SCIENTIFIC ARTICLE

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# Lipid peroxidation levels, total oxidant status and superoxide dismutase in serum, saliva and gingival crevicular fluid in chronic periodontitis patients before and after periodontal therapy

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## ABSTRACT

***Background:*** Recent data have demonstrated increased lipid peroxidation (LPO) levels and oxidative stress in periodontitis. Malondialdehyde (MDA) and superoxide dismutase (SOD) are both increased during oxidative stress. Furthermore, this study examined SOD concentration, total oxidative status (TOS) and MDA levels in periodontal patients and investigated the longitudinal effect of periodontal therapy on the index levels of chronic periodontitis (CP) patients.
***Methods:*** Serum, saliva and gingival crevicular fluid (GCF) samples were obtained from 48 CP patients and 35 healthy control subjects prior to, as well as after 16 weeks following non-surgical post-periodontal therapy. MDA, TOS and SOD and clinical parameters were determined pre- and post-therapy.
***Results:*** The levels of TOS and SOD values were significantly higher in the CP group than in the control group ($p < 0.05$), but only MDA in GCF. Post-periodontal therapy, serum, saliva and GCF TOS and SOD levels significantly decreased compared to basal levels ($p < 0.05$), but only MDA in GCF.
***Conclusions:*** LPO was higher in the periodontal region, with TOS and SOD increasing both locally and peripherally. Non-surgical therapy can restore and control the subject antioxidant capacity by locally and systemically modifying the levels of MDA, TOS and SOD.

***Keywords:*** Chronic periodontitis, malondialdehyde, superoxide dismutase, total oxidative status, gingival crevicular fluid.

***Abbreviations and acronyms:*** CAL = clinical attachment level; CP = chronic periodontitis; GBI = gingival bleeding index; GI = gingival index; HPLC = high performance liquid chromatography; LPO = lipid peroxidations; MDA = malondialdehyde; PD = probing depth; PI = plaque index; PMNL = polymorphonuclear leukocytes; ROS = reactive oxygen species; SOD = superoxide dismutase; TOS = total oxidant status.

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## INTRODUCTION

Periodontitis, which affects the supporting tissues of teeth, is an inflammatory disorder of the periodontium. The interactions between pathogen and host defensive capacity result in periodontal tissue breakdown.[1,2] Many studies have investigated the pathogenesis of periodontitis.[3,4] The detection of oxygen-dependent production of reactive oxygen species (ROS) and antioxidant activity may be involved in the pathogenic mechanisms of the disease.

ROS include oxygen-derived free radicals, such as superoxide ($O_2^-$), hydroxyl (OH), nitric oxide (NO), hydrogen peroxide ($H_2O_2$) and hypochlorous acid (HOCL).[5,6] Several inflammatory cells and fibroblasts, vascular endothelial cells and osteoclasts also produce ROS.[7,8] ROS are highly toxic, not only to the internalized microbial agent, but also to the extracellular structure[6] and can induce lipid peroxidations (LPO) having effects on cells.[9] Redundant production of LPO can result in oxidative stress and consequently, damage to cell integrity. Because LPO results from oxidative stress, numerous markers have been used to monitor this process. Malondialdehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation that can indicate the increase of oxidative stress.[10,11]

Antioxidants, many of which are released locally at sites of inflammation by polymorphonuclear leukocytes (PMNLs) and/or other cells, can provide protection against ROS. In healthy organisms, the balance is maintained by the interaction of oxidants and antioxidants. Under pathological conditions, the balance may be directed towards the oxidative side.[12,13] Within mammalian tissues, the most significant antioxidant is superoxide dismutase (SOD), which catalyses the dismutation of $O_2^-$ to $H_2O_2$ and $O_2$.[6,14]

In recent years, attention has focused on the role of ROS, antioxidant systems, products of oxidative stress and LPO in the pathology of periodontitis.[15–19] The possible association of impaired salivary antioxidants status and increased oxidative injury in periodontal disease has been investigated.[17,20,21] Other studies have demonstrated increased LPO levels in periodontitis.[22] Moreover, significant reduction of SOD activity has been found in saliva, gingival crevicular fluid (GCF) and serum in pre-eclamptic women with periodontal disease.[17] Similar SOD activities have been found in periapical granuloma and healthy gingiva.[23] Other studies[19] have found that gingival SOD activity is significantly higher in chronic periodontitis (CP), which suggested that SOD activity increases with the progression of inflammation.

