Antioxidative Status of Saliva before and after Non-Surgical Periodontal Treatment

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SUMMARY

Introduction Oxidative stress and antioxidants play an important role in the pathogenesis of inflammatory disease, including chronic periodontitis (CP). Saliva contains enzymatic (glutathione peroxidase – GPx, superoxide dismutase – SOD, etc.) and non-enzymatic (albumin – ALB, uric acid – UA, glutathione, etc.) antioxidants.

Objective The aims of this study were to investigate: a) level of SOD, GPx, UA, ALB and total antioxidative status (TAS) of saliva in CP patients before and after non-surgical treatment, and b) correlations between clinical periodontal parameters and levels of salivary antioxidants.

Methods Saliva was collected from 21 CP patients before and after non-surgical treatment. The condition of periodontium was assessed by plaque index, gingival index, bleeding on probing, probing depth and clinical attachment loss. Level of investigated antioxidants (except GPx) and TAS was determined using colorimetric method and commercial kits. GPx activity was determined using UV method and commercial kits.

Results After the treatment significant increase of UA, ALB, GPx, TAS was detected (p<0.01) and decrease of SOD activity (p>0.05). A significant correlation was observed between GPx and PI (r=0.575, p=0.008), SOD and GI (r=0.525, p=0.017) before therapy, and SOD and bleeding on probing (BP) (r=0.59, p=0.006), TAS and BP (r=0.453, p=0.045) after therapy.

Conclusion These data suggest that levels of salivary antioxidants generally increase after non-surgical periodontal treatment. Correlation between some clinical periodontal parameters and level of salivary antioxidants was found.

Keywords: saliva; antioxidants; uric acid; albumin; glutathione peroxidase; superoxide dismutase; total antioxidative status; antichronic periodontitis treatment

INTRODUCTION

Chronic periodontitis is the most prevalent oral inflammatory disease. It appears as a result of imbalance between bacterial colonization of the oral environment and host immune defense. As a part of body immune response inflammatory mediators are released allowing chemotaxis of immune cells in place of inflammation. Polymorphonuclear leukocytes (PMN) are the first line defense of oral tissues from pathogenic microorganisms. Interaction of leukocytes and bacteria trigger the biochemical and physiological processes that cause the host to neutralize pathogens, but also possible damage to local tissues. Polymorphonuclear leukocytes induced by pathogens are characterized by increased consumption of oxygen (respiratory burst) i.e. increasing the production of free radicals (superoxide anion, hydrogen peroxide, hydroxyl radical, etc.). Released radicals, by oxidative mechanism distort the structure of bacterial cell membrane and thus kill bacteria. However, during the defense reaction, especially under the conditions of the overproduction of radicals can lead to oxidative modification of various host biomolecules and damage oral tissue cells, as a kind of collateral damage. Saliva contains enzymatic (superoxide dismutase – SOD, glutathione peroxidase – GPx, peroxidase, catalase, etc.) and non-enzymatic antioxidants (uric acid – UA, albumin – ALB, glutathione, vitamins A, C, etc.) which neutralize free radicals. It has been clamed that there is significant decrease in the concentration of antioxidants in saliva of periodontal patients comparing to healthy individuals [1-5]. There are a lot of systemic disorders giving oral manifestations based on imbalance between free radicals and antioxidants, with consequential destruction of host tissue as a result of oxidative stress (diabetes mellitus, atherosclerosis, multiple sclerosis, etc.). Thereby, recent research is very oriented...
to determine, not only periodontal biomarkers, but also the risk markers of systemic diseases giving oral manifestations, which reflect disease onset, progression and therapy outcome, with emphasis on available and low invasive procedures.

OBJECTIVE

The aim of the study was to investigate the influence of non-surgical periodontal treatment on salivary antioxidants.

METHODS

This controlled clinical study was conducted at the Clinic of Periodontology and Oral Medicine, and biochemical research at the Biochemical Laboratory of the Faculty of Dental Medicine, University of Belgrade. The protocol was approved by the Ethic Committee of the Faculty of Dental Medicine, University of Belgrade.

Patient selection

The study group consisted of 21 systemically healthy non-smokers aged between 25 and 55 years with chronic periodontitis (CP) in the period of exacerbation (radiographic evidence of generalized alveolar bone loss >30%, presence of at least one pocket with PPD >5 mm per quadrant with positive bleeding on probing – BOP). Exclusion criteria were pregnant or lactating women, use of food supplements, such as vitamin C and E supplements over the previous three months or some restrictive diet.

