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ORIGINAL ARTICLE

Periodontal therapy reduces arginase activity in saliva of patients with chronic periodontitis

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Abstract This present study evaluated the salivary arginase activity (SAA) in patients with chronic periodontitis and the effect of periodontal therapy on the activity of such enzyme. Thirty-six patients (mean age, 45.97±14.52), 18 chronic periodontitis subjects (test group), and 18 periodontally healthy individuals (control group) participated in the study. Clinical periodontal examinations included measurements of probing pocket depth (PD), clinical attachment level (CAL), plaque (PI), and gingival (GI) indexes. The test group received periodontal therapy according to individual needs. The saliva sample was collected from all study population at baseline (both groups) and 30 days after periodontal therapy (test group). SAA was determined by measuring the L-ornithine formation from L-arginine and was expressed as mU/ml. The results showed that the mean values of SAA were statistically different between control and test groups. SAA was about 2.5 times higher in test than control groups. Thirty days after periodontal therapy, enzyme levels were 1.56 times lower than before periodontal therapy. We concluded that SAA is increased in chronic periodontitis subjects when compared to periodontally healthy individuals and that periodontal therapy significantly reduced SAA levels. It was suggested that in the near future, SAA may be used as a salivary marker of periodontal status.

Keywords Arginase · Saliva · Pathogenesis · Periodontitis · Periodontal treatment

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Introduction

Chronic periodontitis is recognized as the most frequently occurring form of periodontitis that may progress continuously or by bursts of activity [12, 21]. The disease is characterized by clinical attachment loss, alveolar bone loss, periodontal pocketing, and gingival inflammation. In addition, gingival enlargement or recession and increased mobility, drifting, and/or tooth exfoliation may occur [27]. Current concepts of the etiology of periodontitis implicate a biofilm in the initiation and progression of periodontitis.

Saliva has been showed as an important biological material to the purpose of diagnostic tests in periodontal diseases. Saliva is a fluid, which contains local and systemic derived markers of periodontal disease. It may offer the basis for a patient specific diagnostic test for peri-

odontitis [14]. In addition, the use of saliva for periodontal diagnosis has been the subject of considerable research activity, and proposed markers for disease include enzymes, immunoglobulins, proteins, phenotypic markers, host cells, etc. [23].

The analysis of such enzymes may be used as a subsidy to obtain a diagnosis of the periodontal status and to clearly understand the whole pathogenic process of periodontal diseases. Recently, some investigators have considered the role of salivary arginase activity in the periodontal disease process [26, 33], showing a higher salivary arginase activity in chronic periodontitis patients compared to healthy periodontal control patients.

It is known that arginase and nitric oxide synthetase (NOS) compete for a common substrate, L-arginine [33]. Arginase makes use of L-arginine to produce urea and ornithine, whereas NOS uses L-arginine to produce nitric oxide [18]. The urea cycle is an essential metabolic pathway due to the conversion of ammonia ions, which are highly toxic to the human organism into urea [10]. According to Jenkison et al. [13], the urea cycle acts as a life support because it removes the highly toxic products from the organism. Arginase is one of the five key enzymes of the urea cycle, and it is found mainly in the human liver, but it can also be found in other non-hepatic tissues such as salivary glands.

Nitric oxide is a free radical, produced by L-arginine under the action of an isoenzyme called NOS. Induced NOS, identified in macrophages and neutrophils, is activated by the immune system and in response to inflammatory stimuli including cytokines such as interferon-gamma and bacterial lipopolysaccharides [15, 25]. Nitric oxide produced locally may act as a cytotoxic molecule against cells infected by fungi, protozoa, and bacteria as well as tumor cells and cells close to their production, possibly leading to tissue destruction [19]. In addition, according to Akopov and Kankanian [2], nitric oxide is produced in response to periodontal pathogens and local inflammatory alterations. Due to its antimicrobial activity, it is considered an important molecule against some infections. Furthermore, considering that L-arginine is used as a common substrate by both arginase and NOS, it is believed that an increase in arginase production would lead to a reduction in the production of nitric oxide, consequently increasing the susceptibility to bacterial infection.

In the present study, we hypothesized that a decreased level of salivary arginase activity would be present in patients with chronic periodontitis after periodontal therapy. To test this hypothesis, we compared the salivary arginase activity levels in patients with chronic periodontitis with those in periodontally healthy individuals and evaluated whether scaling and root planing would affect the levels of salivary arginase activity.

