em um hospital público universitário no sul do Brasil. O grupo teste recebeu tratamento periodontal intensivo com uma sessão de raspagem, alisamento e polimento supragengival (RAP) e orientação de higiene bucal, além de até quatro sessões de raspagem e alisamento radicular subgengival (RASUB) por quadrante, em um período máximo de 14 dias. O grupo controle recebeu uma única sessão de RAP, além de orientação de higiene bucal. A função endotelial foi avaliada através da VFM, antes e após três meses do tratamento periodontal. **Resultados:** Nesta análise interina (amostra total 84), foram incluídos 69 pacientes, 31 no grupo teste e 38 no controle. O grupo teste apresentou condição periodontal significativamente melhor aos 3 meses no índice de placa visível (24,58%±23,36 vs. 48,77%±20,62), profundidade de sondagem (2,27±0,51 vs. 3,16±0,73), perda de inserção (4,31%±1,26 vs. 4,91%±1,35) e sangramento subgengival (34.08%±33.32 vs. 71.74%±21.39). Após tratamento, houve melhora das medidas da VFM (hiperemia reativa) nos grupos teste e controle (1,39% vs. 1,37%; p=0,84). **Conclusão:** Resultados preliminares indicam efeito semelhante na função endotelial, independente do tratamento periodontal em pacientes com DAC, durante o seguimento de 3 meses.

Número de Registro: ClinicalTrials.gov (NCT01609725)

Descritores: endotélio vascular; aterosclerose; periodontite.

INTRODUÇÃO

A periodontite é definida como uma doença crônica infecto-inflamatória que afeta os tecidos de suporte do dente, incluindo ligamento periodontal e osso alveolar [1]. O endotélio vascular exerce propriedades regulatórias, secretórias, metabólicas, imunológicas e sintéticas. O desequilíbrio destas funções relaciona-se com processo de disfunção endotelial, aumento de marcadores inflamatórios, aterogênese e risco de eventos cardiovasculares [2]. Estudos epidemiológicos apontam evidências de associação entre periodontite e doença cardiovascular [1,3]. Uma metanálise com estudos de coorte prospectivo avaliou aproximadamente 86000 pacientes em um seguimento de 6 anos. Os resultados indicaram que indivíduos com doença periodontal possuem 14% maior risco de desenvolvimento de doença arterial coronariana (DAC), comparado aos controles (IC 95%, 1,07-1,21; p<0,001) [4]. Os mecanismos envolvidos neste processo referem-se a interações diretas ou indiretas, entre patógenos periodontais, inflamação sistêmica e o endotélio vascular na evolução da aterosclerose [5].

Na avaliação da função endotelial podemos utilizar a aferição da vasodilatação fluxo-mediada (VFM) da artéria braquial [6]. Consistentes dados estabelecem significativa associação inversa entre medidas basais da VFM e risco cardiovascular [7,8]. Uma recente metanálise objetivou avaliar o efeito do tratamento periodontal na função vascular avaliada pela VFM. Em indivíduos com periodontite a diferença da média basal da VFM foi 5,1% (IC 95%, 2,08-8,11; p<0,001) comparados ao grupo controle. Após o tratamento periodontal a diferença da média da VFM entre os grupos teste e controle, foi de 6,64% (IC 95%, 2,83-10,44). Porém, na análise dos estudos incluídos houve elevado nível de heterogeneidade nos resultados, I^2=80,1% e I^2=78% respectivamente [9]. Este mesmo grupo havia publicado um ensaio randomizado, o qual mostrou aumento de 2% na média da VFM (IC 95%, 1,2-2,8; p<0,001) no grupo tratado, 6 meses após a terapia periodontal [10].

Em indivíduos com DAC e periodontite não é de conhecimento dos autores que existam estudos que tenham avaliado o efeito do tratamento periodontal com a função endotelial mensurada pela VFM. Nesta perspectiva, este estudo teve o objetivo de testar o efeito do tratamento da periodontite na função endotelial avaliada pela VFM em pacientes com DAC.

MÉTODOS

Desenho do estudo

Ensaio clínico randomizado controlado, em paralelo, cego para avaliação de desfechos. Conduzido com pacientes cardiopatas crônicos atendidos em hospital público universitário no sul do Brasil, no período de agosto de 2012 a agosto 2014. O estudo foi aprovado pelo comitê de ética e pesquisa (CEP) e todos os pacientes assinaram o termo de consentimento livre e esclarecido (TCLE). O protocolo de pesquisa deste estudo foi registrado no *ClinicalTrials.gov* (NCT01609725) e publicado (Trials 2013 14:283) [11].

Participantes

Foram considerados elegíveis pacientes de ambos os sexos, maiores de 18 anos de idade, com diagnóstico de DAC estável (evento ocorrido há mais de 6 meses) e periodontite crônica severa, todos selecionados em ambulatório especializado. O diagnóstico de DAC foi definido pelo histórico clínico dos pacientes na ocorrência de pelo menos um episódio documentado de síndrome coronariana aguda; revascularização percutânea/cirúrgica; cineangiocoronariografia com evidência de lesões ≥50% em pelo menos uma artéria ou teste de isquemia positivo por método não invasivo [12].

O diagnóstico de periodontite foi definido pelos critérios do *Centers for Disease Control - American Academy of Periodontology (CDC-AAP)* [13], pela presença de pelo menos dois dentes com profundidade de sondagem (PS) ≥5mm e perda de inserção (PI) ≥6mm.

Além disso, os pacientes deveriam apresentar pelo menos 12 dentes presentes. Foram excluídos os pacientes que receberam tratamento periodontal nos últimos seis meses e aqueles que utilizaram antibióticos ou anti-inflamatórios nos últimos três meses anteriores à entrada no estudo.

