Salivary arginase activity after mechanical-chemical therapy

Atividade de arginase salivar após tratamento mecânico-químico

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Resumo
Introdução: Componentes salivares podem ser usados como biomarcadores para diagnóstico e monitoramento de doenças orais. Há evidências de que um potencial biomarcador, arginase, está associado com os processos inflamatórios da doença periodontal, e após o tratamento sua atividade enzimática é reduzida em concordância com a melhora nos parâmetros clínicos. Objetivo: O presente estudo objetivou avaliar a atividade da arginase salivar em pacientes com gengivite e periodontite tratados com procedimentos mecânicos em estágio único combinados ao uso coadjuvante de enxaguatórios com óleos essenciais ou clorexidina, respectivamente. Material e método: Vinte e seis pacientes com gengivite e 16 pacientes com periodontite receberam exame periodontal completo antes e 3 meses após a terapia, em que mensurações de profundidade de sondagem, perda de inserção clínica, índice de placa e índice gengival foram realizadas. Nestas mesmas consultas os níveis de proteína total e a atividade de arginase salivar foram estabelecidos via espectrofotometria. Resultado: Todos os parâmetros clínicos melhoraram, em ambos os grupos, do exame inicial para o de 3 meses (p < 0,05). Adicionalmente, após tratamento para gengivite houve redução da atividade de arginase salivar e do nível de proteína total. Conclusão: Semelhante aos resultados clínicos, ambos os protocolos terapêuticos afetaram positivamente a atividade da arginase salivar; entretanto, estudos futuros são necessários para clarificar seu potencial como biomarcador salivar para o monitoramento periodontal.

Descritores: Arginase; gengivite; periodontite; óleos essenciais; clorexidina.

Abstract
Introduction: Salivary components can be used as biomarkers for diagnosing and monitoring oral diseases. There is evidence that one potential biomarker, arginase, is associated with the inflammatory processes of periodontal disease, and its enzymatic activity is reduced according to the improvement in the clinical parameters after treatment. Objective: The present study aimed to evaluate the salivary arginase activity in gingivitis and periodontitis patients treated with full-mouth mechanical procedures combined with the adjunctive use of essential oils or chlorhexidine mouthwash, respectively. Material and method: Twenty-six gingivitis and 16 periodontitis patients received complete periodontal examinations at the baseline and 3 months after therapy, in which the periodontal probing depth, clinical attachment loss, plaque index, and gingival index measurements were taken. At these same appointments, the salivary total protein level and salivary arginase activity were also established via spectrophotometry. Result: There were improvements in all of the clinical parameters (p < 0.05) evaluated from the baseline to 3 months in both groups. In addition, the salivary arginase activity and total protein levels were reduced after the gingivitis treatment. Conclusion: Similar to the clinical results, both therapeutic protocols positively affected the salivary arginase activity; however, further studies are necessary to clarify its potential as a salivary biomarker for periodontal monitoring.

Descriptors: Arginase; gingivitis; periodontitis; essential oils; chlorhexidine.

INTRODUCTION

Saliva has become a popular diagnostic fluid for research and clinical practice in recent years. Its availability, easy collection, and the possibility of repeated noninvasive sampling make it ideal for screening, diagnosing, and monitoring many diseases1. In addition, using saliva to measure host-derived factors would allow for a highly individualized diagnosis, prognosis and treatment approach in periodontics2-4. The increased salivary arginase levels5 observed in the course of periodontal disease and their subsequent reduction after treatment6 suggest the involvement of arginase in the inflammatory process.
Arginase is an enzyme that catalyzes the hydrolysis of L-arginine into L-ornithine and urea, and it can be found in mammalian tissues in two isoforms: type-I arginase (Arg-I), which is present in the cytosol and a critical hepatic metabolism enzyme, and type-II arginase (Arg-II), which is located in the mitochondria of extrahepatic tissues and modulates the L-arginine levels for the synthesis of nitric oxide (NO), polyamines, and proteins7.

As previously described, there are at least two functionally distinct and inflammatory response-related types of macrophages. M1 macrophages are pro-inflammatory (killer cells), and they are up-regulated by the inducible NO synthase (iNOS) and Arg-II induced by the cytokines (mainly interferon-γ) released from type 1 helper T (Th-1) cells. High levels of iNOS kill infectious agents via their oxidative activity; however, the role of Arg-II is not clear in the processes mediated by M1 macrophages. M2 macrophages are anti-inflammatory (repair-type cells) that do not modulate the iNOS gene, but they are up-regulated by Arg-I, which is induced by the cytokines (mainly interleukin-4) that do not modulate the iNOS gene, but they are up-regulated by Arg-I, which is induced by the cytokines (mainly interleukin-4) released from type 2 helper T (Th-2) cells. Therefore, high Arg-1 levels accelerate the hydrolysis of L-arginine, releasing L-ornithine for the synthesis of polyamines and proline, which are essential for cell growth and collagen synthesis, respectively8,9.

