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ORIGINAL ARTICLE

Lipid peroxidation and antioxidant activity in saliva of periodontitis patients: effect of smoking and periodontal treatment

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Abstract The aim of this study was to measure lipid peroxidation (as an end product of oxidative stress) and corresponding antioxidant activity in patients with periodontitis and assess the influence of smoking and periodontal treatment on these parameters. Thirty healthy subjects (including 15 smokers) were compared to periodontitis patients (n=30, including 15 smokers). Malondialdehyde (MDA), glutathione peroxidase (GSHPx) and the total antioxidant capacity (TAOC) were recorded in saliva. The lowest level of lipid peroxidation (MDA) was measured in saliva in the non-smoking periodontally healthy subjects (0.065±0.05 μmol/l). MDA levels were significantly higher in periodontitis patients who smoked (0.123±0.08 μmol/l) compared to non-smoking controls (0.065±0.05 μmol/l; p<0.05). The periodontally healthy subjects demonstrated significantly lower levels of GSHPx (antioxidative parameter) than the periodontitis group (p<0.05). The TAOC flow rate (delivered antioxidant components within saliva) was significantly lower in patients with periodontitis (0.34±0.26 μmol/ml) in comparison to the controls (0.62±0.24 μmol/ml; p<0.05). Patients with periodontitis demonstrate more lipid peroxidation than healthy subjects, and this effect is enhanced by smoking. Imbalance between oxidative stress and antioxidant capacity may play a role in the pathogenesis of periodontal disease. Non-surgical periodontal treatment leads to a reduction of MDA and GSHPx to levels comparable to healthy controls.

Keywords Periodontitis · Oxidative stress · Lipid peroxidation · Antioxidant capacity · Smoking · Saliva

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Introduction

It is widely accepted that the host response to subgingival bacteria plays a critical role in periodontal pathogenesis [30] and that pathogenic processes are modified by environmental and acquired risk factors such as smoking [24]. For example, smokers demonstrate 2.6–6 times increased prevalence of periodontal diseases compared to non-smokers [1] and a reduced response to periodontal treatment [2]. Reduced success rates for implant placement have also been reported for smokers compared to non-smokers [3].

The polymorphonuclear leucocyte (PMN) constitutes the first line of cellular host defences against bacteria in the gingival sulcus [30]. The antimicrobial activities of PMNs and monocytes include oxygen-dependent and oxygen-independent mechanisms [22]. The oxygen-dependent pathway involves the production of reactive oxygen species (ROS), molecules which are capable of initiating periodontal tissue destruction [5]. The production of ROS by PMNs is primarily focused towards bacterial killing, but extracellular

release of ROS results in destruction of the surrounding tissues [34]. ROS cause tissue damage via multiple mechanisms, e.g. DNA damage, lipid peroxidation, protein damage, enzyme oxidation and the stimulation of pro-inflammatory cytokine release by monocytes and macrophages [11, 20].

Polyunsaturated fatty acids are highly susceptible to attack by ROS [29], leading to lipid peroxidation, which has been shown to cause profound alterations in the structural integrity and function of cell membranes [37]. Lipid peroxidation can be quantified using the thiobarbituric-acid-reactive substances method (TBARS), which evaluates oxidative stress by assaying malondialdehyde (MDA), the final product of lipid breakdown caused by oxidative stress [27].

Several ROS and lipid peroxidation products are produced in physiological quantities in the human body, but an overproduction of ROS occurs at sites of chronic inflammation [22]. The removal of ROS by antioxidant defence systems is essential for maintaining health [21], and antioxidant mechanisms specifically remove harmful oxidants as they form or repair damage caused by ROS [11]. Antioxidant defence systems comprise three main components: (1) preventive antioxidants, which suppress the formation of free radicals (e.g. superoxide dismutase, catalase, glutathione peroxidase), (2) radical-scavenging antioxidants, which scavenge radicals to inhibit chain reactions (e.g. uric acid, vitamins A, C and E, albumin, bilirubin) and (3) repair and de novo enzymes, which repair damage and reconstitute membranes (e.g. DNA repair enzymes, lipase, protease, transferase) [6]. In normal physiology, there is a dynamic equilibrium between ROS activity and antioxidant defense capacity. When there is an imbalance, caused by a reduction in antioxidant defence and/or ROS production or activity, oxidative stress results [14].