Intervenções

O tratamento periodontal foi realizado por dois periodontistas experientes, os quais não avaliaram os desfechos clínicos. O grupo teste recebeu tratamento periodontal intensivo. Inicialmente, foi realizada uma sessão de raspagem, alisamento e polimento supragengival (RAP) com orientação personalizada de higiene bucal. Em seguida, foram realizadas até quatro sessões de raspagem e alisamento radicular subgengival (RASUB) por quadrante, sob anestesia local, em um período de no máximo 14 dias. Os pacientes foram acompanhados através de consultas individualizadas de manutenção periodontal (remoção profissional de biofilme dental e reorientação de higiene bucal), a cada mês durante o seguimento de três meses.

O grupo controle consistiu de uma única sessão de raspagem, alisamento e polimento supragengival (RAP), além de orientação de higiene bucal.

Desfecho primário

Função Endotelial

A mensuração não invasiva da função endotelial foi avaliada por um único profissional enfermeiro treinado, o qual era cego para a randomização e tratamento. Utilizou-se na mensuração da vasodilatação fluxo-mediada (VFM) da artéria braquial um equipamento de ultrassonografia bidimensional (2D), modelo *Philips En visor*, com monitor de eletrocardiograma interno e transdutor vascular de alta frequência (7-12 MHz). Os exames ocorreram em uma sala com temperatura controlada e todos os pacientes estavam descansados. Todas as medicações vasoativas foram suspensas pelo menos 4h antes do

exame. Evitar atividade física, alimentos com cafeína ou fumar pelo menos 4 h antes do exame foram outras orientações. Após 15 minutos de descanso em posição supina com o braço em posição confortável foi iniciado o exame. A imagem da artéria braquial foi verificada acima da fossa antecubital em um plano longitudinal. O fluxo foi monitorado com o Doppler posicionado em um ângulo de 65^{o} e obtido imagens entre o lúmen e a parede do vaso (interface intimal anterior e posterior). Um esfigmomanômetro no antebraço foi inflado por 5 minutos em pelo menos 50 mmHg acima da pressão arterial sistólica para a mensuração da hiperemia reativa. As imagens foram estabelecidas 30 segundos antes (basal) e 60 segundos após a desinsulflação do esfigmomanômetro (hiperemia reativa), sincronizadas com a onda "R" do eletrocardiograma. A VFM foi expressa como variação relativa do diâmetro braquial na fase de hiperemia e definida como: [(diâmetro pós hiperemia – diâmetro basal) / diâmetro basal] x 100.

Após 10 minutos da mensuração da hiperemia reativa e normalização do diâmetro arterial foi novamente mensurado o diâmetro arterial basal. Na sequência, após 5 minutos da administração de uma dose (spray) de Nitroglicerina (NTG) sublingual (*Nitrolingual Pumpspray 0.4mg*) ocorreu a avaliação das imagens da vasodilatação endotélio independente. Da mesma forma, a leitura das imagens foi sincronizada com a onda "R" do eletrocardiograma e o cálculo da variação relativa do diâmetro foi definido como: [(diâmetro pós NTG – diâmetro basal) / diâmetro basal] x 100 [6]. Todas as imagens obtidas foram congeladas para obtenção da média de seis medidas do calibre do vaso, realizadas em pontos diferentes do mesmo.

Marcadores sanguíneos

No início do estudo, foram coletados 15ml de sangue de cada participante, com 8h de jejum, de uma veia da região antecubital para a mensuração de marcadores de risco cardiovascular no sangue. Parte do sangue foi imediatamente levada para o laboratório do

HCPA para a quantificação do perfil lipídico e glicêmico. Outra parte foi centrifugada em uma centrífuga refrigerada (ALC PK 120 R, ALC International, Milan, Italy) a 4^{o}C e 4.000 rpm, durante 10 minutos. O soro foi congelado a -80^{o}C em microtubos *(Eppendorfs)*, devidamente identificados, para quantificação da concentração de proteína C reativa (PCR).

Os marcadores de risco cardiovascular sanguíneos avaliados foram a PCR, glicose, hemoglobina glicada, triglicerídeos (TG), colesterol total (CT), lipoproteina de alta e baixa densidade (HDL-C e LDL-C). Os valores obtidos para os marcadores cardiovascular foram mensurados, registrados e classificados de acordo com as Diretrizes da Sociedade Brasileira de Cardiologia [14,15].

A glicose, TG, CT e HDL-C foram mensurados pelo método colorimétrico-enzimático automatizado (ADVIA 1800, Siemens, Germany). Para a determinação quantitativa da PCR foi realizado o método de imunoturbidimétrico com látex (CRP_2). A LDL-C foi calculada usando a formula de *Friedewald* [LDL-C=CT – HDL-C – (TG/5)]. O valor da hemoglobina glicada foi obtida por cromatografia (Merck-Hitachi L-9100, Merck, Germany).

Cálculo do tamanho da amostra

O tamanho da amostra foi calculado considerando a VFM como o desfecho primário do presente estudo. O tamanho amostral foi estimado considerando uma diferença de 1% na média da VFM entre os grupos e um desvio padrão de 1,5. Além disso, erros alfa e beta de 5% e 20%, respectivamente, foram considerados. Esperando uma perda de 15% durante o seguimento, foi estimado que 42 pacientes em cada grupo seriam necessários para a realização do estudo (n=84). Na presente análise interina, foram incluídos 69 pacientes, 31 no grupo teste e 38 no grupo controle.

Randomização

Os pacientes foram alocados em grupos teste e controle através de randomização em blocos de 10. Um programa específico *(randomization.com)* para a alocação aleatória nos grupos teste e controle foi utilizado.

Cegamento e sigilo de alocação

Todo o procedimento de randomização da amostra foi realizado por um assistente externo ao estudo, garantindo sigilo de alocação dos pacientes utilizando códigos numéricos aos pacientes e envelopes opacos. O avaliador da VFM foi cegado, não sabendo, portanto o grupo o qual o paciente pertencia.

Exame periodontal

O exame clínico periodontal foi conduzido por um examinador calibrado para avaliar as condições periodontais de cada paciente. Foram utilizados espelho bucal e sonda periodontal manual milimetrada tipo *Williams*. Todos os dentes permanentes erupcionados, excluindo-se os terceiros molares, foram examinados em seis sítios por dente. Registraram-se os índices de placa visível (IPV), recessão gengival, profundidade de sondagem (PS) e o sangramento subgengival (SS). A perda de inserção clínica (PI) foi obtida pela soma de PS e recessão gengival.