Mechanical procedures alone are able to reduce the salivary arginase in gingivitis and periodontitis patients10-12. However, the way in which the mechanical procedures are performed seems to influence the results. The studies conducted by our group revealed a decrease in the salivary arginase activity (SAA) after quadrant-wise scaling11,12, despite a tendency toward a salivary nitrite increase (also related to the NO cycle) after full-mouth scaling9. Mechanical procedures can also be combined with chemical agents for the treatment of gingivitis and periodontitis. Clinical, microbiological, and immunological improvements have been reported after full-mouth scaling and the daily use of an essential oil (EO) mouth rinse for gingivitis treatment13. In addition, full-mouth scaling with the adjunctive use of chlorhexidine (CHX) reduced the probing depth, percentage of periodontal disease sites, and total subgingival bacterial counts in periodontitis patients14. However, the effects of mechanical-chemical therapy on the SAA have not yet been investigated. Therefore, this study was designed to evaluate the SAA in gingivitis and periodontitis patients undergoing full-mouth procedures with the adjunctive use of EO or CHX mouthwash, respectively.

MATERIAL AND METHOD

Study Population

Eligible patients of both genders were recruited by convenience for the present study, which was previously approved by the Institutional Committee on Research involving humans at the University of Taubate (protocols #52110 and #52210). All of the patients provided written informed consent before enrolling in the present study, which was composed of baseline and 3-month post-treatment appointments. The study subjects included gingivitis or periodontitis patients15 who were in good general health, between 20 and 50 years of age, who sought dental care in the Dental Clinic at the University of Taubate, SP, Brazil. Each patient’s personal information and medical and dental histories were obtained in an interview.

The initial sample size of at least 20 gingivitis and 12 periodontitis patients was determined based on a previous study that also evaluated the clinical parameters and the SAA15.

Clinical Examination

Two calibrated examiners performed a complete periodontal examination at the baseline and 3 months after the therapy. The agreement between examiners was high [kappa = 0.84 for the pocket depth (PD) and 0.82 for the clinical attachment loss (CAL)]. The PD, CAL, plaque index (PI), and gingival index (GI) measurements were obtained at four sites per tooth using a manual periodontal probe. A panoramic radiograph was obtained for each patient.

Gingivitis Group

Inclusion criteria

Plaque-related gingivitis with no radiographic evidence of periodontal bone resorption and a bleeding site rate of more than 30%13,14, at least 20 natural teeth, and good general health.

Exclusion criteria

Gingival overgrowth, orthodontic devices, extended prosthetic fixed devices, removable partial dentures, overhanging restorations, systemic diseases or other conditions that could influence the periodontal status (such as diabetes and obesity), alcohol abuse, pregnancy or breast-feeding, history of sensitivity or suspected allergies following the use of oral hygiene products, a need for antibiotic prophylaxis, antibiotics and/or anti-inflammatory drug use in the six months prior to the beginning of the study, regular use of chemotherapeutic antiplaque/antigingivitis products, periodontal treatment performed within six months prior to the study initiation, and an unwillingness to return for follow-up.

Treatment

Each of the patients underwent one-stage ultrasonic debridement and received a 3-month supply of mouthwash (20 ml/30 s twice daily) containing a fixed combination of four EOs (0.092% eucalyptol, 0.042% menthol, 0.060% methyl salicylate, and 0.064% thymol) (Johnson and Johnson, São José dos Campos, SP, Brazil). After the first supervised rinse, each patient was instructed to rinse at home in the morning and in the evening. In addition, the patients received a monthly fluoride dentifrice, toothbrush, and dental floss.

Periodontitis Group

Inclusion criteria

Mild to moderate chronic periodontitis15, at least four teeth with one or more periodontal sites with a PD ≥ 4 mm and CAL ≥ 3 mm15, at least 20 natural teeth, and good general health.
Exclusion criteria

Systemic diseases or other conditions that could influence the periodontal status, alcohol abuse, orthodontic devices, extended prosthetic fixed devices, removable partial dentures, overhanging restorations, pregnancy or breast-feeding, history of sensitivity or suspected allergies following the use of oral hygiene products, the need for antibiotic prophylaxis, antibiotics and/or anti-inflammatory drug use in the six months prior to the beginning of the study, regular use of chemotherapeutic antiplaque/antigingivitis products, any furcation lesions, periodontal treatment performed within six months prior to the study initiation, and an unwillingness to return for follow-up.

