



Activities of selected antioxidants in saliva and plasma in patients with periodontal diseases - initial results

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General Note



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ABSTRACT

Background: Periodontal diseases are very common, beginning with inflammation caused mostly by dental plaque bacteria. Persistent infection can lead to soft tissue damage and tooth loss. Oxidative stress is common in all oral inflammatory diseases and may affect systemic changes. **Methods:** The selected enzymes (superoxide dismutase, glutathione peroxidase and reductase) as well as non-enzyme antioxidant (reduced glutathione) parameters in saliva and plasma from patients with diagnosed gingivitis, a chronic and aggressive form of periodontitis were detected and compared to healthy individuals. **Results:** Initial results of the study come from analyses in a small number of patients, which is likely also the reason for the lack of statistical significance in the differences between parameters obtained in experimental groups and the control group. Even so, it is apparent that oxidative stress is indicative of changes in the activities of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, glutathione reductase) relative to reduced glutathione concentrations. **Conclusions:** There are positive correlations between saliva and plasma superoxide dismutase, glutathione peroxidase and glutathione reductase activities. This would allow for the dissemination of results within research groups in favour of analyses of more readily available biological material from patients.

Keywords: antioxidant enzyme, antioxidant marker, parodontitis, periodontal disease, reactive oxygen species, saliva

1. INTRODUCTION

Chronic periodontitis is one of the most common periodontal diseases worldwide, leading to the loss of supportive tissues of the teeth and the teeth themselves (Albandar, 2005; Bueno et al., 2015). The main cause of this disease is the presence of microbial plaques which contain bacteria which cause inflammatory changes in the gums and consequent bone damage (Ma et al., 2018; Shaddox et al., 2010). Symptoms of chronic periodontal disease are gingivitis, alveolar bone resorption and the presence of periodontal pockets. In addition, there are other unconventional symptoms such as dental mobility and pain. Its severity and prevalence vary considerably between populations (Albandar, 2005), which may, in fact, be due to differences in data collection methods and patient selection criteria.

The bacteria considered to be initiating factors of periodontitis include the gram-negative anaerobic bacteria *Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans*. Inflammatory reactions were also observed after stimulation by *Bacteroides forsythus*, *Prevotella intermedia*, *Peptostreptococcus micros* and *Fusobacterium nucleatum* (Kluknavská et al., 2019). Hiranmayi et al. (2017) also found the involvement of other pathogens in the development and progression of periodontitis such as *Cryptobacteriumcurtum*, *Dialisterpneumosintes*, *Filifactoralocis*, *Mitsuokelladentalis*, *Slackiaexigua*, *Selenomonassputigena*, *Solobacteri ummoorei*, *Treponema lecithinolyticum*, and *Synergistes*.

The incidence of periodontitis is also related to other factors, e.g. high blood pressure, high cholesterol, diabetes, genetic factors and obesity (Natto et al., 2018). Periodontitis is considered a classic complication of diabetes (Löe, 1993) due to its action as a metabolic stressor that increases insulin resistance or as a continuous source of secretion of inflammatory markers. These may then enhance the cytokine response mediated by the end products of advanced glycation (Mealey and Oates, 2006). Chronic periodontitis is also an independent risk factor for cardiovascular disease involved in the development of systemic inflammatory mediators. Moreover, some of the same bacteria were found in periodontal pockets and atherogenic plaques (Haraszthy et al., 2000; Humphrey et al., 2008; Paraskevas et al., 2008). Recently, a study of Kamalabadi et al. (2020) has shown that saliva of patients with chronic periodontitis also reflects the state of liver disease. While diet, physical inactivity and smoking are also considered to be risk factors (Reynolds, 2000), individuals exposed to a higher level of stress tend to neglect oral hygiene, change their eating habits, and smoke; all of which affect the entire immune system negatively (Peruzzo et al., 2007). Despite this, age and gender are among the most prominent factors. The production of reactive oxygen species (ROS) and the incidence of chronic inflammatory diseases increase with age. In men, the cause of periodontal disease has been shown to be poor oral hygiene in many cases (Natto et al., 2014; Shiau and Reynolds, 2010).

Periodontitis is a chronic inflammatory process associated with the production of ROS. Factors with a demonstrable link to it are also involved in the production of ROS. The aim of this study was to determine the effectiveness of selected antioxidant markers against ROS in saliva from patients with periodontitis and to compare them with values in healthy individuals.