During gingival inflammation, gingival crevicular fluid (GCF) flow increases, and components of the inflammatory response are detectable in saliva, including lipid peroxidation products [6]. Saliva can be easily collected and hence may offer a basis for patient specific diagnostic tests for periodontitis [25]. Given the importance of lipid peroxidation products in periodontal pathogenesis, the aim of this study was to examine lipid peroxidation and antioxidative parameters in saliva and blood (i.e. measure local and systemic effects) in patients with periodontitis in comparison to healthy subjects. Further, the influence of smoking and the effects of treatment on these parameters were observed.

Materials and methods

Subject recruitment

Thirty subjects with generalised chronic periodontitis characterised by at least 30% teeth with pockets >5 mm were recruited from new patients of the Department of Conservative Dentistry (Section of Periodontology), University of Jena. None of the patients had received any periodontal therapy nor had used antibiotics or immunosuppressive agents in the preceding 6 months. Periodontally healthy control subjects (n=30) with no evidence of periodontal disease (all probing depths ≤3 mm) were also recruited. Test and control groups each contained the same proportion of smokers and non-smokers (each n=15).

Subjects with significant systemic disease (e.g. diabetes mellitus, cancer or coronary heart disease), antibiotic therapy within the last 6 months and pregnant or lactating females were excluded. Further exclusion criteria were vitamin supplementation within the previous 3 months and any special dietary requirements. Inclusion criteria for the smokers were current smoking of at least ten cigarettes per day and a minimum of four packs per year. Subjects were only included into the non-smoker group if they were never-smokers. Former smokers were not recruited.

Ethical approval was obtained from local ethics committee of the University of Jena (no. 08390402). Written informed consent was obtained from each subject before participation. The study was conducted in accordance with the principles of the Declaration of Helsinki (World Medical Association Declaration of Helsinki 1989).

Periodontal assessment

The clinical data were recorded after sample collection (saliva and blood). Probing depths (PD) were measured with a periodontal probe (PCP-UNC 15, Hu Friedy, Leimen, Germany) at six sites per tooth. Bleeding on probing (BoP) was calculated as the percentage of positive sites per subject. All data were recorded in the test group after the hygiene phase, before surgery and 6 months after periodontal treatment

Periodontal treatment

Non-surgical periodontal treatment was performed in the periodontitis patients. This included intensive hygiene phase, full-mouth scaling and root planing (performed in all four quadrants under local anaesthesia at one appointment) and then a period of periodontal maintenance and monitoring of oral hygiene. Sample collection and clinical data were recorded following hygiene phase. Periodontal maintenance visits were at 4, 12 and 24 weeks after initial treatment. At the 6 months appointment, samples and clinical data were recorded again. No early withdrawals during the study period were observed.

Clinical samples

Whole saliva samples were collected from patients and controls between 8:00 and 10:00 A.M. using a sterile glass

funnel into weighed 10-ml sterile polypropylene containers for 10 min. No oral stimulus was permitted for 120 min before collection to exclude any influence of mastication or foods. The seated patients collected the saliva over the period and pooled the saliva in the bottom of the mouth and drained to a collection tube when necessary. The containers were weighed again after sample collection, and flow rate was calculated (ml/min). Fasting venous blood was collected from the antecubital fossa (lithium heparin tube, Monovette, Sarstedt AG, Nümbrecht, Germany). The tube was centrifuged at 4,000×*g* for 10 min to separate serum and plasma. The serum was collected and stored in three 1.5 ml aliquots at −70°C.

Analysis

MDA as an index of lipid peroxidation was measured using the method of Yagi [45]. The proteins in saliva or blood were precipitated with trichloracetic acid (10%) to increase the specificity. Thiobarbituric acid (0.8%) was added for 25 min at 95°C. The developed fluorescent substance was extracted with an n-butanol-hydrochloric acid (HCl 32%). The MDA concentration was determined relative to a standard calibrant. Fluorescence was measured at an excitation length of 515 nm and an emission wavelength of 553 nm (Fluorometer LS 30, PerkinElmer, Fremont, CA, USA).