Materials and methods

Study population

Thirty-six patients, 18 males and 18 females (mean age, 45.97±14.52), who never smoke, participated in the present study. Inclusion criteria stated that (1) they had to have at least 18 teeth, (2) all were systemically healthy, (3) they had not taken antibiotics, anti-inflammatory agents, immunosuppressants, or systemic contraceptives for the past 6 months, (4) they had not had chronic medical disease or conditions, and (5) they had not received periodontal therapy within the previous 6 months.

Out of the 36 subjects, 18 were diagnosed with chronic periodontitis, which was named test group. According to the criteria established by Lopez et al. [22], the periodontitis group included subjects demonstrating at least four teeth with one or more sites with ≥4 mm pocket depth (PD), and ≥3 mm clinical attachment level (CAL). Out of the 18 patients from this group, tow sub-groups were formed according to mean values of periodontal parameters, one considered a group of subjects with more severity of periodontal disease (CAL>6 mm) and the other, subjects with less severity of disease (CAL≤6 mm). The control group was comprised of 18 periodontally healthy individuals with absence of sites presenting >4 mm pocket depth.

Data and personal information related to the medical and dental history of the subjects were obtained by questionnaire. All subjects signed an informed consent, which was previously reviewed and approved by the institutional review board at the University of Taubaté, São Paulo, Brazil (protocol # 482/05).

Periodontal examination

During periodontal examination, one trained and calibrated examiner measured full-mouth periodontal PD and CAL at six sites per tooth, with the aid of a manual periodontal probe (PQWBR, Hu Friedy). For a comprehensive periodontal examination, radiographic analysis was performed, and the same operator investigated oral hygiene status using plaque and gingival indexes, according to the protocol established by Ainamo and Bay [1]. Briefly, the frequency of occurrence of gingival margins bleeding on pressure and tooth surfaces covered with clearly visible plaque was determined as a percentage of the sites examined. All these periodontal parameters were recorded again after completion of periodontal therapy.

Periodontal therapy

The 18 patients from the test group received periodontal treatment according to individual needs including (1)

instructions of daily personal oral hygiene care, (2) at least four sections of scaling and comprehensive periodontal root planing to remove supra and subgingival bacterial plaque, as well as calculus, and to treat root surface irregularities.

Saliva sampling

The saliva sample was collected from all study population. The saliva sample of the control group was collected only once, at baseline, whereas the test group was subjected to two saliva sample collections, one at baseline previous to periodontal therapy and the other 30 days after a comprehensive periodontal therapy. During the procedure, the subjects were instructed to rinse their mouth with water and to chew a standardized paraffin wax to stimulate the saliva, which was collected to yield a total 2.0 ml. Samples were centrifuged for 10 min at 15,000×*g* at 4°C, and the supernatants were immediately stored at −20°C.

Biochemical procedures

Salivary arginase activity was determined by measuring the L-ornithine formation from L-arginine, according to the protocol established by Chinard [4], and was expressed as mIU/ml. One milliunit (mIU) of arginase is defined as the amount of enzyme that produces 1 nmol of ornithine per minute at 37°C. The standard reaction mixture (0.5 ml) contained 60 mM carbonate buffer pH 9.8, 20 mM L-arginine pH 9.8, and 1 mM $MnCl_2$, and the reactions were initiated by adding the 50 μl of salivary samples diluted threefold in sodium chloride solution 0.9% (*p*/*v*). The reaction was stopped with the addition of 1.5 ml of glacial acetic acid and 0.5 ml of ninhydrin solution (0.2 ml H_3PO_4 6M; 0.3 ml glacial acetic acid; 12.5 mg ninhydrin) to each sample. After the samples were boiled at 100°C for 60 min and cooled in a room temperature water bath, the absorbances of the samples were measured at 505 nm. Each sample was analyzed in duplicate.

Results

We analyzed reproducibility of the periodontal PD and CAL measurements using kappa-statistics and obtained a kappa value of 0.81.

The mean values of salivary arginase activity were statistically different among control group, test group before therapy (TO), and test group after therapy (T1). Salivary arginase activity was about 2.5 times higher in T0 (119.38 mIU/ml) compared to control group (46.95 mIU/ml). Thirty days after periodontal treatment, the enzyme levels were 1.56 times lower in T1 (69.98 mIU/ml) compared to T0 (119.38 mIU/ml) groups, and 1.6 times higher in T0 compared to control group (46.95 mIU/ml). In addition, the test group had significantly ($p<0.05$) higher mean values of PD, CAL, PI, and GI compared to control group. Furthermore, test group (TO) had higher mean values of the same periodontal parameters than test group (T1; Table 1).