Treatment protocol

All participants were evaluated clinically and radiographically at the first visit. If they fulfilled the inclusion criteria they were informed about the study and gave a written consent. On the second (baseline) visit the first saliva sample was collected (see sampling technique) before clinical measurements in order to avoid any potential contamination of the specimen by blood originating from periodontal pockets after provocation with clinical measurements. After sampling, full-mouth clinical measurements (see clinical measurements) were performed and recorded into the patient’s study chart. Non-surgical periodontal treatment included oral hygiene instructions and full-mouth scaling and root planing. Treatment was performed by the same periodontist under local anesthesia aiming to avoid painful experience during periodontal debridement that included scaling and root planing using ultrasonic scaler and Gracey curettes. A control visit was scheduled two months after treatment. The second saliva sample was collected and the same clinical measurements were performed and recorded in the chart.

Clinical measurements

The following clinical parameters were assessed at six sites around each present tooth for the whole mouth excluding third molars by a single investigator. Clinical parameters used in this study were gingival index (GI) [6], plaque index (PI) [6], bleeding on probing (BP) [7], probing depth (PPD) distance from the gingival margin to the location of the tip of the graduated periodontal probe (North Carolina-Hu-Friedy, Chicago, IL, USA) inserted in the pocket with moderate probing force and clinical attachment level (CAL) distance from the cemento-enamel junction to the location of the inserted probe tip.

Sampling technique

The samples for biochemical analyses were collected at baseline visit and two months after finished periodontal treatment. Unstimulated whole saliva (2 ml) was collected from each subject by the Salivette (Salivette, Sarstedt, Germany) between 9.00-10.00 a.m. The participants were requested not to eat or drink anything except water on the morning before sampling. This was to ensure that the variability in salivary flow and composition be minimized due to diurnal variation. The subject was asked to rinse the mouth with distilled water thoroughly to remove any food debris. After that the Salivette was placed in the sublingual area for 1 min. the collected samples were immediately transported to the laboratory, and were centrifuged at 4000 g for 10 minutes and frozen at -80°C until analysis.

Biochemical analyses

SOD, ALB, UA, and total antioxidant status (TAS) were determined using the colorimetric method and commercial kits Ransod (Randox Laboratories Ltd., United Kingdom), Albumin (Human Gesellschaft für Biochemica und Diagnostica mbH, Germany), Uric Acid liquicolor (Human Gesellschaft für Biochemica und Diagnostica mbH, Germany) and TAS (Randox Laboratories Ltd., United Kingdom). Glutathione peroxidase activity was determined using UV method and commercial kit Ransel (Randox Laboratories Ltd., United Kingdom).

Statistical analysis

Statistical analysis was performed using commercial statistical software (SPSS 16.0, Inc., Chicago, IL, USA). Biomarkers were expressed as follows: ALB levels as g/L, uric acid and TAS levels as µmol/L, GPX and SOD levels as IU/L. Secondary outcome variables included CAL, GI and PI. Clinical variables (GI, PI, PPD and CAL) were calculated by the patient and visit. All data were expressed as mean and standard deviation. Kolmogorov-Smirnov test was performed for all continuous variables to assess normality of data. In the assess-
ment of correlation between parameters, Pearson’s correlation coefficient was used. Demographic data (gender and age) as nominal variables were analyzed using chi-square test. Statistical significance was established at the 95% confidence level.

RESULTS

In this study 21 participants were included, 14 male (66.67%) and 7 female (33.33%), aged 39.2±11.5 years.

The values of clinical parameters except CAL showed a significant decrease after therapy (Table 1). Also, significant increase of ALB and UA concentrations and GPx activity as well as TAS (p<0.01) was shown in this study. On the other hand SOD activity was decreased after non-surgical periodontal treatment, but it was not statistically significant (p>0.05) (Table 2).

In this study correlations between biochemical and clinical parameters were analyzed. A significant positive correlation was observed between GPx and PI (r=0.575, p=0.008) (Figure 1) and negative correlation between SOD and GI (r=-0.525, p=0.017) (Figure 2) before therapy, as well as a positive correlation between SOD and BP (r=0.59, p=0.006) (Figure 3), TAS and BP (r=0.453, p=0.045) (Figure 4) after therapy.

DISCUSSION

Salivary antioxidants protect the integrity of oral tissues by neutralizing free radicals. Many studies have shown that

Table 1. Clinical parameters in chronic periodontitis patients before and after non-surgical periodontal treatment (mean ±SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before</th>
<th>After</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>1.39±0.55</td>
<td>0.36±0.30</td>
<td>0.01</td>
</tr>
<tr>
<td>GI</td>
<td>1.96±0.41</td>
<td>0.55±0.24</td>
<td>0.01</td>
</tr>
<tr>
<td>BP</td>
<td>1.81±0.52</td>
<td>0.43±0.19</td>
<td>0.01</td>
</tr>
<tr>
<td>PD</td>
<td>3.40±0.66</td>
<td>2.02±0.54</td>
<td>0.01</td>
</tr>
<tr>
<td>CAL</td>
<td>2.95±0.99</td>
<td>2.93±1.01</td>
<td>0.185</td>
</tr>
</tbody>
</table>