GSHPx activity was measured according to the method of Paglia and Valentine [31]. GSHPx affects the degradation of H_2O_2 and organic hydroperoxides. The reducing agent of this reaction is glutathione. The reaction is reversible with oxidised glutathione (GSSG) reductase, which needs nicotinamide adenine dinucleotide phosphate (NADPH). The principle of the GSHPx assay is to measure the consumption of NADPH using the change of extinction length (340 nm). The extinction was measured with a UV–Vis spectrometer UV-2 (UNICAM, Cambridge, UK).

Antioxidant capacity assay

Total antioxidant capacity (TAOC) comprises water- and lipid-soluble components. The total water-soluble antioxidant capacity and the total lipid-soluble antioxidant capacity were measured by photochemical luminescence [35] (Photochem, Analytik Jena AG, Jena, Germany). Using this photochemical luminescence technique, it is possible to examine the antioxidative capacity of both lipid-soluble and water-soluble substances in a single system, and so the total antioxidative capacity as a sum of these parameters can be measured in mixtures such as saliva and blood. The results were presented on equivalent concentration units of ascorbic acid for water-soluble substances or Trolox units for lipid-soluble substances.

In accordance with the approach of Moore et al. [28] and Sculley and Langley-Evans [39], who described that the absolute antioxidant concentrations may be misleading and it is more important to consider the rate of delivery of antioxidant components into the oral cavity, the absolute antioxidant concentrations were calculated into antioxidant flow rates (μmol/ml): antioxidant flow rate = [antioxidant]/saliva flow [28, 39].

Statistical analysis

The periodontal parameters and the levels of the observed parameters in saliva and blood were expressed as means± SD. The primary outcome parameters were MDA and TAOC flow rate. GSHPx activity, PD and BoP were secondary endpoints. Normal distribution of the data was tested with the Kolmogorov–Smirnov test with Liliefors correction. A two-factor analysis of variance was performed for the recorded parameters as well as the effect of both binary factors, smoking habits and disease status, for each parameter examined. A p value of <0.05 was considered to be statistically significant. SPSS 13.0 (SPSS, Chicago, IL, USA) was used for all statistical analyses.

Results

Demographic and baseline clinical data are presented in Table 1. Not unexpectedly, patients with periodontal disease demonstrated significantly higher mouth mean probing depths (3.82±0.68 mm) compared to the healthy control subjects (1.64±0.31 mm; $p<0.05$). However, there were no statistically significant differences in mean probing depths between smokers and non-smokers within the control or test groups. Mean probing depths were significantly reduced after periodontal treatment in the periodontitis patients ($p<0.05$), as would be expected.

When comparing the healthy (control) and periodontitis (test) groups, there was significantly more BoP in the test group (91.81±15.98%) than in the healthy subjects without periodontal disease (6.36±9.20%; $p<0.05$). After periodontal treatment, we recorded a significant reduction in % BoP in both groups ($p<0.05$).

Table 2 presents data relating to lipid peroxidation and antioxidant capacity in saliva. The level of MDA as a value of lipid peroxidation increased progressively from the non-smokers (0.065±0.05 μmol/l) to the smokers (0.085± 0.08 μmol/l) of the periodontally healthy controls and then to the non-smokers (0.095±0.05 μmol/l) and the smokers (0.123±0.08 μmol/l) of the periodontitis group. Patients with periodontitis who smoked demonstrated significantly higher MDA concentrations than the non-smoking controls ($p<0.05$). The treatment of periodontitis led to a significant reduction in lipid peroxidation products ($p<0.05$) in the periodontitis patients, and post-treatment MDA levels in the