Treatment

Each patient underwent full-mouth manual scaling within 24 hours. At the beginning of each session, each patient rinsed with 20 ml of 0.12% CHX (Colgate-Palmolive, São Bernardo do Campo, SP, Brazil) for 30 s (the last 10 s consisted of gargling), and at the end of each session there was 1 min of tongue brushing with CHX gel (1% di gluconate chlorhexidine, oral gel basis for 30 g, sodium saccharin 0.05%, and mint flavoring) followed by an additional mouth rinse. After the first supervised rinse, each patient was instructed to rinse at home in the morning and in the evening. In addition, the patients received a monthly fluoride dentifrice, toothbrush, and dental floss. All of the gingivitis and periodontitis patients received oral hygiene instructions.

Salivary Arginase Activity

The saliva samples were collected in the morning, from 8:00 to 11:00, and the patients were instructed not to eat or drink prior to sampling. Immediately before sampling, the patients rinsed their mouths with water. During collection, they remained seated with their heads tilted forward (approximately 45°) and 2.0 ml of unstimulated whole saliva were collected into sterile Falcon tubes. The samples were centrifuged for 10 min at 15,000g at 4°C, and the supernatants were immediately stored at -80°C. Prior to the analysis, the saliva samples were thawed and centrifuged for 5 min at 12,000 rpm at 4°C; then, 500 µl of the supernatant were transferred to an Eppendorf minitube. To this supernatant, phosphate buffered saline containing 0.05% v/v Tween 20 (PBS-T), 1 mM phenylmethylsulfonyl fluoride (PMSF), and a protease inhibitor cocktail (1:1000 dilution) were added.

The total salivary protein concentration of the homogenates was determined using the bicinchoninic acid method (BCA) with a QuantiPro™ BCA Assay Kit (Sigma-Aldrich, St. Louis, MO, USA). The spectrophotometric readings were performed at 595 nm using a FuoStar Optima microplate reader (BMG Labtech, Ortenberg, Germany). Using a method adapted from Iyamu et al.18, the SAA (ARG, EC 4.2.1.11) was determined by the discontinuous method in a 50 mM glycine buffer (pH 9.5) containing 100 mM of L-arginine (pH 9.5) and 1 mM of manganese chloride. The formation of L-ornithine from L-arginine was measured to determine the activity of the arginase, so that one unit (U) of arginase was defined as the amount needed for the production of 1 µmol of L-ornithine per min at 37°C. The enzymatic reaction was conducted in 96-well microplates, and then, the aliquots were transferred to 384-well microplates and read at 520 nm using a FuoStar Optima microplate reader (BMG Labtech).

**Statistical Analysis**

The statistical analyses were performed using specific Bio Estat 5.0 software. All of the comparisons were made using a paired t test with a significance level of 5% (p < 0.05).

**RESULT**

Twenty-six gingivitis patients concluded this study (15 females, 11 males, mean age = 33.84 ± 11.13 years old). The clinical evaluation showed improvements from the baseline to 3 months (p < 0.05) with regard to the PD, PI, and GI (Table 1). In addition, there were reductions (p < 0.05) in the SAA and total protein levels after the gingivitis treatment (Table 2).

Sixteen periodontitis patients underwent 3-month evaluations (8 females, 8 males, mean age = 49.31 ± 8.19 years old). Clinically, there were statistically significant (p < 0.05) changes in the PD, PI, GI, and CAL mean values (Table 1). Among the periodontitis patients, there were SAA decreases without changes in the total protein levels after 3 months of treatment (Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Clinical variables</th>
<th>PD (mm)</th>
<th>PI (%)</th>
<th>GI (%)</th>
<th>CAL (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gingivitis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>PD (mm)</td>
<td>1.83 ± 0.05</td>
<td>0.49 ± 0.20</td>
<td>1.43 ± 0.26</td>
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<tr>
<td>3 months</td>
<td>PD (mm)</td>
<td>1.52 ± 0.13</td>
<td>0.20 ± 0.05</td>
<td>0.63 ± 0.39</td>
<td>-</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>0.04</td>
<td>0.02</td>
<td>0.03</td>
<td>-</td>
</tr>
<tr>
<td><strong>Periodontitis</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>PD (mm)</td>
<td>3.72 ± 0.95</td>
<td>0.39 ± 0.22</td>
<td>0.35 ± 0.34</td>
<td>5.00 ± 1.92</td>
</tr>
<tr>
<td>3 months</td>
<td>PD (mm)</td>
<td>2.11 ± 0.79</td>
<td>0.22 ± 0.25</td>
<td>0.13 ± 0.17</td>
<td>3.50 ± 1.84</td>
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<tr>
<td>p value</td>
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<td>0.02</td>
<td>0.04</td>
<td>0.03</td>
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</table>