2. MATERIALS AND METHODS

The study was approved by the Ethics Committee of Louis Pasteur University Hospital in Košice under no. 2018/EK/2010. Potential study participants were familiarised with the aim of the research prior to enrolment, and sampling took place after informed consent

was signed (January 2019 –December 2019). Blood samples were taken from the antecubital vein in order to determine standard and biochemical parameters and selected antioxidant markers. The collection of saliva (50 patients) and blood (44 patients) took place in the periodontology department of the 1st Dental Clinic of the University hospital in the morning between 7:00 – 9:00. Prior to collection, patients fasted without drinking fluids or brushing their teeth. During the collection, the patients were seated upright with their head slightly bent and spitting free-forming saliva in the mouth for 10 minutes. Saliva was then transported to the biochemical laboratory on ice. Patients were grouped according to clinical signs.

The first group consisted of healthy individuals (13 saliva and 12 plasma samples). Patients with gingivitis were the second group (12 saliva and 9 plasma samples). In the clinical picture, gingival bleeding and possible calculus deposits were observed. In the CPITN index examination, values of 1:(indicative of any bleeding), and 2:(indicative of calculus deposits), were reported in most sextants. The third group (CP) consisted of patients with chronic periodontitis and were found to have periodontal pockets during the clinical examination (16 saliva and 15 plasma samples). Values of 3: (pocket depth up to 6 mm), and 4: (more than 6 mm), were measured according to the CPITN (Community Periodontal Index of Treatment Needs). Alveolar bone resorption with concomitant gingivitis was observed via X-ray. The fourth group (AP) consisted of patients with aggressive periodontitis (9 saliva and 8 plasma samples). We observed deep periodontal pockets (pocket depth over 6 mm) predominantly in relatively young individuals. The gingiva was pale pink with possible point-like bleeding upon stimulus and the widespread damage to the periodontal tissues did not respond to oral hygiene. Groups with periodontitis, either chronic or aggressive, represented a generalised form.

Protein concentration in saliva and plasma was determined by abicinchoninic assay. The activities of glutathione peroxidase (GPx, EC 1.19.1.9), glutathione reductase (GR, EC 1.8.1.7) were determined according to the kit manufacturer procedures (Sigma-Aldrich, Germany) and superoxide dismutase (SOD, EC 1.15.1.1) by the SOD Assay Kit- WST (Fluka, Japan). The reduced glutathione (GSH) concentration was determined by the method of Floreani et al. (1997). After testing the normal distribution of values in groups, the Kruskal-Wallis test was used to determine the differences in the groups within each parameter and the Mann-Whitney U test to compare the individual parameters between groups. Results were considered significant at $p < 0.05$. The Spearman correlation test was used to determine the possible relationship between saliva and plasma measured parameters.

3. RESULTS

Tables 1 and 2 provide descriptive characteristics of the four measured parameters in the four saliva and plasma patient groups. The median SOD activity value was the lowest in the saliva of control group. The highest activities were found in patients with CP and AP. These values were comparable to plasma SOD activities. However, in plasma, median values were highest in the control group and in patients with gingivitis. Inter- and intragroup differences in SOD activities were not statistically significant. The median GPx activity in the control group was higher in saliva than plasma. It was also higher in the saliva of patients with gingivitis and AP, in contrast to plasma, where GPx was higher in CP patients. There were no statistically significant values within or between groups.

Table 1: Activities of superoxide dismutase and glutathione peroxidase in saliva and plasma of patients with periodontal disease

Group/ parameter	SOD ($\mu\text{kat}/\text{mg prot}$) med (min – max)		P saliva vs. plasma	GPx ($\mu\text{kat}/\text{mg prot}$) med (min – max)		P saliva vs. plasma
	saliva	plasma		saliva	plasma	
Control	0.0580 (0.004 – 0.151)	0.1870 (0.1300 – 0.2700)	1.7068	0.8740 (0.2743 – 7.4479)	0.2485 (0.0146 – 4.1228)	0.2460
Gingivitis	0.0640 (0.0170 – 0.1040)	0.2420 (0.0680 – 0.3200)	0.3371	1.2743 (0.2469 – 2.2531)	0.2778 (0.1754 – 0.9064)	0.3271
CP	0.1440 (0.0210 – 0.3390)	0.1600 (0.1170 – 0.2910)	0.7718	0.5190 (0.0439 – 1.7105)	1.0965 (0.1170 – 3.9474)	0.4593
AP	0.1570 (0.1090 – 0.1980)	0.1490 (0.1300 – 0.1560)	0.7949	1.7105 (0.0439 – 3.6988)	0.9503 (0.1023 – 2.1199)	0.7263
P	0.4796	0.8556		0.6902	0.7805	

AP – aggressive periodontitis, CP – chronic periodontitis, GPx – glutathione peroxidase, med (min – max) – median (minimum – maximum), P – statistical significance, SOD – superoxide dismutase.

