Influence of treatment of periodontal disease on sirtuin 1 and mannose-binding lectin in individuals with coronary artery disease

Pérola Michelle Vasconcelos Caribé [1,2]
Antonio de Padua Mansur [1]

1. Clinical Department, Heart Institute (InCor), University of São Paulo Medical School, São Paulo, Brazil
2. Division of Periodontology, Stomatology Department, Dental School, University of São Paulo, São Paulo, Brazil

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Advisor: Prof. Dr. Antonio de Pádua Mansur

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The present study does not have NCT

Abstract
Introduction: Increased levels of periodontal pathogens disrupt the homeostasis between the host and its microbiota and increase susceptibility to periodontal disease (PD). PD increases the serum concentration of mannose binding lectin (MBL), which exacerbates local inflammatory processes. In animal studies, sirtuin-1 (SIRT1) was associated with protection against inflammation. This study analyzed the influence of nonsurgical treatment of PD on serum levels of MBL and SIRT1.
Methods: Forty patients with PD and 38 without PD (aged 45-79 years) were evaluated. PD was confirmed by the presence of at least 6 teeth with at least one noncontiguous interproximal site with probing pocket depth and clinical attachment loss ≥ 5 mm, 30% of sites with probing pocket depth and clinical attachment loss ≥ 4 mm, and bleeding on probing. MBL and SIRT1 concentrations were analyzed by ELISA.
Results: For all patients, an inverse correlation was observed between the serum concentrations of MLB and SIRT1 ($r = -0.30$; $p = 0.006$). PD treatment reduced the serum concentration of MBL from 1099.35 ± 916.59 to 861.42 ± 724.82 ng/mL ($p < 0.001$), and CRP showed a reduction from 6.05 ± 8.99 to 2.49 ± 2.89 mg/L ($p=0.009$). By contrast, PD increased the serum SIRT1 concentration from 1.06 ± 1.03 to 1.66 ± 1.64 ng/mL ($p<0.001$).
Conclusion: PD treatment was associated with decreased serum concentrations of MBL and CRP and increased serum levels of SIRT1. Prospective studies are needed to assess the impact of these biomarkers on the progression and prognosis of PD.

Keywords: periodontal disease, atherosclerosis, mannose binding protein, sirtuin, periodontitis, inflammation.

Clinical Relevance

Periodontal disease (PD) or periodontitis is a highly prevalent chronic inflammatory disease that affects the protective and supporting structures of the teeth. [1] Clinically, PD is characterized by the presence of bleeding, edema and reabsorption of alveolar bone tissue. These changes result from the inflammatory activity of bacteria, endotoxins and the cellular/humoral response of the host, which is mediated by polymorphonuclear leukocytes, lymphocytes, immunoglobulins and the complement system. [2] The delicate balance between the host and oral bacteria is controlled by salivary immunoglobulins and lactoferrin, antimicrobial peptides in the epithelium and the oxidative and nonoxidative death systems of neutrophils, as well as the classical complement system pathway with its lectin, which form the first line of defense against bacteria. [3, 4] Mannose binding protein (MBL) is a serum protein synthesized in the liver [5] and can bind mannose residues or other carbohydrates commonly present in various pathogens. [6] After binding, MBL activates the complement system via lectins and mediates phagocytosis. [7] Unlike other collectins, MBL can activate the complement system in the absence of antibodies. Important periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, present mannose rich polysaccharides on their cell surfaces that are potentially capable of binding MBL and undergoing phagocytosis. Thus, MBL promotes defense against invading pathogens, but at high levels, it can be harmful, causing too much local and systemic inflammation through the activation of the complement system, aggravating inflammatory diseases and increasing tissue damage. [8]