Paired t test (p < 0.05).
Table 2. Baseline and 3-month comparative values [mean ± standard deviation (SD)] of the total protein and salivary arginase activity (SAA) in the saliva of the patients with gingivitis and periodontitis

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein</th>
<th>SAA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/ml</td>
<td>U/ml</td>
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<tr>
<td>Baseline</td>
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<td>32.49 ± 17.61</td>
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<tr>
<td>Gingivitis</td>
<td>0.94 ± 0.38</td>
<td>17.52 ± 25.66</td>
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<tr>
<td>3 months</td>
<td>0.0003</td>
<td>0.0126</td>
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<tr>
<td>Periodontitis</td>
<td>1.22 ± 0.46</td>
<td>30.30 ± 18.47</td>
</tr>
<tr>
<td>3 months</td>
<td>0.3530</td>
<td>0.0228</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Paired t test (p < 0.05).

DISCUSSION

Salivary analyses have shown promise as diagnostic tools given the advantages of saliva collection, such as facility, reproducibility, and noninvasiveness, as well as the possibility of evaluating a wide variety of useful components (biomarkers) for monitoring health and disease processes. In dentistry, the biochemical analysis of saliva is important for estimating the risk of onset or recurrence of a given disease, determining the severity of an oral disease, and monitoring the host response to different oral treatments. Based on its increase in the presence of inflammatory processes, and the expected decrease with therapeutic treatment, several authors have been evaluating the role of the enzyme arginase as a salivary biomarker of periodontal disease. Although promising, its role in the response to nonsurgical periodontal treatment requires deeper clarification. Moreover, as in other health areas, periodontists are looking for more sensitive and reliable means of diagnosing periodontal diseases in the early stages.

In the present study, both groups (gingivitis and periodontitis) showed a statistically significant reduction in the SAA (p < 0.05), when comparing the initial data with a 3-month post-treatment evaluation. Our results were consistent with those of Özmeriş et al., Ash, Gheren et al., and Pereira et al., who observed reductions in the arginase levels following clinical improvements. Several proteins can be found in the saliva, and they are related to several biological processes. Although not completely understood, a decrease in the total protein levels can be expected following the successful treatment of periodontal disease. This expectation is based on the higher levels of proinflammatory cytokines observed when the periodontal tissues are diseased. In the present study, 3 months after treatment, the total protein level was only reduced in the gingivitis group, corroborating the data of Nieminen et al. and Vieira et al. However, the total protein level remained unchanged in the periodontitis group. Contrarily, Cortelli et al. observed reductions in the total protein levels in the saliva of patients with mild periodontitis treated by full-mouth scaling and an EO mouthwash. However, in the present study, the individuals in the periodontitis group underwent full-mouth scaling plus CHX, as described by Quirynen et al., instead of EO. Despite some undesirable side effects due to its higher anti-plaque effectiveness, CHX is the first option for the short-term chemical dental biofilm control of periodontitis. Thus, whether a longer period of time is needed to observe the total protein level reduction in the presence of periodontitis is a subject for further studies, especially when considering the improvements in the classical clinical parameters. Furthermore, it should be keep in mind that oral therapeutic procedures could, per se, trigger an inflammatory host response. Recently, Morozumi et al. reported a moderate systemic acute-phase response in addition to clinical and microbiological improvements after full-mouth procedures.

In the present study, the gingivitis patients underwent full-mouth ultrasonic scaling during one appointment. Apatzidou and Singh et al. previously demonstrated good clinical results for this type of treatment. Moreover, this group received oral hygiene instructions and rinsed with an EO mouthwash, which, according to Raslan et al., provided additional benefits when compared to isolated mechanical procedures. Studies that have compared EOs with CHX demonstrated that EOs are a viable option for gingivitis patients due to their appropriate clinical benefits with less side effects. The present gingivitis patients showed statistically significant improvements at 3 months, as revealed by the PD, PI, and GI reductions. The results from the present study corroborate the previous findings regarding the effects of EOs on gingivitis patients. However, Van der Sluijs et al. did not observe any additional benefits when the EOs were combined with ultrasonic scaling.

CONCLUSION

According to our results, the effectiveness of the mechanical-chemical treatment on the periodontal diseases studied was proven, given the clinical improvement found in the groups evaluated. In addition, arginase was shown to be a useful salivary biomarker after full-mouth procedures, with the adjunctive use of EOs or CHX, given the significant reduction in the arginolytic activity associated with the improvement in the clinical parameters in the study groups.
REFERENCES


CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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