Table 2: Activities of glutathione reductase and concentrations of reduced glutathione in saliva and plasma of patients with periodontal disease

Group/ parameter	GR (μ kat/ mg prot) med (min - max)		P saliva vs. plasma	GSH (nmol SH/ mg prot) med (min - max)		P saliva vs. plasma
	saliva	plasma		saliva	plasma	
Control	0.7407 (0.1608 – 2.1138)	1.1015 (0.3129 – 1.9583)	0.5961	0.1652 (0.0835 – 0.7498)	0.3536 (0.2206 – 1.2629)	0.2263
Gingivitis	0.6902 (0.4321 – 10.3175)	1.2391 (0.1681 – 4.1209)	0.6455	0.1260 (0.0414 – 0.9926)	0.3863 (0.1671 – 1.1029)	0.5755
CP	0.8602 (0.7373 – 1.1311)	0.9619 (0.5459 – 1.6588)	0.8415	0.2834 (0.0189 – 0.5980)	0.4322 (0.2057 – 1.4117)	0.6101
AP	1.2118 (0.1449 – 4.3016)	0.8184 (0.2083 – 1.4035)	0.5552	0.5181 (0.2280 – 1.8919)	0.2783 (0.1992 – 1.8615)	0.7795
P	0.9667	0.9337		0.7975	0.9126	

AP – aggressive periodontitis, CP – chronic periodontitis, GR – glutathione reductase, GSH – reduced glutathione, med (min – max) – median (minimum – maximum), P – statistical significance.

GR activities were very similar in saliva and plasma in patients with CP. However, median values were higher in healthy individuals and patients with gingivitis in plasma. A similar trend in median values was also observed in saliva and plasma for GSH concentrations. There were no statistically significant differences in both parameters. The results presented in Tables 1 and 2 show that the median of SOD, GPx, GR and GSH concentrations were consistently higher in the plasma of AP patients. In contrast, these are lower in plasma of AP patients.

Concentrations of some biochemical parameters are given in Table 3. There were no statistically significant differences between groups. The values examined mostly fall within the reference range (as for creatinine, albumin, alkaline phosphatase, sodium, potassium, chloride, calcium and magnesium). The exceptions were the highest C-reactive protein assayed in the CP patient and the lowest phosphorus concentration detected in the AP patient, both outside the reference range. Higher total cholesterol levels were found in each group.

Table 3: Serum concentrations of some biochemical parameters

	Control med (min - max)	Gingivitis med (min - max)	CP med (min - max)	AP med (min - max)	P
Creatinine (μ mol/l)	62.8 (53 – 85.6)	66 (53 – 85.6)	68.95 (46.9 – 85.6)	58 (49.8 – 81.9)	0.9832
Albumin (g/l)	46.1 (43 – 49.9)	47 (43.3 – 47.6)	42.3 (38.7 – 43.6)	42.75 (42.5 – 45.6)	0.4247
ALP (μ kat/l)	1.07 (0.45 – 1.75)	1.02 (0.81 – 1.52)	1.35 (0.88 – 1.6)	1.54 (0.82 – 1.91)	0.9205
Cholesterol (mmol/l)	4.25 (3.42 – 6.56)	4.1 (3.67 – 6.14)	5.86 (3.79 – 8.89)	4.71 (3.86 – 6.81)	0.6771
CRP (mg/l)	0.57 (0.22 – 9.71)	1.15 (0.3 – 5.69)	1.81 (0.71 – 16.31)	1.28 (0.93 – 5.31)	0.9486
Na (mmol/l)	138.3 (136.2 – 140.9)	137.9 (135.8 – 140)	139.1 (135.7 – 142.5)	137.6 (136.3 – 144)	0.9844
K (mmol/l)	4.4 (4 – 4.7)	4.2 (3.8 – 4.8)	4.3 (3.9 – 4.6)	4.4 (4.2 – 4.8)	0.8677
Cl (mmol/l)	103.8 (99.4 – 107.1)	103.4 (99.7 – 107)	104.5 (101.8 – 108.1)	102.3 (99.4 – 107.3)	0.9662

Ca (mmol/l)	2.47 (2.41 – 2.56)	2.48 (2.42 – 2.55)	2.38 (2.3 – 2.49)	2.52 (2.37 – 2.58)	0.8619
Mg (mmol/l)	0.84 (0.77 – 0.95)	0.84 (0.78 – 0.88)	0.82 (0.75 – 0.91)	0.85 (0.07 – 0.88)	0.9927
P (mmol/l)	1.11 (0.76 – 1.31)	1.05 (0.97 – 1.21)	1.08 (0.94 – 1.21)	0.86 (0.65 – 1.24)	0.9975

AP – aggressive periodontitis, ALP – alkaline phosphatase, CP – chronic periodontitis, CRP – C-reactive protein, med (min – max) – median (minimum – maximum), P – statistical significance.