Oxidative stress has been variably determined by the measurement of a decrease in total antioxidant capacity, or more often, by estimation of the products of oxidative damage to lipids, proteins and DNA. Measurement of the products of oxidative damage can provide the most direct assessment of oxidative stress.[24,25] However, as the measurement of different oxidant molecules is not practical and their oxidant effects are additive, measurement of total oxidant status (TOS) in a sample can provide a new and practical approach.[26]

To date, there are few studies relating to the association between antioxidant systems and inflammation in the oral cavity or periodontium. Studies are also limited with regard to ROS and/or antioxidants mechanisms in oral fluids.

The purpose of this study was to examine SOD, TOS and MDA levels in periodontal patients, and to investigate the longitudinal effect of periodontal therapy on these indices in CP patients with varying degrees of periodontal destruction and inflammation.

## SUBJECTS AND METHODS

### Study groups

A total of 83 individuals were recruited into this randomized, longitudinal and interventional study; 48 were CP patients (27 males and 21 females) with an average age of 40.1 ± 7.3 years (mean ± standard deviation). These subjects were chosen from individuals who were referred to the Department of Stomatology, Nanjing Traditional Chinese Medical University Affiliated Changzhou Hospital, China due to periodontal problems or for routine periodontal controls. The patients were clinically and radiographically evaluated for CP according to the criteria accepted by the American Academy of Periodontology in 1999.[27] It was ensured that patients had teeth with 30% periodontal bone loss and with ≥5 mm deep pockets. The gingivae showed bleeding on probing and had the characteristics of chronic inflammation. The patients had poor oral hygiene, and the amount of accumulated plaque was measured along with the amount of clinical attachment loss. No evidence of rapid and aggressive periodontal breakdown was observed.

The control group included 19 males and 16 females with an average age of (42.1 ± 7.7) years (mean ± standard deviation) who presented with first or second premolars scheduled for extraction for orthodontic reasons. They had periodontal health and good oral hygiene with no clinical signs of gingival inflammation (bleeding on probing, hyperaemia and oedema), deep pockets, or tooth mobility.[28]

Criteria for being included in the study were having no systemic disease, having received no periodontal treatment, antibiotics, anti-inflammatory or other drugs in the last six months, being never-smokers, not being alcohol or antioxidant vitamin consumers and not going through menopause, menstruation, pregnancy or lactation at the time of the study. All subjects lived in the same geographic area (Changzhou city, Jiangsu Province, China) and were of a middle-class socioeconomic status and had similar traditional nutrition habits.

The periodontal sites in patient groups received conventional periodontal treatment consisting of oral hygiene insctructions, scaling and root surface debridement. At 16 weeks following treatment, the dentition received supragingival polishing with a rubber cup and pumice. Subjects in the control group received no periodontal treatment during the study. Prior to, as well as 16 weeks following periodontal therapy, measurement of periodontal status, collection of samples and the assay of biochemical indices were performed in the patient group and the control group.

The research protocol was reviewed and approved by the institutional ethical committee of the Nanjing Chinese Traditional Medical University. One of the authors and several assistants explained the objectives and procedures of the study to the selected subjects and informed written consent was obtained. Participation was voluntary and subjects could withdraw from the study at any time.

### Clinical measurement

The periodontal status of all individuals was detected by measurements of probing depth (PD), clinical attachment level (CAL), gingival index (GI),[29] gingival bleeding index (GBI)[30] and plaque index (PI)[31] prior to and after the therapy in CP and control groups. Full-mouth periapical radiographs were taken to determine the level of periodontal bone loss.[32,33] PD and CAL were measured on six sites of teeth (mesial, median and distal points at buccal and palatal aspects). All clinical measurements and radiographic examinations were performed by a single investigator. It was ensured that the total number of teeth in the mouth was ≥20.