PI – plaque index; GI – gingival index; BP – bleeding on probing; PD – probing depth; CAL – clinical attachment loss; SD – standard deviation

Table 2. Biochemical parameters in chronic periodontitis patients before and after non-surgical periodontal treatment (mean ±SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before</th>
<th>After</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>UA (µmol/l)</td>
<td>153.95±41.87</td>
<td>198.43±87.74</td>
<td>0.01</td>
</tr>
<tr>
<td>ALB (g/l)</td>
<td>1.31±0.07</td>
<td>1.43±0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>GPx (IU/l)</td>
<td>1842.95±157.76</td>
<td>3310.75±169.24</td>
<td>0.01</td>
</tr>
<tr>
<td>SOD (IU/l)</td>
<td>0.45±0.12</td>
<td>0.39±0.24</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>TAS (µmol/l)</td>
<td>0.40±0.24</td>
<td>0.66±0.35</td>
<td>0.01</td>
</tr>
</tbody>
</table>

UA – uric acid; ALB – albumin; GPx – glutathione peroxidase; SOD – superoxide dismutase; TAS – total antioxidative status

Figure 1. Correlation between plaque index (PI) and glutathione peroxidase (GPx) in chronic periodontitis patients before non-surgical periodontal treatment

Figure 2. Correlation between superoxid dismutase (SOD) and gingival index (GI) in chronic periodontitis patients before non-surgical periodontal treatment

Figure 3. Correlation between superoxide dismutase (SOD) and bleeding on probing (BP) in chronic periodontitis patients after non-surgical periodontal treatment

Figure 4. Correlation between total antioxidative status (TAS) and bleeding on probing (BP) in chronic periodontitis patients after non-surgical periodontal treatment
disruption of relations of free radicals and antioxidants play an important role in the pathogenesis of inflammatory diseases of oral tissues. Most authors compared the levels of antioxidants in the saliva of CP patients, as the most common oral inflammatory disease, with their levels in individuals with the clinically healthy periodontium [2, 8, 9]. In recent studies the levels of antioxidants in the saliva of CP patients before and after the therapy have been compared [10, 11].

UA is the most common antioxidant of saliva. In this study, conducted after the non-surgical therapy of periodontitis, the concentration of this antioxidant increased significantly. Results of previous studies indicated similar results. In an extensive study involving 129 subjects in whom periodontal health was evaluated by CPIITN (Community Periodontal Index of Treatment Needs), a direct proportion between the level of UA and CPIITN was found. The concentrations of uric acid in patients with high values of CPIITN index (diseased periodontal tissue) were significantly lower than in those with low values of this index [12]. The aforementioned research provided information of lower concentration of uric acid in women. The level of UA in our study was also lower in women than in men but the difference did not reach statistical importance. A study conducted on patients with periimplantitis a significantly lower concentration of uric acid in saliva was found than in patients who had implants but without periimplantitis [13]. A study involving 17 patients with CP (pocket depth ≥3 mm) and 20 individuals with clinically healthy periodontal tissue failed to find significant difference in the concentration of UA between CP patients and healthy individuals [14].

Non-enzymatic salivary antioxidant ALB is the main protein of blood plasma. This protein is able to neutralize the peroxide radical, binds free fatty acids and thus protects against lipid peroxidation. In the study, conducted after the completion of non-surgical therapy, statistically significant increase in ALB concentration in the saliva was found. It was found that albumin in saliva of patients with chronic periodontal disease does not originate from the salivary glands than from plasma [9, 15]. This research is pointing out that the concentration of albumin, in the saliva of CP patients is significantly lower than in individuals with a healthy periodontium [16]. A study involving 26 subjects (12 smokers and 14 nonsmokers) with CP shows an increase in albumin concentration after non-surgical treatment. Many studies have shown the decrease in the concentration of ALB with deterioration of periodontal tissue [12, 14, 17, 18].

GPx has a primary role in neutralizing hydrogen peroxide and the prevention of H$_2$O$_2$, it must interact with enzymes such as GPx, which removes H$_2$O$_2$. This is necessary because the accumulation of substances with OH group may be more dangerous than substances with O$_2$. [19, 24, 25]. In our study, after treatment of periodontitis, SOD activity was reduced, but this reduction was not statistically significant. Bacterial lipopolysaccharide stimulates the release of O$_2$ from tissue fibroblasts during inflammation [26]. Increased creation of O$_2$ can lead to an increase in SOD activity in order to establish a balance between oxidative stress and antioxidant protection. Also, increased activity of SOD, that as a final result of its reactions has the creation of H$_2$O$_2$, for the same reasons can lead to an increase in GPx activity, which eliminates H$_2$O$_2$, hence the increase in GPx activity after the therapy. Conducted surveys using standard deviation have found a significant and progressive reduction of SOD activity with increasing depth of periodontal pockets [27].