4. DISCUSSION

Patient samples obtained so far are relatively low, but taking blood samples from patients at the dentist has been problematic. If patients did not wish to provide blood, we proceeded to evaluate and compare parameters at this stage, as further such comparison is unlikely to be possible. No significant differences were found in individual parameters between groups, or between the given parameters in saliva and plasma. However, according to median values there is a trend of increased SOD activities in patients with chronic periodontitis in both saliva and plasma (Table 1). A similar trend can be seen in GSH concentrations (Table 2), while GPx and GR activities do not show same increasing shift in activities for both chronic and aggressive periodontitis. Positive correlation has been demonstrated between the progression of periodontitis and serum SOD activity in experimental animals (Sobaniec and Sobaniec-Lotowska, 2000). This has been confirmed in human serum, gingival sulcular fluid and saliva (Akalin et al., 2005; Wei et al., 2010; Canakci et al., 2009; Guentsch et al., 2010; Karim et al., 2012). As observed in an animal model under experimental conditions, induction of SOD resulted in the inhibition of periodontitis (Petelin et al., 2000). Thus, SOD is considered a potential diagnostic marker of periodontitis.

We determined the selenium isoforms of glutathione peroxidase according to the kit composition. Glutathione peroxidase is an enzyme that catalyses the reduction of hydrogen peroxide using glutathione as a reducing agent (Patel et al., 2012). Almerich-Silla et al. (2015) found the highest values of glutathione peroxidase in the group of patients with chronic periodontitis compared to the healthy group and patients with gingivitis.

The initial results of our study point to a difference from these studies, although the number of patients examined so far is low. Nevertheless, the nature of the enzymes' action suggests glutathione peroxidase inhibition due to excess substrate (peroxides). Glutathione reductase activity is then affected as a compensatory mechanism for the production of reduced glutathione as a direct reducing agent. A second explanation can be found in the assay methodology used to determine the activity of the selenium isoforms of glutathione peroxidase and not the total, so the overall activity of glutathione peroxidases could be increased. However, a positive correlation was found between SOD activities in plasma and saliva ($r = 0.677$), and GPx ($r = 0.5234$) and GR ($r = 0.6417$).

Also, the biochemical parameters determined in routine examinations (Table 3) do not show statistically significant differences between groups, especially given the small numbers of patients examined. Nevertheless, some of the value distribution can be pointed out. First, the median of albumin values is higher in patients with chronic and acute periodontitis. Studies by Kaur et al. (2015) and Kolte et al. (2010) showed that there is association between chronic periodontitis and serum albumin concentrations in terms of inflammatory conditions in an organism, and with nutritional deficiency resulting also from the number of teeth and condition of the oral cavity. Second, median values of alkaline phosphatase (ALP) are shifted higher in patients with chronic and aggressive periodontitis. Recently, Malhotra et al. (2010) confirmed that ALP positively correlates with probing depth and is a suitable marker for identifying inflammatory sites in the oral cavity. Compared to healthy individuals, ALP levels are higher not only in saliva but also in the serum of patients with chronic periodontitis (Jeyasree et al., 2018). Third, it is evident that the medians of the C-reactive protein values in patients with periodontal disease (also the maximum value in chronic periodontitis) are higher than in healthy individuals. This would be consistent with a recent study by Bolla et al. (2017), confirming higher serum CRP values in patients with chronic and aggressive periodontitis as compared to healthy subjects. Also noteworthy is the shift of the median to higher values for total cholesterol, more significantly in patients with chronic periodontitis, which would correspond to the confirmed association between hyperlipidemia and periodontal disease (Hagh et al., 2014).

5. CONCLUSION

The nature of the effect of antioxidant enzymes points to the conditions of oxidative stress in patients with periodontal diseases, which is compensated by the reduced glutathione concentration. However, the sample size needs to be expanded. Maintaining a

balance between increased production of reactive oxygen species and an adequate response to antioxidant mechanisms is important through the course of the disease, both against bacterial infection and in preventing tissue destruction. Recent results indicate a positive correlation between SOD, GPx and GR activities in saliva and plasma, which could facilitate the collection and analysis of biological material in favour of something more readily accessible to patients.

Informed Consent

Potential participants were enrolled the study after signing the informed consent.

Conflict of Interest

None of the authors has financial/commercial conflicts of interest with the published data.

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Ethical approval for the study protocol

The study was approved by the Ethics Committee of Louis Pasteur University Hospital in Košice under no. 2018/EK/2010.

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