### Collection of samples

All the samples, prior to and after periodontal therapy, were collected within 48 hours after the clinical measurements in the morning, following an overnight fast. All participants were told not to eat or drink anything or chew gum that morning. The subjects were asked whether they had followed these instructions before samples were collected.

Unstimulated whole saliva samples were used in this study. Saliva samples were obtained in the morning, over five-minute periods. Seated patients were instructed to allow saliva to pool in the bottom of the mouth and drain into a collection tube. Subjects were asked not to swallow any saliva for the duration of the collection to allow the calculation of salivary flow rate. Upon completion of the timed collection, the saliva volume was measured. The salivary flow rate was calculated by dividing the volume collected by time. Before analysis, the saliva was centrifuged at 4000 g for 10 minutes at 4°C. The supernatant fraction was then aliquotted into storage vials and kept in liquid nitrogen until analysis.

The GCF samples were collected between 8 and 10 am from the regions with ≥5 mm PD, ≥4 mm CAL and ≥30% bone loss. Ten GCF samples were collected from each patient. The sites were isolated using cotton rolls and were gently air dried before sampling. It was ensured that the samples were not contaminated by saliva. Collections were performed over 30 seconds with standardized paper strips (Periopaper), and volume was measured on a precalibrated Periotron 8000 with serum. GCF samples were collected in the same way from subjects in the control group. Ten Periopaper strip samples belonging to each subject were pooled in glass tubes and immersed in 500 ml of PBS (pH 6.5). Samples were eluted for 30 minutes at room temperature before removing the Periopaper strips and storage of the eluent in liquid nitrogen until analysis.

Venous blood was collected in plain tubes (for serum). Plain tubes were maintained at 4°C for a further 30 minutes before centrifugation at 1500 g for 10 minutes (room temperature). Serum samples were aliquotted into cryogenic vials and stored in liquid nitrogen. Each patient was used as the unit of analysis.

### Laboratory studies

#### *Lipid peroxidation (MDA) assay*

MDA levels in serum, saliva and GCF were determined by the method of Young and Trimble[25] with slight modification using the high performance liquid chromatography (HPLC) method. Briefly, 100 µl of samples were added to 10 µl of 0.2% butylated hydroxytoluene and mixed with 600 µl of 0.46M $H_3PO_4$, and left to stand at room temperature for 10 minutes. Two hundred microlitres of 0.6% TBA were added to all tubes, vortexed and heated for 30 minutes at 90°C. After cooling the tubes on ice, 400 µl of sample was mixed with 720 µl of methanol and 80 µl of 1 M NaOH in order to neutralize the acid and precipitate the protein content. Forty microlitres of supernatant, after centrifugation at 12 000 g for five minutes, were applied to an HPLC column (ZORBAX Eclipse XDB-C18; 4.6 × 150 mm; Agilent Technologies, Agilent 1100 series HPLC systems, Waldbronn, Germany). The column was equilibrated with an eluent consisting of 35:65 (v/v) methanols: 25 mM phosphate buffer, pH 6.4, for 60 minutes at 1 ml/min. A fluorescence detector at excitation 536 nm and emission 555 nm was used. MDA standards (0.035–1.25 mM) were prepared from tetraethoxypropane and were included in parallel with all samples.