We considered the salivary arginase activity in both subgroups of the test group separately, before (Table 2) and after periodontal therapy (Table 3). No statistically significant differences were observed regarding the amount of salivary arginase activity between subjects with more (128.02± 19.15 mIU/ml) and less (110.74±24.40 mIU/ml) severity of disease before periodontal therapy (Tables 2 and 3).

Discussion

Clinical periodontal parameters such as PD, CAL, bleeding on probing, plaque index, and radiographic evidence of alveolar bone loss are used to assess the severity of peri-

Table 1 Salivary arginase activity (mIU/ml) related to periodontal parameters PD, CAL, PI, and GI for control and test groups at baseline (T0) and 30 days after periodontal treatment (T1)

Groups	Parameters				
	Arginase Mean values mIU/ml±SD	PD Mean values mm±SD	CAL Mean values mm±SD	PI Mean values %±SD	GI Mean values %±SD
Control group	46.95	2.02	2.2	14.62	15.57
	19.72	0.19	0.17	13.35	13.79
Test group TO	119.38	5.36	6.07	85.35	88.36
	23.06*	1.58*	1.89*	15.66*	16.98*
Test group T1	68.98	4.29	5.75	38.72	42.33
	16.75**	1.22**	1.82**	11.52**	13.09**

Statistical analysis: ANOVA and Student *t* test

PD Pocket depth; *CAL* clinical attachment level; *PI* plaque index; *GI* gingival index; *TO* Baseline; *T1* 30 days after periodontal therapy

*$p<0.05$ significant differences between test (T0) and control group

**$p<0.05$ significant differences between test groups T0 and T1 group

Table 2 Salivary arginase activity (mIU/ml) related to periodontal parameters PD, CAL, PI, and GI according to severity of periodontal disease before periodontal treatment

Groups	Parameters				
	Arginase Mean values mIU/ml±SD	PD Mean values mm±SD	CAL Mean values mm±SD	PI Mean values %±SD	GI Mean values %±SD
Less severity[a]	110.74	4.06	4.61	75.12	79.33
	24.40	0.55	1.01	14.87	20.28
More severity[b]	128.02	6.66	7.52	96.18	97.39
	19.15	1.10*	1.36*	5.94*	4.27*

Statistical analysis: ANOVA and Student *t* test
PD Pocket depth; *CAL* clinical attachment level; *PI* plaque index; *GI* gingival index
[a] CAL≤6 mm
[b] CAL>6 mm
*Significant differences between the subgroups (less and more severity), $p<0.05$

odontal disease; they do not characterize the disease activity or provide information on the risk for future breakdown. Therefore, microbiological examination and analysis of the host response have been proposed to identify individuals at risk for progressive disease [20, 28].

Nowadays, different markers in saliva have been proposed as diagnostic tests for periodontal disease [3, 24]. Salivary-based tests are advantageous compared to other diagnostic fluids such as blood and crevicular fluid, as saliva can be collected more easily and less invasively than blood, and in larger amounts, and with less patient discomfort, relative to gingival crevicular fluid.

Analysis of the whole salivary components rather than gland-specific fluids covers a great spectrum of potential diagnostic markers or risk factors for periodontal disease [29]. One of them is represented by arginase, which is able to lead to a decrease in NO synthesis. Consequently, less amount of NO in saliva reduces its antimicrobial properties and makes periodontal tissues more susceptible to existent pathogens. Taking all this into account and considering that only a few number of papers addressing this issue have been published up to now [9, 26, 33], the present study was conducted to further evaluate the role played by salivary arginase on periodontal status of patients.

First, the present study demonstrated an increase in salivary arginase activity in the group of chronic periodontitis when compared to the control group. Accordingly, Özmeriç et al. [26] showed higher salivary arginase activity in periodontitis patients compared to healthy controls. These authors justified their results based on the fact that arginase in saliva might originate from host cells, such as macrophages [5, 7, 8, 16, 30], or from oral bacteria, such as periodontal pathogens [11]. However, Özmeriç et al. [26] found no statistically significant correlation between biochemical and clinical variables.