Dados clínicos e demográficos

Um questionário estruturado foi aplicado para todos os participantes para obtenção dos dados clínicos e demográficos com a finalidade de caracterizar a amostra do estudo. Os dados clínicos incluíram: idade, peso, altura, história da doença atual e pregressa, comorbidades e tratamento farmacológico atual. Os dados demográficos incluíram: situação socioeconômica e nível escolar.

Classificou-se como paciente diabético aquele que recebeu tratamento medicamentoso para esse fator de risco, referiu que tem a doença e/ou o diagnóstico descrito no prontuário. A

pressão arterial foi verificada usando um esfigmomanômetro colocado no braço esquerdo, após o paciente permanecer sentado e descansado por 15 minutos antes da avaliação da VFM. Classificou-se como fumante aquele paciente que fumava regularmente, no mínimo 01 cigarro ao dia ou fumou no período <6 meses à inclusão do estudo; não fumante aquele indivíduo que nunca fumou ou parou de fumar a 10 anos anteriores à inclusão do estudo; ex-fumante o indivíduo que não fuma a pelo menos 6 meses à inclusão do estudo. Definiu-se como indivíduo que realiza atividade física aquele que referiu exercer esta prática de forma moderada, pelo menos 5 vezes por semana, durante 30 minutos. O Índice de Massa Corporal (IMC) foi calculado através da divisão do peso (kg) pelo quadrado da altura (m) [peso (kg)/ altura (m^2)].

Reprodutibilidade dos examinadores

Antes da realização do estudo, o examinador periodontal realizou procedimento de calibragem com exames repetidos de PS e recessão gengival, com intervalo de uma hora, em 10 pacientes periodontais que não participaram do estudo. Os valores de Kappa ponderado (considerando erro de ±1mm) para PS e PI foram de 0,91 e 0,88, respectivamente.

Um único examinador treinado realizou as medidas de VFM. A correlação interobservador das medidas (% de variação) na VFM da hiperemia reativa e da vasodilatação endotélio independente foi de 0,89 e 0,93 respectivamente. Considerando um nível de significância de $\alpha = 0,05$, um poder de 80% e uma diferença de 3% na média da VFM, assumindo um desvio padrão de 5%, um total de 11 indivíduos da amostra foram avaliados aleatoriamente.

Análise estatística

O desfecho primário do presente estudo foi a VFM, após hiperemia reativa e a avaliação da vasodilatação endotélio independente. Essas duas variáveis apresentaram distribuição assimétrica e, portanto, testes não-paramétricos foram aplicados (teste de Mann-

Whitney e Wilcoxon). As variáveis categóricas foram comparadas no basal entre os grupos usando testes de quiquadrado ou exato de Fisher quando apropriado.

Médias para cada indivíduo de IPV, PS, PI e SS foram calculadas. Essas variáveis periodontais foram comparadas entre os grupos com testes de Wald.

Um pacote estatístico (STATA-versão 10) foi utilizado nas análises. Um valor de p <0,05 bicaudal foi considerado estatisticamente significativo.

RESULTADOS

No total de 428 pacientes elegíveis, 262 foram excluídos por não preencherem os critérios de inclusão, 60 não quiseram participar do estudo, 19 não compareceram na primeira avaliação periodontal e 11 apresentaram um novo evento ou morte antes da randomização. Assim sendo, foram randomizados 76 pacientes, 36 no grupo teste e 40 no grupo controle. Destes, 5 pacientes do grupo teste e 2 do controle não receberam a intervenção, pois apresentaram um novo evento, morreram ou não compareceram na primeira avaliação. No final de 3 meses de seguimento, 31 pacientes do grupo teste e 38 do grupo controle foram analisados (Figura 1).

Características basais e fatores de risco cardiovascular

As características basais dos pacientes estão descritas na Tabela 1. Não foram observadas diferenças significativas entre os grupos em nenhuma das variáveis. A média de idade da amostra foi de 61,7±8,3 anos e 58,6±8,5 anos nos grupos controle e teste respectivamente. A maioria dos pacientes era do sexo masculino nos dois grupos e estavam bem controlados para triglicerídeos, colesterol total, LDL-C e hemoglobina glicada. O controle do HDL-C foi alcançado em aproximadamente um terço dos pacientes em cada grupo. O diâmetro basal da artéria braquial foi igual a 0,41cm e 0,43cm nos grupos controle e teste (p=0,07), respectivamente.

Resposta periodontal

O grupo teste apresentou condição periodontal significativamente melhor aos 3 meses comparado ao grupo controle (Tabela 2). Não foram observadas diferenças significativas entre os grupos controle e teste nos níveis basais de placa, PS, PI e SS. Nos dois grupos houve redução significativa de placa visível após 3 meses, porém esta redução foi significativamente maior no grupo teste do que no controle. Ao final de 3 meses, não foram observadas diferenças significativas na PS e PI nos pacientes do grupo controle. Diferentemente, PS e PI reduziram significativamente no grupo teste. Aos 3 meses, PS e PI foram significativamente menores no grupo teste do que no controle. Ambos os grupos reduziram SS após 3 meses, porém a redução foi de magnitude maior no grupo teste, sendo o SS final significativamente menor neste grupo do que no grupo controle.

Função endotelial

A Figura 2 ilustra os resultados obtidos para VFM ao longo do estudo. Não houve diferença significativa entre os grupos na avaliação basal da hiperemia reativa (7,10±6,09 e 7,05±5,6 controle e teste, respectivamente). Após tratamento, houve melhora não significativa de 1,37% e 1,39% nos grupos controle e teste, respectivamente, sem diferença significativa entre os grupos (p=0,84). O mesmo padrão foi observado na mensuração da vasodilatação endotélio independente. Não houve diferença significativa entre os grupos nas medidas basais (12,3±6,9 e 13,9±7,6 controle e teste, respectivamente) e nas finais (11,9±8,4 e 13,3±6,8 controle e teste, respectivamente; p=0,68). Quando a amostra foi avaliada como um todo sem considerar os grupos de alocação, também não foram observadas diferenças significativas na melhora da vasodilatação após 3 meses (redução de 1,38%, p=0,15).