#### *Total oxidant status assay*

TOS in serum, saliva and GCF samples was measured using a new measurement method developed by Erel.[26] Briefly, 225 µl reagent 1 (xylenol orange 150 µM, NaCl 140 mM and glycerol 1.35M in 25 mM $H_2SO_4$ solution, pH 1.75) was mixed with 35 µl of samples (serum, saliva and GCF) and the absorbance of each sample was read spectrophotometrically at 560 nm as a sample blank. Subsequently, 11 µl reagent 2 (ferrous ion 5 mM and o-dianisidine 10 mM in 25 mM $H_2SO_4$ solution) was added to the mixture and about 3–4 minutes after the mixing, the absorbance was read at 560 nm. The analytical sensitivity of the method was found to be 0.0076 absorbance/amount (AX (µM)).[1] The assay was calibrated with $H_2O_2$ and the results were expressed in terms of micromolar $H_2O_2$ equivalent per litre (µmol $H_2O_2$ Equiv/l). The detection limit of the method was determined by evaluating the zero calibrator 10 times. The detection limit, defined as the mean TOS value of the zero calibrator +3 SD, was

1.13 μmol $H_2O_2$ Equiv/l. The total TOS in GCF was measured in the total amount of GCF that was collected in 30 seconds. TOS concentration was calculated by dividing the total TOS to volume of GCF.

### *Superoxide dismutase activity assay*

Serum SOD activity was measured by the reduction of nitroblue tetrazolium (NBT) by xanthine–xanthine oxidase system.[34] Briefly, 0.5 ml serum was treated with ethanol–chloroform (5:3) mixture and vigorously vortex-mixed for 1 minute. Treated samples were centrifuged at 18 000 g for 60 minutes and the supernatant was used for the assay. A total of 0.250 ml supernatant was mixed with 1.25 ml of SOD assay reagent (this reagent consisted of 40 ml of 0.3 mmol/l xanthine, 20 ml of 0.6 mmol/ml EDTA, 20 ml of 150 μmol/l NBT, 12 ml of 400 mmol/l $Na_2CO_3$ and 6 ml of 0.1% bovine serum albumin). Twenty-five microlitres of xanthine oxidase solution (167 U/l) was added and tubes were incubated for 20 minutes at 25°C. The reaction was terminated by adding 0.5 ml of 0.8 mmol/l $CuCl_2$. The formed formazan was determined spectrophotometrically at 560 nm. Enzyme activity leading to 50% inhibition was taken as one unit and bovine erythrocytes (SOD) were used as the external standard. SOD activity of GCF was measured with the same method, except ethanol–chloroform treatment. The concentration results were expressed in terms of U/ml for serum and GCF. The range of standards that were used for SOD assay in the present study was 0–10 U/ml (0, 0.5, 1, 1.5, 2, 4, 6, 8 and 10 U/ml).

### Statistical analysis

Using Kormogorov–Smirnov goodness-of-fit test, the distribution of each set of data was tested for normality prior to analysis. Where necessary, data were normalized using natural-log transformations. Normally distributed data were expressed as mean ± standard deviation (X ± SD) and non-normally distributed data were expressed as median (minimum–maximum). Tests of significance were two-tailed and $p < 0.05$ was considered statistically significant. The differences between the patient group and the control group before and at the end of treatment were investigated with independent student's *t* test for data with normal distribution, and otherwise with the Mann–Whitney U-test. In addition, to compare the different concentrations of MDA, TOS and SOD in the three samples of serum, saliva and GCF before therapy, multiple comparisons were undertaken with Student-Newman-Keuls (SNK) test (normal and homogeneous data) or Student-Newman-Keuls rank test (nor-normal data), and SNK grouping letters were provided. Finally, the correlation coefficient was used to study the correlation between MDA, TOS and SOD levels and clinical parameters before therapy. Pearson's correlation coefficient was used for normally distributed data or Spearman's correlation coefficient for non-normally distributed data, while a Bonferroni correction was also made.

## RESULTS

### Clinical findings

The values of the clinical parameters are listed in Table 1. All the parameters were found to be significantly higher in the CP group compared with the control group ($p < 0.05$) (Table 1) before periodontal therapy. The mean saliva flow rate was measured as (0.44 ± 0.13) ml/min in the CP group and (0.42 ± 0.11) ml/min in the control group before therapy.

After periodontal therapy, no marked difference of clinical parameters were found between the CP group and the control group ($p > 0.05$) (Table 1). Similarly, the mean saliva flow rate in the CP group and the control group was (0.46 ± 0.15) ml/min and (0.43 ± 0.12) ml/min, respectively. There was no significant difference of saliva flow rate between the CP group and the control group regardless of periodontal therapy ($p > 0.05$).