Studies dealing with measuring the activity of antioxidant enzymes in plasma, erythrocytes and gingival tissues of patients with chronic periodontitis and those with clinically healthy periodontal tissue showed a significantly reduced activity in all tested substrates in patients with the clinically healthy periodontal tissue. The same study found a reduced activity of GPx in CP patients. GPx should remove products of SOD catalyzed reactions [28]. Some studies suggest reduced activity of SOD in patients with chronic periodontitis compared to those with the clinically healthy periodontal tissue [29, 30]. In a study that had a similar design as ours, the SOD activity was significantly higher after the therapy of CP [31]. The diversity of the results of various studies on the activity of salivary enzymes can be explained by a rather difficult process of determining these enzymes in saliva when compared to blood. This is due to the lack of diagnostic tests for saliva.
The subject of this and many previous studies was the analysis of the total antioxidant capacity of saliva (TAS) of CP patients. In our study, after non-surgical treatment of CP, a statistically significant increase of saliva TAS was found. This result is consistent with results from the literature [2, 5] and with the data of this study that was obtained for the concentration of uric acid which represented 70% of the antioxidant capacity of saliva.

In this study, the correlation of clinical parameters with biochemical parameters, before and after the therapy of CP was also examined. Before therapy a positive correlation was established between PI and GPx activity, and negative correlation between BP and SOD activity. After treatment we found a positive correlation between TAS and BP, SOD and BP. Only a few previous researches dealt with the correlation between antioxidant capacity of saliva and clinical parameters of periodontal health.

The study that included CP patients (19) and individuals with the clinically healthy periodontium (8) focused on the correlations between GPx activity in GCF and clinical indicators of periodontal tissue: PI, GI, probing pocket depth and the level of CAL. Positive correlation between GPx activity and all of the observed clinical parameters was found [32]. Differences from the results of our study originate from the fact that, as shown by many studies, the activity of GPx in GCF is higher than in saliva. In the study of TAS in the saliva and serum from CP patients no statistically significant correlations between biochemical and clinical parameters were found [2]. The study, which examined the correlations GPx activity in saliva of CP patients before and after therapy and clinical periodontal parameters (PI, GI, BP, PD, CAL) showed a statistically significant correlation. [10]. The average values of GPx activity in aforementioned study were much higher than in our study, probably due to different collection and storage of saliva even though in both studies mixed unstimulated saliva was used.

CONCLUSION

The importance of such studies could be in the use of saliva as a valid diagnostic fluid in monitoring periodontal disease, due to the fact indicated by numerous studies reporting that the secretion is in continuous and intimate contact with tissues of the oral environment, thus accurately reflecting all events in them, such as physiological and pathological, as well as those on the cellular molecular level.

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КРАТАК САДРЖАЈ
Увод Оксидативни стрес и антиоксиданси играју важну улогу у патогенези запаљења зубних болоба, укључујући и хроничну пародонтопатију. Пљувачка садржи ензимске антиоксидансе, као што су глутатион-пероксидаза (GPx) и су- пероксид-дисмутаза (SOD), као и неензимске антиоксидансе, под хемиксолиметричним и комерцијалним реагенсама.

Циља рада Циљ истраживања био је да се испитају нивои SOD, GPx, UA и ALB и утврдити укупан антиоксидантни статус пљувачке (TAS) код особа с хроничном пародонтопатијом према каузалним пародонтопатијама. Утврдити корелације између клиничких показатеља стања пародонтодисаза и нивоа антиоксидансе у пљувачком садржима.

Методе рада Пљувачка је сакупљена од 24 пацјената с хроничном пародонтопатијом и нивоа каузалне индексе.

Ниво испитиваних антиоксиданса (осим GPx) i TAS одређиван је помоћу колориметријске методе и комерцијалних реагенса. GPx је одређиван применом УВ методе и комерцијалних реагенса.

Резултати Након терапије установљени су значајно по-већа концепција UA, GPx и TAS (p < 0,01) и смањење активности SOD (p > 0,05). Примењена је и значајна корелација између GPx i PI (r = 0,575; p = 0,008), те SOD i GI (r = 0,525; p = 0,017) према терапији, односно SOD i BP (r = 0,59; p = 0,006), као и i TAS i BP (r = 0,453; p = 0,045) после ње.

Заклучаки Добијени налази показују да се нивои антиоксиданса у пљувачком садржима по-већа у пародонтопатији.

Кључне речи: пљувачка; антиоксиданси; мокраћна киселина; албумин; глутатион-пероксидаза; супероксид-дисмутаза; терапија хроничне пародонтопатије.