DISCUSSÃO

O presente estudo objetivou determinar o efeito da terapia periodontal intensiva na função endotelial de pacientes com DAC. O tratamento periodontal intensivo foi significativamente superior na redução do quadro inflamatório periodontal e no ganho de inserção clínica comparado a uma única sessão de raspagem supragengival. Por outro lado, não foi observado um efeito positivo na VFM que tenha sido superior no grupo de tratamento periodontal teste comparado ao tratamento controle.

A resposta ao tratamento periodontal observada no presente estudo foi semelhante, ou até mesmo superior, àquela observada em estudos anteriores [10,16]. A retomada da saúde periodontal em estudos de intervenção na área da medicina periodontal é de fundamental importância metodológica, uma vez que a ausência de um impacto sistêmico após tratamento periodontal não pode ser explicado pela ausência de resposta terapêutica periodontal. Neste sentido, cabe salientar que a falta de diferença significativa entre os grupos na VFM observada no presente ensaio controlado não deve ser atribuída a falha do tratamento periodontal teste, mas sim a outros aspectos a serem discutidos.

A literatura é consistente quanto a existência de correlação positiva direta e indireta entre periodontite e DAC. Em um estudo de metanálise evidenciou que indivíduos com doença periodontal possuem 14% maior risco de desenvolvimento de DAC, comparado aos controles [4]. A infecção periodontal contribui para inflamação sistêmica indicada pela elevação de biomarcadores inflamatórios como PCR, fibrinogênio e interleucinas, os quais são determinantes para o desenvolvimento da aterosclerose [17]. Nosso grupo publicou um estudo transversal, o qual encontrou associação significativa de periodontite severa com elevados níveis de PCR em pacientes com DAC estável [18]. O perfil de risco cardiovascular em pacientes tratados para periodontite foi avaliado em outro recente estudo. Destacadamente, pacientes com periodontite e outras co-morbidades (doença cardiovascular,

síndrome metabólica) apresentaram melhor benefício após a terapia periodontal na redução da PCR, interleucina-6, triglicerídeos, colesterol total, HDL-C e hemoglobina glicada [1].

O endotélio vascular atua como uma barreira de permeabilidade seletiva entre os meios extra e intravascular. Além disso, exerce propriedades anti-inflamatória, antitrombótica e anticoagulante [19]. A disfunção endotelial se mostra como um preditor independente para eventos cardiovasculares em estudos epidemiológicos [20,21]. Neste sentido, tem sido demonstrado na literatura que a periodontite pode alterar a função endotelial avaliada por diversos desfechos, incluindo a VFM. Orlandi et al. [9] encontraram em uma revisão sistemática três estudos observacionais transversais tendo como desfecho a VFM e, após meta-analise, demonstraram uma redução significativa na VFM de 5,1% em pacientes com periodontite comparados aos periodontalmente saudáveis. Nesta mesma revisão sistemática, foram encontrados 6 ensaios controlados do efeito do tratamento periodontal na VFM, sendo três incluídos em meta-análise que demonstrou um aumento na VFM de 6,64% com alta heterogeneidade.

Os achados do presente ensaio clínico randomizado não estão de acordo com os dados acima reportados na literatura, tanto na ausência de diferença entre grupos teste e controle, quanto na magnitude do efeito na VFM. Por outro lado, há que se considerar que o presente estudo é único no que se refere à população estudada de pacientes cardiopatas crônicos. Todos os estudos até então publicados incluíram somente pacientes sistemicamente saudáveis sem doença cardiovascular. Além disso, os pacientes do presente estudo já estavam em acompanhamento cardiovascular, recebendo medicações e outros cuidados, o que pode explicar a melhora na VFM também observada no grupo controle.

As melhoras na VFM observadas nos dois grupos de tratamento periodontal do presente estudo podem ser, por outro lado, discutidas na perspectiva do cuidado cardiovascular provido aos pacientes. Isto inclui uma abordagem para modificações no estilo

de vida com impacto em redução do risco cardiovascular, já demonstrada em estudos anteriores do grupo [22]. Neste sentido, uma revisão sistemática de estudos prospectivos [7] mostrou que a VFM foi significativamente e inversamente associada com risco de eventos cardiovascular em diversas populações, com uma estimativa de risco de 0,90 para 1% de aumento dos valores da VFM (IC 95%, 0,86-0,94; p<0,01). Além disso, foi discutido nesta revisão que este 1% de aumento na VFM em populações doentes possa ser relativamente mais importante em termos de risco cardiovascular do que em populações com endotélio vascular saudável. Estabelecendo um comparativo com esses achados, no presente estudo em pacientes com DAC, os valores da VFM melhoraram em 1,37% e 1,39% nos grupos controle e teste, respectivamente. Dessa forma, do ponto de vista clínico houve benefício na redução de risco cardiovascular em toda amostra.

Os resultados dos diversos estudos e cenários não podem provar que a VFM é um fator de risco causal para doença cardiovascular, mas podemos levantar a hipótese de que a piora da função endotelial contribui para a deterioração vascular e acelera o processo aterosclerótico. Com isso, o impacto da terapia periodontal em pacientes com DAC na avaliação de desfechos cardiovasculares é ainda inconsistente. Futuros estudos são necessários para determinar se o tratamento da periodontite poderá contribuir para a prevenção da aterosclerose e eventos cardiovasculares. Nosso grupo vem trabalhando na inclusão de novos pacientes. O objetivo é seguí-los por um ano e avaliar outros parâmetros de risco cardiovascular como marcadores inflamatórios e moléculas de adesão.