**Table 1. Comparison of clinical parameters between CP and control groups before and after therapy**

| Parameters | CP 1 (before therapy) (n = 48) | Control 1 (n = 35) | CP 2 (after therapy) (n = 48) | Control 2 (n = 35) |
|---|---|---|---|---|
| PD | 3.81 ± 0.44† | 1.21 ± 0.23 | 1.23 ± 0.19‡§ | 1.22 ± 0.26 |
| CAL | 4.65 ± 0.91† | 0.49 ± 0.33 | 0.52 ± 0.29‡§ | 0.48 ± 0.31 |
| GI | 1.76 (0.61–2.49)† | 0 (0–0.3) | 0 (0–0.2)‡§ | 0 (0–0.24) |
| GBI | 2.71 (0.97–3.81)† | 0 (0–0.3) | 0 (0–0.28)‡§ | 0 (0–0.3) |
| PI | 1 (0–3)† | 0 (0–0) | 0 (0–0)‡§ | 0 (0–0) |

Normally distributed data are expressed as x ± SD and non-normally distributed data are expressed as median (minimum–maximum).
PD = probing depth; CAL = clinical attachment level; GI = gingival index; GBI = gingival bleeding index; PI = plaque index; CP = chronic periodontitis.
†The difference was significant between CP group and control group before therapy ($p < 0.05$).
‡No difference was found between CP group and control group after therapy ($p > 0.05$).
§The difference was significant before and after therapy ($p < 0.05$).

## Laboratory findings

### *Lipid peroxidation (MDA)*

The values of MDA levels in serum, saliva and GCF in the CP and control groups before and after therapy are shown in Table 2. While serum and saliva MDA concentrations were almost the same in the CP and control groups regardless of therapy, the values obtained in GCF were significantly higher in the CP group compared with the control group ($p < 0.05$) before therapy. After therapy, the concentrations of MDA in GCF decreased to the lower levels compared with the basal levels ($p < 0.05$) and showed no significant difference with the control group ($p > 0.05$). And the concentration of MDA in GCF was the highest among three samples ($p < 0.05$) in CP patients before therapy and that in saliva was the lowest ($p < 0.05$) regardless of group and therapy.

### *Total oxidative status*

The values of TOS for the CP and control groups are listed in Table 2. TOS concentrations in serum, saliva and GCF were found to be significantly higher in the CP group compared with the control group ($p < 0.05$). After therapy, the concentrations of TOS in serum, saliva and GCF decreased to the lower levels compared with the basal levels ($p < 0.05$) and showed no significant difference with the control group ($p > 0.05$). Similarly to MDA, the concentration of TOS in GCF was the highest among three samples ($p < 0.05$) in CP patients before therapy and that in saliva was the lowest ($p < 0.05$) regardless of group and therapy.

### *Superoxide dismutase*

The changes of the concentration of SOD were similar to that of TOS in CP and control groups before and after therapy. The concentration of SOD in GCF was the highest among three samples ($p < 0.05$) in CP patients before therapy and that in serum was the lowest ($p < 0.05$) regardless of group and therapy.

## Correlation between clinical parameters and laboratory findings

Correlations between clinical parameters and MDA, TOS and SOD levels in serum, saliva and GCF were investigated together for all individuals before therapy. Positive correlations were observed between clinical parameters and MDA, TOS and SOD levels in saliva and GCF with statistical significance ($p < 0.05$). At the same time, correlations were also observed between clinical parameters and serum TOS and SOD values ($p < 0.05$). However, no significant relation of serum MDA level with clinical parameters was found ($p > 0.05$) (Table 3).