Sirtuins are a class of proteins that regulate a variety of cellular functions, such as genome integrity and cellular metabolism, and are associated with longevity. [9] Most sirtuins (SIRT1, SIRT2, SIRT3 and SIRT5) catalyze NAD^+ deacetylation that is dependent on protein lysine residues. SIRT1 is the most studied sirtuin and is involved in several central metabolic pathways. [10] The beneficial effects of SIRT1 on inflammation have been well documented in preclinical and animal studies. [11] Mansur et al. [12] showed an increase in serum levels of SIRT1 in normal subjects undergoing caloric restriction and after resveratrol administration. According to Moschen et al., [13] weight loss that increases the expression of SIRT1 is probably a consequence of reduced inflammation. Several studies have suggested that metabolic dysfunction in obesity is associated with inflammation in adipose tissue. [14] Our present study is the first to assess the impact of PD treatment on serum levels of SIRT1. In vitro, Lee et al. [15] identified the role of SIRT1 in human periodontal ligament cells by measuring the gene expression and SIRT1 protein during the process of human periodontal ligament cell formation. These authors used pieces of periodontal ligament obtained from extracted dental roots and studied the effects of overexpression and lower expression of SIRT1 during the differentiation of human periodontal ligament cells, as well as the signaling mechanisms involved. Lee and colleagues concluded that SIRT1 is a potent regulator of the differentiation of human periodontal ligament cells and may have clinical implications for periodontal bone regeneration.

In our study, we utilized periodontal treatment focused on the elimination of pathological oral microorganisms that avoided exacerbation of inflammatory

processes, with the aim of increasing the levels of SIRT1 and reducing serum levels of MBL.

Methods

This prospective study analyzed 78 patients (aged 45-79 years old, 38 women and 42 men) from October 2016 to September 2018. Patients were distributed in two groups according to the presence (N=40) or absence of PD (N=38). The inclusion criteria for PD were the presence of at least 15 teeth (excluding third molars) and clinical diagnosis of PD. This diagnosis was confirmed by the presence of at least 6 teeth with at least one noncontiguous interproximal site with probing pocket depth (PPD) and clinical attachment loss (CAL) ≥ 5 mm, as well as 30% of sites with PPD and CAL ≥ 4 mm and bleeding on probing (BOP).[16] PD is currently classified as Stage III Degree B. [17] Individuals who presented a periodontium without loss of insertion, with PPD ≤3 mm, BOP in less than 10% of sites and no radiographic bone loss were classified as periodontal healthy. [18]

The exclusion criteria of the study were uncontrolled diabetes, chronic renal disease, smoking, HIV and hepatitis B and C, pregnancy, edentulous jaws, orthodontic brachytherapy using specific drugs known to affect periodontal tissues, previous periodontal treatment (minimum of 6 months), anti-inflammatory drugs and corticosteroids, and allergies to the antibiotics prescribed in this protocol. The study was approved by the Ethics Committee for Analysis of Research Projects-CAPPesq, Hospital das Clínicas, Faculty of Medicine, University of São Paulo (HC-FMUSP), CAAE: 55556116.0.0000.0068. All participants signed a consent form.

Nonsurgical Periodontal Therapy

Nonsurgical periodontal treatment was performed, including oral hygiene education, scaling, smoothing and coronal-radicular polishing (RAR). Six sites were evaluated in each tooth (mesiobuccal, buccal, distobuccal, distolingual/palatal, lingual/palatal and mesiolingual/palatal surfaces). Scaling and root planing were performed using mechanical devices—ultrasound and manual instruments. The treatment was administered with local anesthesia, 3% lidocaine with a vasoconstrictor, for PPD ≥ 5 mm. The objective of each session was to achieve a smooth surface devoid of biofilm and calculus.

The following parameters were evaluated during clinical examination: PPD, distance of the enamel-cementum line at the gingival margin, CAL, plaque index (IP)[19] and BOP.[20] PPD and CAL measurements were rounded to the nearest millimeter using a North Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA). Patients in this study also received the antibiotics metronidazole (1.2 g/d/14 days) and amoxicillin (1.5 g/d/14 days).

The calibration of the examiner was aimed at establishing consistency and obtaining confidence in the results of periodontal exams. After performing two periodontal exams in 10 individuals with a one-week interval, the result was submitted to the intraclass correlation test. The intraexaminer agreement of CAL was verified by the examination of 1074 sites of 8 patients in 2 exams with an interval of 2 weeks. Reproducibility was assessed using the intraclass correlation coefficient (Table 1).

A significant correlation was found between the two exams [(R = 0.95 (95% CI: 0.94-0.96, p <0.001).) According to Evans, [21] a correlation between 0.80 and 1.0 is considered strong.