Then, we observed that arginase activity decreased from119.38 mIU/ml before scaling and root planing to 68.98 mIU/ml, 30 days after periodontal therapy. In addition, the clinical parameters before and after periodontal therapy were statistically different, suggesting that arginase

Table 3 Salivary arginase activity (mIU/mL) related to periodontal parameters PD, CAL, PI and GI according to the severity of periodontal disease after periodontal treatment

Groups	Parameters				
	Arginase Mean values mIU/ml±SD	PD Mean values mm±SD	CAL Mean values mm±SD	PI Mean values %±SD	GI Mean values %±SD
Less severity[a]	60.43	3.42	4.43	30.90	34.18
	11.95	0.36	0.98	8.22	8.83
More severity[b]	78.26	4.69	7.32	46.41	52.01
	15.58	1.08*	1.20*	8.93*	10.38*

Statistical analysis: Kruskal Wallis test
PD Pocket depth; *CAL* clinical attachment level; *PI* plaque index; *GI* gingival index
*Significant differences between the subgroups (less and more severity), $p<0.05$
[a] CAL≤6 mm
[b] CAL>6 mm

activity might be a precise marker of clinical status. This effect is probably due to the reduction of the inflammatory condition, which was verified after the therapy and most probably due to the reduction of microorganisms involved in periodontal disease. Accordingly, a previous study from Debats et al. [6] has showed that infected chronic wounds have increased arginase levels compared with non-infected chronic wounds.

A previous study from Sosroseno et al. [31] highlighted the importance of bacteria in arginase activity. They showed that *Actinobacillus actinomycetemcomitans*-lipopolysaccharide is able to stimulate arginase activity by murine macrophages. The increase in arginase levels may be explained as a survival need of the bacteria. It is well known that microorganisms have developed several strategies to colonize the host and evade the immune response. The induction of arginase by the host leads to the depletion of L-arginine, common substrate for nitric oxide synthase, which results in reduced levels of nitric oxide radicals. Therefore, the arginase increase can be seen as a mechanism by which microorganisms are able to avoid nitric oxide toxicity, decreasing the defensive barrier.

The effect of scaling and root planing and modified Widman flap procedures on nitric oxide synthase and arginase activity in patients diagnosed with chronic periodontitis was previously studied by Gullu et al. [9]. This study compared in a split-mouth design the correlation between arginase and NOS activity in 13 patients, showing that induced NOS expression was decreased after periodontal therapy. However, differently from our study, arginase level was significantly increased in both scaling and root planing and modified Widman flap groups. One possible explanation for these contradictory results is the fact that Gullu et al. [9] evaluated the arginase activity present locally in the gingival tissues, whereas in our present study, the arginase activity was observed in the saliva of periodontitis patients. The degradation of arginine by asaccharolytic anaerobic Gram-positive rods, including *Eubacterium minutum*, *Filifactor alocis*, *E. infirmum*, *E. sulci*, and *E. saphenum* has been shown in periodontal pockets [32]. Several bacterial enzymes such as arginine deiminase have been implied in arginine metabolization to citrulline. Whether the activity of such periodontal bacteria enzymes is able to decrease the arginine availability for arginase, therefore decreasing L-ornithine production within the periodontal pocket, remains speculative and deserves further clarification in the future.

The influence of periodontal disease severity on the salivary arginase activity was also considered in our study. However, in spite of an observed trend for higher levels of salivary arginase activity in higher PD, CAL, PI, and GI parameters, there were no statistically significant differences regarding the amount of salivary arginase activity between subjects with more (128.02±19.15 mIU/ml) and less (110.74±24.40 mIU/ml) severity of disease before periodontal therapy.

In conclusion, the present study demonstrates that salivary arginase activity is increased in chronic periodontitis subjects when compared to control healthy individuals and that this enzyme decreased after periodontal therapy. Therefore, we suggest that salivary arginase activity could be an additional tool to connect salivary markers with periodontal status.