Table 1 Demographic and clinical data

	Healthy patients		Periodontitis patients	
	Non-smokers (n=15)	Smokers (n=15)	Non-smokers (n=15)	Smokers (n=15)
Age (mean±SD, years)	34.1±11.8	30.6±8.7	46.3±13.1	41.0±8.8
Gender (m/f)	7:8	7:8	6:9	8:7
Smoking (cigarettes per day)	0	18.3±6.2	0	19.0±4.7
Baseline PD (mean±SD, mm)	1.60±0.34	1.67±0.29	3.85±0.66[a,b]	3.79±0.72[a,b]
Post-treatment PD (mm)	–	–	2.30±0.75[a,b,c]	2.59±0.75[a,b,c]
Baseline BoP (mean±SD, %)	7.11±10.84	5.34±6.72	90.02±21.16[a,b]	93.87±6.67[a,b]
Post-treatment BoP (%)	–	–	21.62±9.86[a,b,c]	17.95±14.98[a,b,c]

[a] Significantly different from non-smoking control group (p<0.05)
[b] Significantly different from smoking controls (p<0.05)
[c] Significantly different from baseline (p<0.05)

periodontitis patients (0.060±0.11 μmol/l) were similar to those seen at baseline in the healthy controls (0.075± 0.06 μmol/l; Fig. 1).

Patients with periodontitis (whether smokers or non-smokers) demonstrated significantly elevated glutathione peroxidase activity compared to periodontally healthy patients (p<0.05). In both the non-smoking and smoking periodontitis groups, we measured significantly higher GSHPx activity (approximately threefold elevation) compared to the periodontally healthy control groups. The elevated GSHPx activity was significantly reduced after periodontal therapy (p<0.05) such that post-treatment values in the periodontitis patients (6.63±3.53 U/l) were similar to those seen at baseline in the control group (6.75± 3.44 U/l; Fig. 2).

Patients with periodontitis both smokers (0.29± 0.21 μmol/ml) and non-smokers (0.37±0.24 μmol/ml) showed significantly lower salivary antioxidant capacity than the non-smoking (0.52±0.20 μmol/ml) and smoking (0.75±0.24 μmol/ml) controls. Smoker with periodontitis had a tendency for lower salivary antioxidative capacity (not significant).

TAOC flow rates were significantly lower in the periodontitis group in comparison to healthy patients (p<0.05). Notably, TAOC flow rates did not increase in the periodontitis patients following treatment (Fig. 3).

MDA levels in serum of periodontally healthy subjects who were non-smokers (1.57±0.35 μmol/ml) or smoker (1.67±0.46 μmol/ml) were comparable to the MDA level in serum of non-smoking periodontitis patients (1.63± 0.46 μmol/ml). Except for the significantly higher value of MDA for smoking subjects with periodontitis (1.93± 0.47 μmol/ml) compared to the non-smoking control group (p<0.05), we did not identify any other significant differences for the measured parameters between the four groups. No differences of GSHPx activity were observed in serum of non-smoking (213.90±28.70 U/l) and smoking (214.35± 22.12 U/l) controls as well as non-smoking (191.36± 58.41 U/l) and smoking (202.00±39.00 U/l) subjects with periodontitis.

Table 2 Lipid peroxidation and antioxidative parameters in saliva (mean±SD)

	Healthy patients			Periodontitis patients	
	Non-smokers (n=15)	Smokers (n=15)		Non-smokers (n=15)	Smokers (n=15)
Flow rate (ml/min)	0.60±0.28	0.45±0.20	Pre	0.59±0.29	0.55±0.25
			Post	0.65±0.19	0.63±0.21
MDA (μmol/l)	0.065±0.05	0.085±0.08	Pre	0.095±0.05	0.123±0.08[a]
			Post	0.060±0.09[c]	0.060±0.05[c]
GSHPx (U/l)	5.78±3.77	7.72±2.70	Pre	16.08±13.34[a,b]	21.10±18.65[a,b]
			Post	6.54±2.84[c]	6.74±4.33[c]
TAOC flow rate (μmol/ml)	0.52±0.20	0.75±0.24	Pre	0.37±0.24[a,b]	0.29±0.21[a,b]
			Post	0.44±0.22[a,b]	0.42±0.17[a,b]

Malondialdehyde (MDA) is presented as a parameter of lipid peroxidation, while glutathione peroxidase (GSHPx) and antioxidant flow rate (TAOC flow rate) are antioxidative parameters, including pretreatment (Pre) and post-treatment (Post) data in the periodontitis groups
[a] Significantly different from non-smoking control group (p<0.05)
[b] Significantly different from smoking controls (p<0.05)
[c] Significantly different from the pretreatment values (p<0.05)