Laboratory Tests

A 10 ml sample of blood from the peripheral vein was collected at baseline and at the end of the study after 8-12 hours of fasting. Laboratory tests analyzed triglycerides, total cholesterol, HDL-cholesterol, glucose and CRP. Plasma glucose, triglycerides and HDL-cholesterol were obtained by a commercial calorimetry-enzymatic method. Measurements were performed with Dimension RxL (Siemens Healthcare Diagnostic Inc., Newark, DE, USA) with dedicated reagents. The LDL-cholesterol value was calculated by the Friedewald equation. The determination of ultrasensitive C-reactive protein was performed by immunonephelometry with dedicated reagents on Siemens Healthcare BN-II equipment (Marburg, Hessen, Germany). Serum MBL levels were determined by enzyme linked immunosorbent assay (ELISA) using anti-MBL monoclonal antibody HYB 131-01 (BioPorto Diagnostics A/S, Copenhagen, Denmark). Sirt1 concentrations were determined using an ELISA kit (Usin Life Science, Wuhan, Hubei, China). Before and after interventions, SIRT1 samples were analyzed in duplicate on the same ELISA plate using a Multiscan FC plate reader (Thermo Scientific), with a coefficient of variation of 12%, according to the manufacturer's instructions.

Statistical Analyses

Sample size was calculated by the difference in serum levels of SIRT1 pre- and postperiodontal treatment. The difference between the means for the control group was 1 mg, and the standard deviation was 1 mg. For the group of

patients with PD, the difference was 2 mg, with a standard deviation of 1 mg. The power of the test was $\beta = 0.90$ and $\alpha = 0.05$. The estimated sample was 20 individuals for each group. The expected values were based on a previous study performed in our service in normal individuals.[22] The Chi-square test ($\chi 2$) was used for categorical variables. Correlations between studied variables were performed by the Spearman correlation test. Paired Student's t test was used for intragroup analysis between the initial and final values of the protocol. Unpaired Student's t test was used for comparisons between groups. Student's t test was performed for variables with a normal distribution, which was verified by analysis of the equality of variances (folded F method). Depending on the result of this analysis, we used the pooled method (variances with $p \geq 0.05$) or the Satterthwaite method (variances with $p < 0.05$). The SAS statistical program (version 9.2, Institute, Inc., Cary, NC) was used.

RESULTS

Eighty-eight individuals were included in the study. Of these individuals, 8 were lost to follow-up, and 2 were on dialysis and were excluded. The study follow-up time was 80.4 ± 63.3 days. The age, body mass index, and laboratory tests of the 78 patients are shown in Table 1. Of the 78 patients who participated in the study, 40 (51%) were male.

Table 1

In the 78 patients studied, SIRT1 and MBL mean concentrations were 0.92 ng/mL and 899.8 ng/mL, respectively, at the beginning of the protocol and 1.30 ng/mL and 905.5 ng/mL, respectively, at the end of the study.

An inverse correlation was observed between concentration changes (final minus initial values) in MLB and SIRT1 ($r = -0.30$; $p = 0.006$). There was also a positive correlation between MBL concentrations and changes in total cholesterol ($r = 0.30$ and $p = 0.006$), non-HDL ($r = 0.27$ and $p = 0.014$) and LDL ($r = 0.25$ and $p = 0.024$). There was a significant difference in glucose (126.21 ± 56.12 vs. 114.66 ± 32.72 mg/dL, $p = 0.025$) and triglycerides (155.42 ± 105, 71 vs. 128.42 ± 80.51, $p = 0.016$) between the initial and final time points of the protocol (Table 2). In the group without PD, an increased MBL concentration ($p = 0.014$) was observed at the end of the study. No significant changes in SIRT1 values were observed between the initial and final time points of the group without PD ($p = 0.324$).

Table 2

In the group with PD, the serum concentration of SIRT1 increased from 1.06 ± 1.03 to 1.66 ± 1.64 ng/mL, $p <0.001$), and the serum concentration of MBL decreased from 1099.35 ± 916.59 to 861.42 ± 724.82 ng/mL ($p <0.001$) from the initial and final time points of periodontal treatment. After periodontal treatment, there was a reduction in the IP (63.9% ± 5.7% vs. 37.8% ± 10.5%, $p <0.001$), BOP (34.2% ± 6.9% vs. 16.9% ± 5.1%, $p <0.001$), CAL (5.9 ± 0.7 vs. 4.5 ± 0.8 mm; $p <0.001$) and PPD (5.3 ± 0.8 vs. 3.3 ± 0.7 mm, $p <0.001$).