LIMITAÇÕES

Os achados do presente estudo devem ser interpretados a luz das possíveis limitações metodológicas presentes. Um primeiro aspecto se refere ao tamanho amostral que, neste momento, é menor do que aquele *a priori* calculado. Mesmo assim, deve-se ressaltar que o

efeito do tratamento periodontal parece ser pequeno nesta amostra e que o aumento do tamanho amostral não altere o resultado do estudo. Apesar de o impacto sistêmico do tratamento periodontal acontecer em um período de até três meses, é objetivo deste estudo seguir o acompanhamento dos pacientes por até um ano, pois neste período ainda podem acontecer mudanças inflamatórias sistémicas que possam contribuir com a melhora na função endotelial.

CONCLUSÃO

Resultados preliminares indicam efeito semelhante na função endotelial, independente do tratamento periodontal em pacientes com DAC, durante o seguimento de 3 meses.

AGRADECIMENTO

Os autores agradecem à contribuição de todos os pacientes que fizeram parte deste estudo.

CONFLITO DE INTERESSE

Não declarado

FINANCIAMENTO

Fundo de Incentivo a Pesquisa e Eventos do Hospital de Clinicas de Porto Alegre. CNPq Edital Universal 2010.

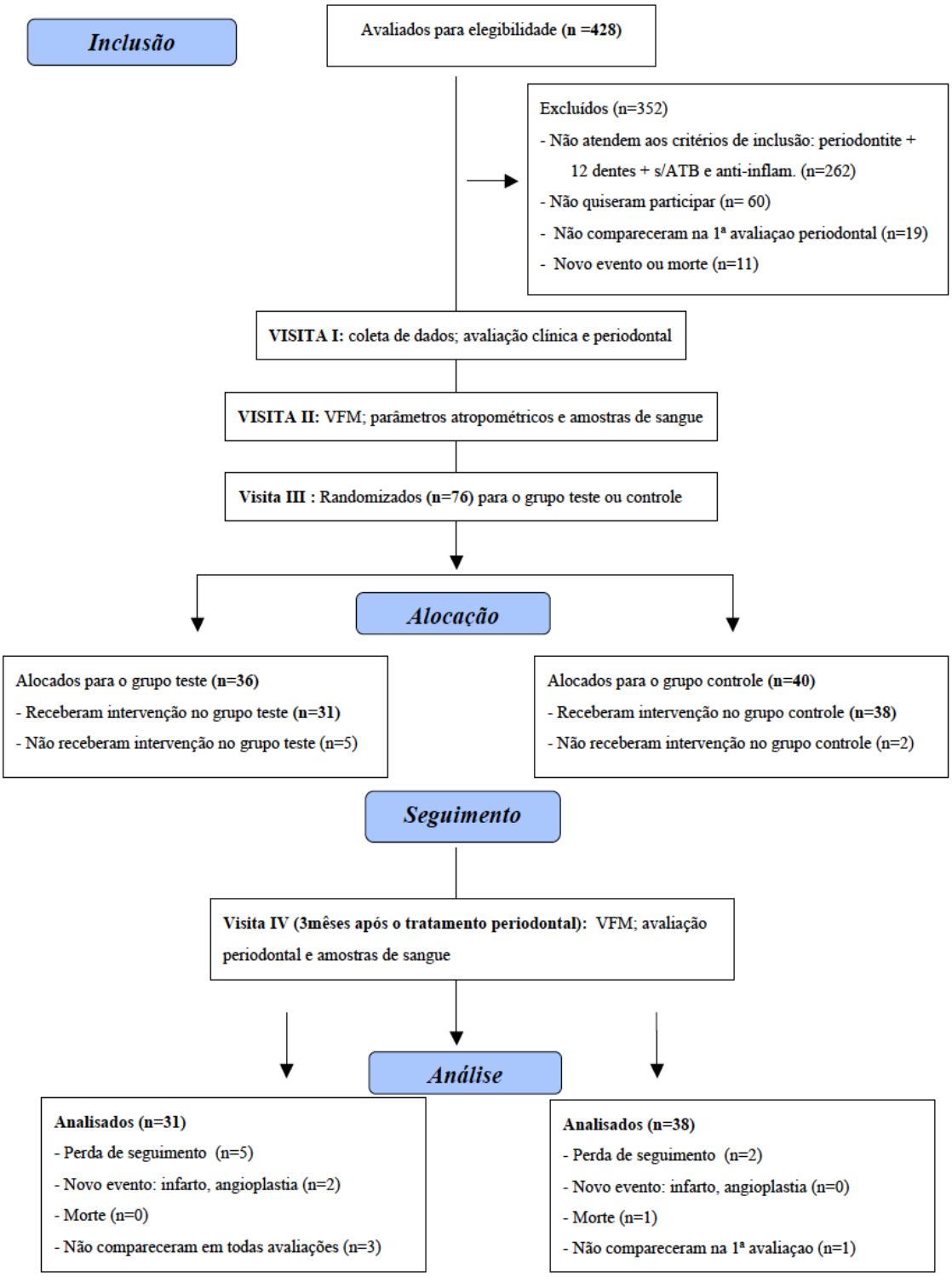

Figura 1. Diagrama de fluxo dos pacientes alocados (Consolidated Standards of Reporting Trials-CONSORT).