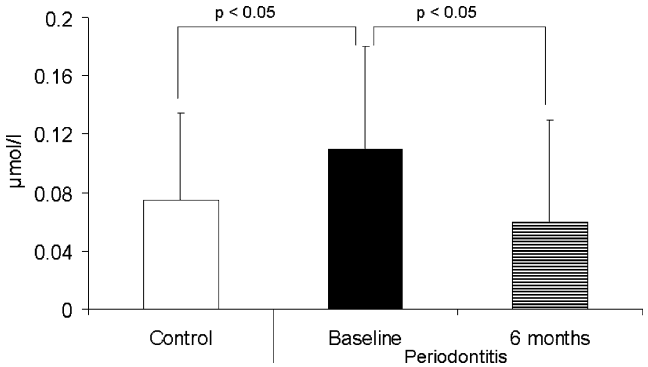

Fig. 1 MDA as parameter of oxidative stress in saliva of periodontally healthy subjects (*open square*; n=30) and patients with periodontitis (*filled square*; n=30, pre- and post-treatment). Patients with periodontitis demonstrated significantly more lipid peroxidation than the control group (p<0.05). Periodontal treatment results in a decrease of MDA values and reached levels comparable to periodontal health (*shaded columns*)

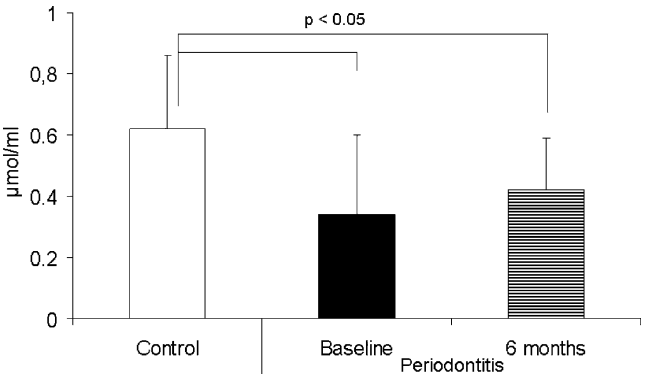

Fig. 3 Total antioxidative capacity (TAOC flow rate) in saliva. Patients with periodontitis (*filled square*; n=30) had a significantly less TAOC flow rate in comparison to the controls (*open square*; n=30; p<0.05). Interestingly, this TAOC flow rate was not affected by periodontal treatment (*shaded columns*)

Discussion

This study evaluated parameters of oxidative stress and antioxidant mechanisms in saliva and blood in a cohort of patients with periodontitis who were either smokers or non-smokers compared to corresponding control groups. Smoking has been clearly shown to be a risk factor for periodontitis, and many studies have compared clinical status in smokers and non-smokers. In this study, we observed no differences in periodontal clinical parameters between smokers and non-smokers within the control groups and the test groups, though of course, this study was not conducted to investigate clinical differences between smokers and non-smokers.

Our study focused on lipid peroxidation and antioxidant mechanisms in periodontitis and the effect of smoking and periodontal treatment on these parameters. Increased levels of lipid peroxidation have previously been reported in inflamed periodontal tissue and may play a role in the destructive processes of periodontitis [43]. Thus, excessive local production of ROS, leading to increased lipid peroxidation, may play a role in periodontal pathogenesis. Furthermore, lower levels of total antioxidant capacity in periodontitis patients have previously been reported [12]. Therefore, the balance between ROS and antioxidant mechanisms is likely to be important in periodontal pathogenesis, and imbalance can be caused by (1) increased ROS and inhibited antioxidant mechanisms [19, 41] and/or (2) decreased antioxidant capacity in diseased subjects [8, 12].