There was also a significant reduction in the serum concentration of total cholesterol (191.28 ± 52.14 vs. 175.78 ± 53.17 mg/dL, p = 0.001), LDL (111.28 ± 44.48 vs. 102, 90 ± 45.19 mg/dL, p = 0.044), non-HDL (139.20 ± 47.02 vs. 125.68 ± 48.90 mg/dL, p = 0.003), triglycerides (138.25 ± 78.66 vs. 117, 40 ± 65.38 mg/dL, p = 0.048) and CRP (6.05 ± 8.99 vs. 2.44 ± 2.89 mg/L, p = 0.009) (Table 3). In these individuals, there was an inverse correlation between MBL and CRP values (r = -0.418; p = 0.009).

Table 3

DISCUSSION

In our study, we observed a significant reduction in the IP, BOP, CAL and PPD in patients who received periodontal treatment. Compared to surgical approaches, nonsurgical treatment, comprising scaling and root planning, was proven effective in PD. [23] According to a previous study, both techniques promoted a reduction in PPD and an increase in CAL. [24] In our study, we chose antibiotic therapy in association with nonsurgical treatment of PD due to the results of recent articles that showed the additional efficacy of antibiotic therapy in nonsurgical treatment of PD. [25, 26, 27] Moreover, our study showed that nonsurgical treatment of PD was associated with a reduction in the serum concentration of MBL and increased serum concentration of SIRT1. After PD treatment, a reduced serum concentration of CRP was also observed for mean values lower than 3 mg/dL. In our study, PD treatment was associated with a significant reduction in MBL serum levels. Periodontal treatment contributed to up to a 25% reduction in the initial MBL concentration. In contrast to our present findings, Maffei et al. [28] did not show elevated serum levels of MBL in periodontitis, and MBL deficiency was also not related to increased susceptibility to PD. However, these authors defined MBL deficiency arbitrarily as serum levels of MBL below 800 ng/ml. By contrast, in most studies, serum MBL levels below 100 ng/ml are defined as deficiency. [29, 30, 31, 32, 33] Additionally, in a study by Louropoulou et al., [34] PD was associated with increased serum MBL levels in individuals deficient in MBL production. Similarly, our study showed that PD treatment was associated with reduced serum MBL levels. These results are consistent with those of previous studies in which MBL was

identified as an acute phase reagent. [35, 36, 37] This finding in part explains the reduction in serum MBL levels observed in our study after periodontal treatment.

In our study, another finding showed that nonsurgical treatment of PD was associated with increased serum concentrations of SIRT1. The beneficial effects of SIRT1 on inflammation, lipid metabolism and atherosclerosis have been well documented in preclinical and animal studies. 11 Using the partial deletion of SIRT1 in atherosclerotic mice, Stein et al. [38] showed that SIRT1 protects against atherosclerosis by reducing the formation of foam cells. SIRT1 increases the production of nitric oxide and promotes vasodilation. [39] Interventions that increase SIRT1 production, such as caloric restriction and resveratrol, significantly attenuate age and inflammation-related vascular oxidative stress and improve endothelial function. [40] In obese rats, Zucker showed greater progression of periodontitis with increased oxidative stress induced by obesity. [41] In other animal studies, resveratrol and curcumin led to a reduction in experimentally induced periodontal degradation by reducing oxidative stress; these effects were possibly mediated by the activation of the sirtuin pathway. [42, 43, 44] Additionally, cell culture studies have shown a reduction in proinflammatory substances, such as matrix metalloproteinases and interleukins, mediated by SIRT1. [45, 46] However, this study is the first showing the interaction between the serum concentration of SIRT1 and the treatment of PD in humans. Increased serum SIRT1 concentrations were probably a consequence of PD treatment and may have had a positive influence on the PD treatment response. According to a recent study, SIRT1 is a potent regulator of the differentiation of human periodontal ligament cells and may have clinical

implications in periodontal bone regeneration. Increased serum concentrations of SIRT1 reduce oxidative stress, and this increase may be beneficial in patients with PD. [15] Machida et al. [47] showed that a reduction in the clinical level of insertion is associated with a reduction in the plasma levels of reactive oxygen species and with less PD progression.

In conclusion, PD treatment was associated with decreased serum MBL and CRP concentrations, as well increased serum SIRT1 levels. Nonetheless, prospective studies are needed to assess the impact of changes in these biomarkers on the progression and prognosis of PD.

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