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References

1. Ainamo J, Bay I (1975) Problems and proposals for recording gingivitis and plaque. Int Dent J 25:229–235
2. Akopov SE, Kankanian AP (1996) Nitric oxide (NO) inactivation by polymorphonuclear leukocytes as mechanism for the development of periodontal disease. Stomatologgia 75:12–14
3. Aurer A, Stavljenic-Rukavina A, Aurer-Kozelj J (2005) Markers of periodontal destruction in saliva of periodontitis. Acta Med Croatica 59:117–122
4. Chinard FP (1952) Photometric estimation of proline and ornithine. J. Biol. Chem 199:91–95
5. Currie GA (1979) Activated macrophages kill tumour cells by releasing arginase. Nature 273:758–759
6. Debats IBJG, Booi D, Deutz NEP, Buurman WA, Boeckx WD, van der Hulst RRWJ (2006) Infected chronic wounds show different local and systemic arginine conversion compared with acute wounds. J Surg Res 134:205–214
7. Farram E, Nelson DS (1980) Metabolism of mouse macrophages as antitumor effector cells: role of arginase. Cell Immunol 55: 283–293
8. Gopalakrishna R, Nagarajan B (1978) Arginase in saliva. Indian J Biochem Biophys 15:488–490
9. Gullu C, Özmeric N, Tokman B, Elgün S, Balos K (2005) Effectiveness of scaling and root planning versus modified Widman flap on nitric oxide synthase and arginase activity in patients with chronic periodontitis. J Periodontal Res 40:168–175
10. Guoyao WU, Morris SM (1998) Arginine metabolism: nitric oxide and beyond. J Biochem 336:1–17
11. Henskens YM, van der Weijden FA, van der Keijbus PA, Veerman EC, Timmerman MF, van der Velden U, Amerogen AV (1996) Effect of periodontal treatment on the protein composition of whole and parotid saliva. J Periodontol 67:205–212
12. Jeffcoat MK, Reddy MS (1991) Progression of probing attachment loss in adult periodontitis. J Periodontol 62:185–189
13. Jenkinson CP, Grody WW, Cederbaum SD (1996) Comparative properties of arginases. Comp Biochem Physiol 114B:107–132
14. Kaufman E, Lamster IB (2000) Analysis of saliva for periodontal diagnosis: A review. J Clin Periodontol 27:453–465
15. Kendall HK, Marshall RI, Bartold PM (2001) Nitric Oxide and tissue destruction. Oral Disease 7:2–10
16. Khramov VA (1997) The utilization of amino acids and urea by human oral fluid. Stomatologia 76:13–15
17. Konarska L, Tomaszewski L, Colombo J, Terheggen HG (1985) Human salivary arginase and its deficiency in argininaemia. J Clin Chem Clin Biochem 23:337–342

18. Kossel A, Dakin HD (1904) Uber die arginase. Z Physiol Chemie 41:321–331
19. Kroncke K, Fehsel K, Kolb-Bachofen V (1997) Nitric oxide: cytotoxicity versus citoprotection—how, when and where. Nitric Oxide Biol Chem 1:107–120
20. Lamster IB, Novak MJ (1992) Host mediators in gingival crevicular fluid: implications for pathogenis of periodontal disease. Crit Rev Oral Biol Med 3:31–60
21. Löe H, Anerud A, Boysen H, Morrison E (1986) Natural history of periodontal disease in man. Rapid, moderate and no loss of attachment in Sri Lankan laborers 14 to 16 years of age. J Clin Periodontol 13:431–440
22. López NJ, Smith PC, Gutierrez J (2002) Higher risk of preterm and low birth weight in women with periodontal disease. J Dent Res 81:58–63
23. Mandel ID (1991) Markers of periodontal disease susceptibility and activity derived from saliva. In: Johnson NW (ed) Risk markers of diseases. Periodontal diseases: markers of disease susceptibility and activity. vol. 3. Cambridge University Press, Cambridge, pp 228–253
24. Miller CS, King CP Jr, Langub MC, Kryscio RJ, Thomas MV (2006) Salivary biomarkers of existing periodontal disease: a cross-sectional study. J Am Dent Assoc 137:322–329
25. Nathan C (1997) Inducible nitric oxide synthase: what difference does it make. J Clin Invest 100:2417–2423
26. Ozmeriç N, Elgun S, Uraz A (2000) Salivary arginase in patients with adult periodontits. Clin Oral Investig 4:21–24
27. Page RC, Schroeder HE (1976) Pathogenesis of inflammatory periodontal disease. A summary of current work. Lab Invest 33:235–249
28. Polson AM, Goodson JM (1985) Periodontal diagnosis—current status and future need. J Periodontol 56:25–34
29. Sahingur SE, Cohen RE (2004) Analysis of host response and risk for disease progression. Periodontol 2000 34:57–83
30. Schneider E, Dy M (1985) The role of arginase in the immune response. Immunol Today 6:136–140
31. Sosroseno W, Musa M, Ravichancran M, Fikri Ibrahim M, Bird PS, Seymour GJ (2006) Arginase activity in a murine macrophage cell line (RAW264.7) stimulated with lipopolysaccharide from *Actinobacillus actinomycetemcomitans*. Oral Microbiol Immunol 21:145–150
32. Uematsu H, Sato N, Hossain MZ, Ikeda T, Hoshino E (2003) Degradation of arginine and other amino acids by butyrate-producing asaccharolytic anaerobic Gram-positive rods in periodontal pockets. Arch Oral Biology 48:423–429
33. Ugar-Çankal D, Ozmeric N (2006) A multifaceted molecule, nitric oxide in oral and periodontal diseases. Clin Chim Acta 366:90–100