Tabela 1. Características da amostra na avaliação inicial.*

Variável	Grupo controle (n=38)	Grupo teste (n=31)	p†
Idade (média±DP)	61,7±8,3	58,6±8,5	0,14
Sexo masculino (n/%)	28 (74)	24 (77)	0,78
Fumo			
Não fumante	10 (26)	7 (23)	
Ex-fumante	24 (63)	20 (64)	
Fumante	4 (11)	4 (13)	0,18
Raça branca	33 (87)	24 (77)	0,35
Ocupação (inativos)	28 (74)	20 (64)	0,44
Educação			
≤8 anos de estudo	25 (66)	16 (52)	
≥9 anos de estudo	13 (34)	15 (48)	0,33
Diabetes	16 (42)	13 (42)	1,00
Sedentarismo	25 (66)	19 (61)	0,80
Colesterol total (mg/dl)	163±42	181±49	0,10
Triglicerídeos (mg/dl)	172±117	217±140	0,14
LDL-C (mg/dl)	92±40	98±39	0,57
HDL-C (mg/dl)	39±10	40±11	0,80
Hemoglobina glicada (%)	6,7±1,8	6,6±1,7	0,70
Glicose (mg/dl)	115±41	130±61	0,23
Pressão arterial sistólica (mmHg)	126±18	123±19	0,39
Pressão arterial diastólica (mmHg)	77±8	77±9	0,67
Índice de massa corporal (Kg/m^2)	28,2±4,1	27±3,6	0,20
Diâmetro arterial (cm)	0,41±0,06	0,43±0,06	0,07
Metas terapêuticas			
PCR <3 mg/dl	16 (51,6)	15 (48,4)	1,00
Triglicerídeos <150mg/dl	23 (60)	15 (48)	0,34
Colesterol total <200mg/dl	29 (76)	22 (71)	0,78
LDL-C <100mg/dl	30 (79)	22 (71)	0,57
HDL-C (homens >40mg/dl - mulheres >50mg/dl)	12 (32)	11 (35)	0,80
Hemoglobina glicada <7%	30 (79)	24 (77)	1,00
Índice de massa corporal (18,5-25 Kg/m^2)	17 (45)	17 (55)	0,50

LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; PCR: Proteína C Reativa; *Variáveis expressas em média±desvio padrão (DP) ou números (%); † Teste t-Student para amostras independentes.

Tabela 2. Condição periodontal nos dois grupos experimentais ao longo do estudo

	Inicial	**3 meses**	**p***
Placa visível (%)			
Controle	75.39±15.54	48.77±20.62	<0.001
Teste	78.20±21.60	24.58±23.36	<0.001
p**	0.54	<0.001	
Profundidade de sondagem			
Controle	3.07±0.54	3.16±0.73	0.25
Teste	3.22±0.54	2.27±0.51	<0.001
p**	0.25	<0.001	
Perda de inserção (%)			
Controle	4.89±1.33	4.91±1.35	0.79
Teste	5.12±1.46	4.31±1.26	<0.001
p**	0.49	<0.001	
Sangramento subgengival (%)			
Controle	88.21±11.56	71.74±21.39	<0.001
Teste	92.49±10.10	34.08±33.32	<0.001
p**	0.10	<0.001	

*Comparação intra-grupo; **Comparação inter-grupos; P-valores do teste de Wald.

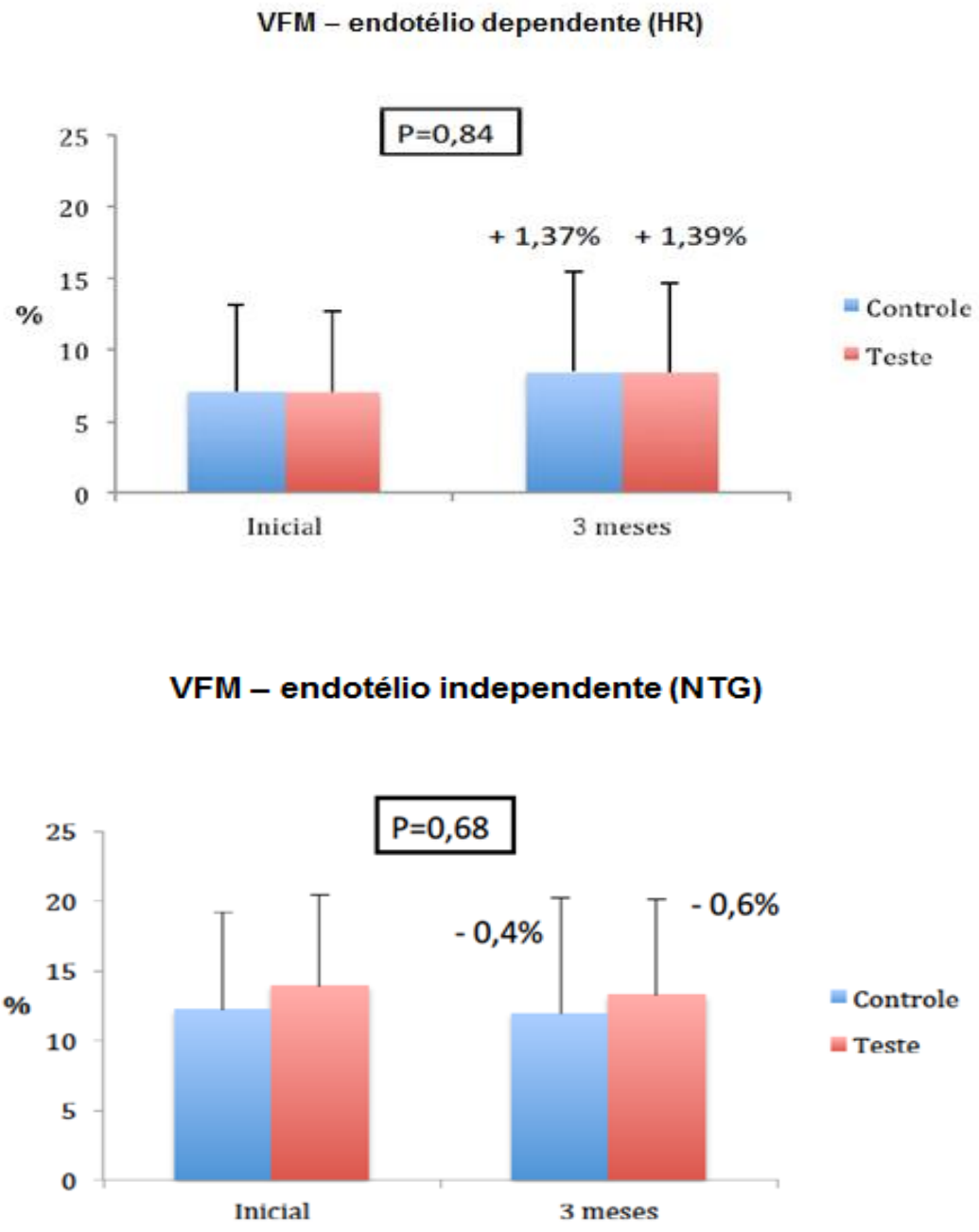

Figura 2. Vasodilatação fluxo-mediada e vasodilatação endotélio dependente ao longo de três meses nos dois grupos.