The findings of our study suggest that both periodontitis and smoking lead to significant changes in lipid peroxidation (MDA) in saliva and blood and salivary antioxidant mechanisms (GSHPx, TAOC flow rate).The combination of periodontitis and smoking tended to result in significant differences in these parameters compared to those recorded in periodontally healthy control patients (specifically MDA and GSHPx in saliva TAOC flow rate and MDA in serum). Lipid peroxidation is one of the most important reactions of free radicals. Tissue destruction by oxidative stress can be measured by the final end products of lipid peroxidation, such as MDA, one of many aldehydes formed during lipid peroxidation [23]. Various studies have demonstrated that increased MDA and GSHPx levels correlate with presence of periodontal disease [7, 33, 43], and both parameters are oxidative stress markers in patients with periodontal disease [7]. TBARS, notably MDA, as a measure for ROS damage is questioned in the literature [14, 23]. However, e.g. Borges et al [7], recently demonstrated a clear association between an increase of TBARS and periodontitis [7], as well as Garg et al. [18] who reported increased MDA levels in gingival tissue in periodontitis patients especially if they smoked [18]. MDA as a biomarker is the principal and most studied product of lipid peroxidation, demonstrating

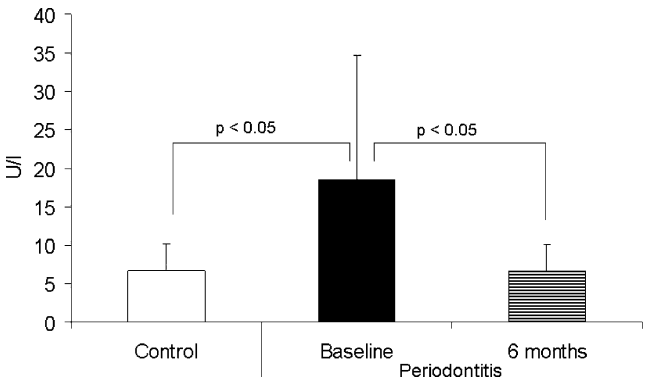

Fig. 2 GSHPx activity as antioxidative parameter in saliva. Periodontally healthy controls (*open square*; n=30) had a significantly lower GSHPx activity than subjects with periodontitis (*filled square*; n=30; p<0.05). GSHPx activity was in subjects with periodontitis 6 months after periodontal treatment reduced (*shaded columns*; p<0.05)

that levels of oxidative stress higher in pathological than in healthy conditions [16].

Local oxidative damage and antioxidant activity can be measured in GCF, gingival tissue or saliva [14]. TAOC is higher in GCF than in saliva (stimulated or unstimulated) [8], but saliva is easily collectable and is of interest as a possible future diagnostic test [25]. Further, different studies have confirmed the practicability of investigating oxidative stress or antioxidants in saliva [8, 17, 28, 38–40].

Tsai et al. [43] reported that lipid peroxidation in GCF and saliva was higher in diseased sites than in healthy sites and concluded that the balance between oxidative stress and antioxidant levels failed in periodontitis, resulting in increased tissue damage by ROS. Similar to this, we also measured higher MDA levels in periodontitis patients compared to healthy controls, and MDA concentrations in smokers with periodontitis (0.123±0.08 μmol/l) were significantly higher than those in non-smoking controls (0.065±0.05 μmol/l; $p<0.05$). Thus, smoking may enhance the effect of ROS in periodontitis, thereby increasing the tissue destruction resulting from oxidative stress. Furthermore, we also identified significantly higher MDA concentrations in the serum of smoking periodontitis patients (1.93 ±0.47 μmol/l) compared to non-smoking controls (1.57± 0.35 μmol/l). Similar findings of increased lipid peroxidation in periodontitis patients have also been described for saliva [26], blood and gingival tissue [33].

Panjamurthy and co-workers [33] investigated lipid peroxidation and antioxidant status in patients with periodontitis. The level of TBARS (MDA) in plasma, erythrocytes, erythrocyte membranes and gingival tissues were significantly higher in periodontitis patients than in the healthy controls. Further, these patients showed also higher levels of the tested enzymatic and non-enzymatic antioxidants. They concluded that disturbances in the endogenous antioxidant system due to overproduction of lipid peroxidation products at inflammatory sites can be related to a higher level of oxidative stress in patients with periodontitis. Our data, in which we found higher MDA and higher GSHPx levels in the saliva of periodontitis patients, support this conclusion that continuous production of ROS in periodontal inflammation adversely affect antioxidant mechanisms leading to tissue damage, measurable in tissue [33] or in saliva [26]. Celec et al. [10] demonstrated increased salivary MDA levels in patients with periodontitis and found no correlation between MDA concentration in saliva and serum. They suggested that local oxidative stress in periodontitis may lead to increased MDA levels [10].