## DISCUSSION

It is suggested that patients with periodontal disease are more susceptible to an imbalance of antioxidant-oxidative stress situation.[20,22] ROS have a very short life and are therefore not easy to detect. However, ROS-related tissue destruction can be measured by the final product of LPO, such as MDA,[11,35] which is the principal and most studied product of polyunsaturated fatty acid peroxidation. It is worth noting that some studies[36,37] have indicated that the measurement of 4-hydroxynonenal, acrolein and isoprotane might

**Table 2. Comparison of serum, saliva, and GCF MDA, TOS and SOD levels between CP and control groups before and after therapy**

| Parameters | CP 1 (Before therapy) (n = 48) | Control 1 (n = 35) | CP 2 (After therapy) (n = 48) | Control 2 (n = 35) |
|---|---|---|---|---|
| Serum MDA (Mm) | 0.72 ± 0.13[b] | 0.68 ± 0.11 | 0.70 ± 0.12‡ | 0.67 ± 0.11 |
| Saliva MDA (Mm) | 0.11 ± 0.05[c] | 0.10 ± 0.02 | 0.09 ± 0.01‡ | 0.11 ± 0.03 |
| GCF MDA conc (Mm) | 1.03 ± 0.22†[a] | 0.51 ± 0.19 | 0.49 ± 0.10‡§ | 0.48 ± 0.11 |
| Serum TOS (Mm $H_2O_2$ Equivalent) | 21.45 (10.33, 42.56)†[b] | 15.44 (7.12, 35.08) | 13.11 (5.97, 24.36)‡§ | 14.66 (8.73, 30.07) |
| Saliva TOS (Mm $H_2O_2$ Equivalent) | 9.12 ± 1.77†[c] | 6.75 ± 1.02 | 5.61 ± 0.95‡§ | 5.69 ± 1.03 |
| GCF TOS conc (Mm $H_2O_2$ Equivalent) | 50.91 ± 6.33†[a] | 42.76 ± 7.94 | 39.66 ± 3.08‡§ | 40.47 ± 3.33 |
| Serum SOD (U/ml) | 134.3 ± 22.19†[c] | 100.4 ± 19.23 | 95.2 ± 17.96‡§ | 98.6 ± 19.08 |
| Saliva SOD (U/mg protein) | 216.4 ± 36.78†[b] | 174.9 ± 21.07 | 169.8 ± 23.65‡§ | 177.6 ± 24.61 |
| GCF SOD (U/mg protein) | 561.2 ± 67.01†[a] | 476.3 ± 79.73 | 486.7 ± 81.34‡§ | 462.9 ± 76.3 |

Normally distributed data are expressed as X ± SD and non-normally distributed data are expressed as median (minimum–maximum).
GCF = gingival crevicular fluid; MDA = malondialdehyde; TOS = total oxidant status; CP = chronic periodontitis; $H_2O_2$ = hydrogen peroxide. SOD = superoxide dismutase.
†The difference was significant between CP group and control groups before therapy ($p < 0.05$).
‡No difference was found between CP group and control groups after therapy ($p > 0.05$).
§The difference was significant before and after therapy ($p < 0.05$).
a, b, c Student-Newman-Keuls test or Student-Newman-Keuls rank test for multiple comparison: same letters are not significantly different.

**Table 3. Correlation between clinical parameters and serum, saliva and GCF MDA, TOA and SOD values before therapy**

| Parameters | MDA | | | TOS | | | SOD | | |
|---|---|---|---|---|---|---|---|---|---|
| | Serum | Saliva | GCF | Serum | Saliva | GCF | Serum | Saliva | GCF |
| PD | 0.147† | 0.287†‡ | 0.327†‡ | 0.217‡ | 0.481†‡ | 0.234†‡ | 0.196†‡ | 0.412†‡ | 0.201†‡ |
| CAL | 0.052† | 0.319†‡ | 0.330†‡ | 0.401‡ | 0.379†‡ | 0.219†‡ | 0.227†‡ | 0.263†‡ | 0.303†‡ |
| GI | 0.119 | 0.337‡ | 0.219‡ | 0.302‡ | 0.313‡ | 0.354‡ | 0.291‡ | 0.294‡ | 0.229‡ |
| GBI | 0.095 | 0.251‡ | 0.164‡ | 0.287‡ | 0.278‡ | 0.377‡ | 0.186‡ | 0.173‡ | 0.492‡ |
| PI | 0.076 | 0.211‡ | 0.245‡ | 0.193‡ | 0.330‡ | 0.331‡ | 0.304‡ | 0.221‡ | 0.117‡ |