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CONCLUSÃO E CONSIDERAÇÕES FINAIS

O tratamento periodontal intensivo foi superior na redução da inflamação periodontal e no ganho de inserção clínica comparado ao tratamento comunitário convencional. Os resultados desta análise preliminar permitem concluir que independente do tratamento periodontal, o efeito na função endotelial foi semelhante para ambos os grupos estudados no seguimento de 3 meses.

É importante salientar que o presente estudo é único no que se refere à população de pacientes cardiopatas crônicos. Todos os pacientes já estavam recebendo tratamento otimizado em um ambulatório especializado, além de orientações para controle de fatores de risco cardiovascular o que pode explicar a melhora na função endotelial em ambos os grupos.

O período de seguimento dos pacientes talvez tenha sido curto para que as diferenças entre os grupos na função endotelial pudessem ser identificadas. Como perspectivas futuras do grupo iremos finalizar a amostra total, acrescentado a análise de marcadores inflamatórios e moléculas adesão.

ANEXOS E APÊNDICE

ANEXO 1- CARTA DE APROVAÇÃO DO PROJETO

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
GRUPO DE PESQUISA E PÓS-GRADUAÇÃO

COMISSÃO CIENTÍFICA

A Comissão Científica do Hospital de Clínicas de Porto Alegre analisou o projeto:

Projeto: 120265
Data da Versão do Projeto:

Pesquisadores:
ENEIDA REJANE RABELO DA SILVA
MARCO AURELIO LUMERTZ SAFFI

Título: ESTUDOS DA RELAÇÃO ENTRE DOENÇA PERIODONTAL E FUNÇÃO ENDOTELIAL EM PACIENTES COM DOENÇA ARTERIAL CORONARIANA

Este projeto foi APROVADO em seus aspectos éticos, metodológicos, logísticos e financeiros para ser realizado no Hospital de Clínicas de Porto Alegre.
Esta aprovação está baseada nos pareceres dos respectivos Comitês de Ética e do Serviço de Gestão em Pesquisa.

- Os pesquisadores vinculados ao projeto não participaram de qualquer etapa do processo de avalição de seus projetos.

- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao Grupo de Pesquisa e Pós-Graduação (GPPG)

Porto Alegre, 14 de agosto de 2012.

Profª Nadine Clausell
Coordenadora GPPG

ANEXO 2- TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Estamos realizando um estudo para avaliar a relação entre doenças de gengiva e doença do coração. O estudo está sendo realizado por professores e pesquisadores das Faculdades de Odontologia e de Medicina da Universidade Federal do Rio Grande do Sul, em conjunto com o Hospital de Clínicas de Porto Alegre. As pessoas a participarem do estudo são aqueles pacientes que estão em acompanhamento no Ambulatório de Cardiopatia Isquêmica (CPI) e do ambulatório da faculdade de Odontologia.

Durante a pesquisa, serão realizados exames bucais avaliando a presença de doenças de gengiva. Além disso, será realizada uma entrevista sobre dados pessoais, socioeconômicos e comportamentais. Estes dados serão cruzados com os dados médicos presentes no prontuário do Ambulatório CPI onde você é atendido para seu problema cardíaco.

Caso seja detectada doença de gengiva grave, será oferecido tratamento odontológico. Você será sorteado para receber um tratamento periodontal intensivo ou um tratamento periodontal comunitário. O tratamento comunitário consiste do mesmo tratamento realizado no SUS, sendo realizada uma única consulta de raspagem superficial de tártaro e orientação de limpeza dos dentes. Caso ocorra piora dos problemas de gengiva em qualquer dente ao longo do estudo, você será removido do estudo e receberá tratamento de acordo com suas necessidades seguindo o protocolo intensivo. Ao final de 12 meses, os pacientes do grupo de tratamento comunitário receberão o tratamento intensivo. O tratamento intensivo consiste de uma consulta de raspagem superficial de tártaro seguida de quatro consultas de raspagem profunda em um período de no máximo 15 dias, com anestesia local para evitar dor. Os tratamentos serão realizados por dentistas especialistas no tratamento das doenças gengivais. Ao longo de 12 meses após o tratamento, você receberá consultas de manutenção uma vez ao mês. Juntamente com os exames clínicos bucais serão realizados exames laboratoriais comumente utilizados para a avaliação de substâncias relacionadas a doenças cardíacas. Será

feita a coleta de 15mL de sangue para a avaliação de colesterol, HDL/LDL, triglicerídeos, glicemia em jejum e marcadores de função endotelial. Essa coleta de sangue será realizada no início do estudo e 6 meses após a primeira intervenção. Além disso, será avaliado, por meio de um aparelho de ecografia, o seu vaso sanguíneo. Este exame deverá ser realizado antes do tratamento periodontal, após 30 dias, 3 e 6 meses após o início da intervenção.

Os possíveis desconfortos associados à participação neste estudo são aqueles decorrentes da realização de um tratamento das doenças da gengiva, da coleta de sangue e do exame a ser realizado. Todas as medidas de biossegurança necessárias tais como uso de materiais descartáveis e instrumentais esterilizados, serão adotadas. Adicionalmente toda e qualquer ocorrência durante o tratamento estará sendo avaliada.

Os benefícios relacionados à participação neste estudo são o diagnostico de problemas de gengiva e o tratamento da doença caso seja detectada, bem como encaminhamento para o tratamento de outras condições bucais, quando necessário. Fica ainda assegurado o direito ao sigilo de todas as informações coletadas, não sendo permitido acesso por outra pessoa que não o próprio participante ou responsável. O tratamento da doença de gengiva será gratuito. Tratamentos outros, como próteses, quando solicitados pelo paciente, terão o custo que normalmente é cobrado pela Faculdade de Odontologia.