As mentioned above, imbalance between oxidative stress and antioxidant mechanisms could also result from reduced antioxidant capacity in periodontitis. Chapple and co-workers [12] observed a reduction of glutathione and antioxidative capacity in patients with chronic periodontitis. These findings were confirmed in a further study in which TAOC was lower in both GCF and plasma in periodontitis subjects compared to healthy controls [8]. A decrease in systemic (serum) and local (GCF) antioxidant capacity (TAOC and SOD) was also observed in post-menopausal women and women with periodontitis by Baltacioglu and co-workers [4].

Studies of whole saliva in periodontitis patients revealed lower antioxidative capacity in the saliva [28, 39]. Sculley and Langley-Evans identified in 129 patients that poor periodontal health was associated with increased oxidative injury and decreased salivary antioxidants. These findings were confirmed by Diab-Ladki et al. [17] who also described a lower level of total antioxidant activity in saliva of patients with periodontitis [17]. In our study, we also observed less antioxidant capacity (TAOC flow rate) for periodontitis patients compared to healthy controls.

Glutathione peroxidase catalyses the reduction of H_2O_2 and various hydroperoxides using glutathione as a reducing agent. Glutathione metabolism is one of the most important antioxidative defence mechanisms, and GSHPx is the major source of protection against low levels of oxidative stress [27]. Wei et al. [44] observed lower GSHPx activity in GCF samples from healthy subjects (17.21±12.67 ng/μl) compared to patients with gingivitis (23.90±28.35 ng/μl) and periodontitis (35.70±35.89 ng/μl) [44]. Similar to this, we recorded increased levels of salivary GSHPx activity in periodontitis patients compared to healthy controls. The increase in antioxidant GSPHx in periodontitis occurs in response to the oxidative stress which develops in inflamed periodontal tissues.

Cigarette smoke contains a large amount of oxidative species, and so smoking is a source of oxidative stress [32, 36]. Smoking also increases the level of free radicals in periodontal tissues [18]. Tobacco smoke is not only a source of free radicals; antioxidant capacity is also affected. We have previously shown that periodontitis patients have reduced plasma ascorbic acid levels, especially if they smoke [42]. In the present study, we determined an additional effect of smoking on lipid peroxidation and GSHPx activity in saliva in periodontal disease. This is supported by data that suggest that extracellular GSHPx gene expression is induced by chronic exposure to cigarette smoke [15]. No effect of smoking on MDA, GSHPx and antioxidant capacity levels were observed in periodontally healthy patients, and similar findings were previously reported in which smoking and gingival inflammation had no effect on salivary antioxidants [9]. Our observation suggests that smoking elevated the oxidative stress parameters in patients with periodontitis.

The non-surgical periodontal treatment led not only to clinical improvements but also to a reduction parameters of oxidative stress. Tsai et al. [43] reported that lipid

peroxidation concentration was significantly correlated with clinical parameters or periodontal disease, and periodontitis patients showed significantly lower lipid peroxidation concentrations after treatment (0.41±0.26 μM) than before therapy (0.63±0.49 μM).

Chapple et al. [11] reported in a recently published study that local antioxidant capacity in patients with chronic periodontitis seems to reflect increased oxygen radical activity during periodontal inflammation. The antioxidant capacity was restored to control subject levels after successful non-surgical therapy. The periodontal treatment did not alter plasma TAOC [13].

Within the limitations of this study, we can conclude that patients who smoke and suffer from periodontitis demonstrate more lipid peroxidation in saliva than healthy subjects. We also observed an increase of glutathione peroxidase activity in periodontal disease, and in both cases, an additive effect of smoking was identified. A reduced antioxidant capacity in periodontitis patients was noted if the absolute antioxidant concentrations were calculated as antioxidant flow rates. Successful periodontal therapy has an effect on MDA and GSHPx activity in saliva, but not the antioxidant flow rate.

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