PD = probing depth; CAL = clinical attachment level; GI = gingival index; GBI = gingival bleeding index; PI = plaque index; GCF = gingival crevicular fluid; MDA = malondialdehyde; TOS = total oxidant status; SOD = superoxide dismutase.
†Pearson's correlation coefficient, others are Spearman's correlation coefficient.
‡$p < 0.05$.

be more logical than MDA because MDA is only one of many aldehydes formed during lipid peroxidation and can also arise from free radical attack on sialic acid and deoxyribose. In the present study, although the serum and saliva MDA levels in the CP group did not differ significantly compared with control subjects, GCF values displayed significant increases, and the highest MDA concentration was observed in GCF. Moreover, we found that both prior to and following treatment the total amount of GCF MDA was significantly lower in CP compared with healthy controls. Our results are partially in accordance with other studies demonstrating an increased LPO level in GCF of periodontitis patients.[22,38] No significant difference of serum and saliva MDA concentration was found between CP and healthy control groups. Nevertheless, Sobaniec and Sobaniec-Lotowska[39] found that rats with periodontitis had higher blood lipid and peroxidation concentrations than periodontally healthy ones. The manifestation of the significant changes of MDA in GCF but not in serum and saliva, regardless of the therapy, indicates that the LPO levels of GCF is both qualitatively and quantitatively distinct from that of saliva, plasma or serum.

Concentrations of different oxidant species in serum (or plasma) can be measured separately, but the measurements are time-consuming, labour-intensive and costly, and require complicated techniques. Since the measurement of different oxidant molecules separately is not practical and their oxidant effects are additive, the total oxidant status of a sample (which is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and the measurement of the ferric ion by xylenol orange[26]), was measured in the present study. These data appear to support various earlier studies which revealed the increasing TOS level, i.e., the declining antioxidant capacity in serum and GCF of periodontitis patient.[18,40] Panjamurthy *et al.*[41] reported that disturbance in the antioxidant defence system due to overproduction of LPO products at inflammatory sites could be related to a higher level of oxidative stress in periodontitis patients. In addition, our data showed that TOS in CP patients was reduced compared to basal levels before therapy and presented no significant difference to the control group after therapy. Chapple *et al.*[21] suggested that non-surgical therapy with improvements in clinical parameters can increase the antioxidant defence in CP patients.

The present data also suggest that MDA concentration in GCF was significantly higher than that in saliva and serum. This is in agreement with Tsai *et al.*[22] who described a possible association of higher LPO concentrations with an increased percentage of GCF in the saliva of periodontitis patients. There were several explanations about the highest levels of MDA in GCF but in saliva or serum. Firstly, Battino *et al.*[15] suggested that higher GCF flow related to increased PMN levels, which in turn contributed to overall peroxidase enhancement by myeloperoxidase activity. Secondly, the increased MDA level in GCF of CP patients in the present study could partly be due to an increase of LPO in the periodontium itself against the increasing amounts of bacteria and their products. Considering that a disturbance of the balance between ROS and antioxidants may contribute to the development of inflammatory oral diseases, the increased MDA level in GCF detected in our study suggests an increase in the level of LPO in periodontium and oral environment in periodontitis. Local decreases in antioxidant capacity only in GCF, which was reflected by the local increases of MDA levels in GCF in the present study, have been reported to be of greater significance in the aetiology of periodontitis and was associated with the damage of periodontium than the more systemic changes observed in whole saliva.[16,20] However, it remains unclear whether changes in the GCF compartment in periodontitis reflect predisposition to or the results of ROS-mediated damage. MDA levels in GCF, which were higher than those in serum and saliva in our study, showed that a local increase in the LPO level was more prominent in the periodontal region/pocket of CP, and was more significant than the systemic increase in terms of pathology of periodontal disease. The significant

decreases of MDA after therapy also suggested that local total antioxidant capacity in CP appeared to be restored to control subject levels by successful non-surgical therapy.[21]