Fica, ainda, assegurada a liberdade dos participantes de recusarem-se a participar ou retirarem-se do estudo a qualquer momento que desejarem, sem que isso traga prejuízos na assistência médica. A continuidade do tratamento da doença gengival será garantida mesmo que os participantes desejem se retirar do estudo.

Toda e qualquer dúvida no decorrer do estudo poderá ser esclarecida pelos envolvidos nesta pesquisa através dos telefones (51) 96965291 e (51) 33085318. Os pesquisadores Marco Saffi, Carisi Polanczyk e Eneida Rejane Rabelo da Silva estarão sempre à disposição

para esclarecimentos. Possíveis problemas podem ser reportados diretamente ao Comitê de Ética Central da UFRGS 33083629 ou Comitê de Ética do HCPA 33597640.

Eu, _____ (participante), declaro que fui informado dos objetivos e procedimentos que serão realizados nesta pesquisa, bem como sei dos meus direitos e dos deveres dos pesquisadores. Declaro, ainda, que recebi uma cópia deste Termo.

_____

Participante:

Porto Alegre, _____ de _____ de 201_.

_____

Pesquisador

Porto Alegre, _____ de _____ de 201_.

ANEXO 3 – APROVAÇÃO DO PROJETO NA PLATAFORMA BRASIL

Plataforma Brasil - Ministério da Saúde

Hospital de Clínicas de Porto Alegre - HCPA / UFRGS

PROJETO DE PESQUISA

Título: ESTUDOS DA RELAÇÃO ENTRE DOENÇA PERIODONTAL E FUNÇÃO ENDOTELIAL EM PACIENTES COM DOENÇA ARTERIAL CORONARIANA

Área Temática:

Pesquisador: Eneida Rejane Rabelo da Silva **Versão:** 2

Instituição: Hospital de Clínicas de Porto Alegre - HCPA / UFRGS **CAAE:** 04999712.8.0000.5327

PARECER CONSUBSTANCIADO DO CEP

Número do Parecer: 65342

Data da Relatoria: 01/08/2012

Apresentação do Projeto:

Pacientes com cardiopatia isquêmica (DAC) e periodontite apresentam alteração na função endotelial em comparação com pacientes com DAC e sem periodontite. Além disso, o tratamento periodontal intensivo em pacientes com DAC, através de intervenções e consultas de manutenção melhora a função endotelial mensurada pela vasodilatação fluxo mediada e promove a redução dos níveis de marcadores endoteliais comparados ao tratamento convencional comunitário.

Objetivo da Pesquisa:

Estudar as associações entre doença periodontal e função endotelial em pacientes com doença arterial coronariana crônica.

Avaliação dos Riscos e Benefícios:

Não são conhecidos riscos aos participantes. Benefícios: Avaliação e tratamento da condição periodontal e da função endotelial nos pacientes pertencentes ao estudo ao longo do seguimento.

Comentários e Considerações sobre a Pesquisa:

Hipótese pertinente. Estudo bem delineado.

Considerações sobre os Termos de apresentação obrigatória:

Os pesquisadores apresentam Termo de Consentimento Livre e Esclarecido (TCLE) convidando os participantes para o estudo e TCLE para avaliação do endotélio através de ecografia.

Recomendações:

Para a aprovação final do projeto é necessário que além da aprovação ética e metodológica pela Plataforma Brasil o projeto esteja aprovado na WebGPPG (orçamento e logística).

Conclusões ou Pendências e Lista de Inadequações:

Não foi apresentado orçamento. Incluir.
PENDÊNCIA ATENDIDA.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Projeto aprovado versão 25/06/2012.
TCLE aprovado versão 26/06/2012.

PORTO ALEGRE, 01 de Agosto de 2012

Assinado por:

APÊNDICE A- INSTRUMENTO DE COLETA DE DADOS DO ESTUDO

Estudos da relação entre doença periodontal e função endotelial em pacientes com doença arterial coronariana

Número ID: ☐☐☐

1-IDENTIFICAÇÃO

Nome: Idade:

Prontuário:

Data de nascimento: __/__/____

Telefone: Cor: branca ☐ preta ☐

Sexo: M ☐ F ☐ Ocupação: Inativo ☐ Ativo ☐

Estado Civil: solteiro ☐ casado ☐ separado/divorciado ☐

2-DOMÍNIO SÓCIO-DEMOGRÁFICO

2.1 Anos completos de estudo? _____

2.2 Renda familiar? 1 salário ☐ 2 salários ☐ 3 salários ☐

4 salários ☐ + de 4 salários ☐

3-ANTECEDENTES PATOLÓGICOS E FATORES DE RISCO

DM ☐ HF ☐ HAS ☐ Dislipidemia ☐

Tabagismo: nunca ☐ passado ☐ atual ☐

Álcool: ☐ Atividade Física: ☐

Peso: _____ Kg Altura: _____ cm IMC:_____

PA/DIAS	BASELINE	30 DIAS	3 MESES	6 MESES
Pressão Arterial				

4-MEDICAÇÕES EM USO

AAS ☐ Clopidogrel ☐ Antagonista Cálcio ☐

Beta Bloqueador ☐ Diurético ☐ Estatina ☐ Hipoglicemiante ☐

IECA ☐ Nitrato ☐ Antiarrítmico ☐

5-BIOQUÍMICA

Exame/Data	BASAL (1)	FINAL (2)
CT		
HDL		
LDL		
TG		
Glicemia		
Hb Glicada		
V-CAM		
I-CAM		
P- Selectina		
PCR		

6- PARÂMETROS DA VFM

VFM/DIAS	BASELINE	30 DIAS	3 MESES	6 MESES
BASAL				
PÓS HIPEREMIA				
% MUDANÇA				
BASAL NTG				
PÓS NTG				
% MUDANÇA				