TOS levels in serum, saliva and GCF, which were all significantly higher in the CP group compared with the controls, suggested that not only a local but also a systemic increase occurred in oxidant status of CP which was supported by a large, analytical epidemiological study of plasma antioxidant levels in periodontitis.[42] While the TOS level in the serum was found to be higher than that in the saliva, the highest levels were also observed in GCF. Our results are in agreement with the study by Akalin *et al.*[43] which not only revealed that TOS was higher in the periodontium of CP patients, but also displayed a significant systemic increase in peripheral TOS compared with that in saliva. Considering the changes of MDA in GCF serum and saliva, these findings suggest that LPO displays a higher local increase in CP, while other events, which are related to oxidative stress, such as protein carbonilation,[44] increase in ROS production and decrease in AO capacity, can be more effective in the systemic increase of oxidant status in CP.

The present findings indicated higher SOD activity in inflamed GCF from CP patients than in healthy gingiva. This finding confirms several observations in the literature about oxidant–antioxidant imbalance in the pathological process of periodontitis.

In contrast to our finding, Ellis *et al.*[45] found a significant and progressive reduction in SOD activity within gingiva adjacent to deeper pockets. Akalin *et al.*[46] also found that gingival SOD activity was lower in periodontitis groups than the matched control groups and type 2 diabetes mellitus patients with healthy periodontal ligament had the highest but the CP group had the lowest SOD levels. However, consistent with our finding, other studies[41,47] have shown that SOD activity increases with the progression of inflammation in CP patients. The human periodontal ligament has been shown to possess the enzyme SOD, which might afford biological protection against ROS, particularly $O_2^-$ during the inflammatory response.[48] Bacterial lipopolysaccharide was also shown to stimulate $O_2^-$ release from gingival fibroblast, suggesting that the induction of SOD may represent an important defence mechanism of the fibroblast during inflammation.[49] In the present study, increased gingival SOD activity level in CP seems to support the above findings. Moreover, increased SOD activity level in inflamed gingiva from CP patients may indicate the increased $O_2^-$ generation by PMNLs invading at the disease situation. This increase in $O_2^-$ production may have led to the occurrence of oxidative stress, which in turn produced an increased need for SOD production to establish the ROS-antioxidant balance to protect the tissue.

The findings of the correlation of periodontium clinical parameters and the concentrations of MDA, TOS and SOD are partially in accordance with the findings of Tsai *et al.*[22] who measured LPO levels in GCF and detected higher correlation coefficients between total LPO (GCF/30 s) level and periodontal parameters compared with those with LPO concentration. Akalm *et al.*[43] observed strong and positive correlations between clinical parameters and MDA and TOS levels in saliva and GCF, but weak and TOS in serum; particularly, no marked correlation was observed between clinical parameters and MDA in serum. Such results with the current study, though the relatively weak correlation in our data, still suggested LPO level was more prominent in the periodontal region/pocket in CP than systemic effect. All in all, the correlations found in this study not only display significant interactions between serum, saliva and GCF in terms of LPO and oxidant status but also suggest a close relation between the TOS and LPO in the pocket/sulcus.

## CONCLUSIONS

The results of the present study suggest that a significant oxidative stress may occur in periodontitis accompanying higher LPO in the oral environment, predominantly in the periodontal region, with TOS and SOD increasing both locally and peripherally. Non-surgical therapy seems to restore and control the subject antioxidant capacity by locally and systemically modifying the levels of MDA, TOS and SOD. The findings also suggest that significant relations are present between oxidant status and periodontal status, and that oxidative stress may play an important role, prominently being a consequence from periodontitis according to our data, in the pathology of periodontitis. However, further studies are needed to confirm whether oxidant status is a cause of periodontitis which might be targeted to the therapy of periodontitis.

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