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# Lipid peroxidation levels and total oxidant status in serum, saliva and gingival crevicular fluid in patients with chronic periodontitis

**Ferda Alev Akalın[1], Esra Baltacıoğlu[2], Ahmet Alver[3] and Erdem Karabulut[4]**

[1]Department of Periodontology, Faculty of Dentistry, Hacettepe University, Ankara, Turkey; [2]Department of Periodontology, Faculty of Dentistry, Karadeniz Technical University, Trabzon, Turkey; [3]Department of Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey; [4]Department of Biostatistics, Faculty of Medicine, Hacettepe University, Ankara, Turkey

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***Abstract***

**Objectives:** Increased levels of reactive oxygen species lead to oxidative stress. Recent data suggest increased lipid peroxidation (LPO) levels and oxidative stress in periodontitis. Malondialdehyde (MDA), a significant LPO product, increases in oxidative stress. In this study, MDA levels and total oxidant status (TOS) in serum, saliva and gingival crevicular fluid (GCF) were investigated in patients with chronic periodontitis (CP).

**Materials and Methods:** Thirty-six CP patients and 28 periodontally healthy controls were included in the study. Following clinical measurements and samplings, MDA and TOS levels were measured by high-performance liquid chromatography and a novel automatic colorimetric method, respectively.

**Results:** While the saliva and GCF MDA levels, and serum, saliva and GCF TOS values were significantly higher in the CP group than the control group ($p<0.05$), no significant difference in serum MDA levels was found ($p>0.05$). Strong positive correlations were observed between periodontal parameters and MDA and TOS levels ($p<0.05$).

**Conclusions:** The results revealed that LPO significantly increased locally in the periodontal pocket/oral environment, while TOS displayed both systemic and local increases in periodontitis. The findings suggest that increased LPO and TOS may play an important role in the pathology of periodontitis, and are closely related to the clinical periodontal status.

Key words: chronic periodontitis; gingival crevicular fluid; lipid peroxidation; malondialdehyde; oxidative stress; saliva

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Generation of reactive oxygen species (ROS) is an integral feature of normal cellular metabolism (Luqman & Rizvi 2006). These species include oxygen-derived free radicals, namely superoxide ($\overline{O}_2$), hydroxyl (OH) and nitric oxide ($NO^-$), and non-radical derivatives of oxygen, namely hydrogen peroxide ($H_2O_2$) and hypochlorous acid (HOCL). Another free radical, singlet oxygen, is capable of oxidizing a number of biological molecules (Chapple 1997, Halliwell 2000, Waddington et al. 2000). The generation of ROS is an important pathogenic mechanism for diseases associated with phagocytic infiltration as host defence mechanisms against the invading pathogen (Lunec 1990, Halliwell 1996, Chapple 1997, Halliwell 2000, Waddington et al. 2000). However, they are also highly toxic and impair a wide variety of biological molecules including lipids, proteins and DNA (Lunec 1990, Halliwell & Gutteridge 1999, Waddington et al. 2000, Valko et al. 2004). Cells have developed several antioxidant (AO) defence mechanisms to neutralize these harmful ROS (Halliwell & Gutteridge 1990, Maxwell 1995, Halliwell 1996, Chapple 1997, Luqman & Rizvi 2006). AOs are classified as chain-breaking AOs, preventative AOs and enzymes (Maxwell 1995). An excess of ROS can cause oxidative stress and damage to critical biomolecules resulting in deleterious biological effects

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(Luqman & Rizvi 2006). Oxidative stress has been implicated as a major contributor in over 100 disorders and more recently in periodontitis (Pryor 1986, Halliwell & Gutteridge 1990, Chapple 1997, Sies 1997, Bauer & Bauer 1999).

ROS induce lipid peroxidations (LPO), with related effects on cells (Guérin et al. 2001). When ROS interact with the polyunsaturated fatty acids in membranes or lipoproteins, the process of LPO begins. In the resulting LPO chain, the fatty acids are converted to the primary product of lipid peroxides and to secondary metabolites (Little & Gladen 1999). Uncontrolled production of lipid peroxides can result in oxidative stress, with significant damage to cell integrity (Little & Gladen 1999, Marnett 1999). Because LPO is an outcome of 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 be shown to increase following oxidative stress (Marnett 1999, Del Rio et al. 2005).

In recent years, more attention has been focused on the role of ROS, AO systems, oxidative stress, and LPO products in the pathology of periodontitis (Moore et al. 1994, Shapira et al. 1994, Chapple et al. 1997, 2000, Asman 1998, Battino et al. 1999, 2002, Sobaniec & Sobaniec-Lotowska 2000, Sheikhi et al. 2001, Sculley & Langley-Evans 2002, 2003, Brock et al. 2004, Wei et al. 2004, Akalin et al. 2005, Mashayekhi et al. 2005, Panjamurthy et al. 2005, Takane et al. 2005, Tsai et al. 2005, Baltacioğlu et al. 2006). Chapple et al. (1997) reported that periodontal disease was associated with reduced salivary AO status and increased oxidative damage within the oral cavity. The possible association of periodontal disease with impaired salivary AO status and increased oxidative injury has been investigated by a limited number of studies (Moore et al. 1994, Chapple et al. 1997, Battino et al. 2002, Sculley & Langley-Evans 2002, 2003, Brock et al. 2004). Other studies have also demonstrated an increase in LPO levels in periodontitis. (Sobaniec & Sobaniec-Lotowska 2000, Sheikhi et al. 2001, Mashayekhi et al. 2005, Panjamurthy et al. 2005, Tsai et al. 2005).

Oxidative stress has been variably determined by measurement of a decrease in total AO capacity, or more often, by estimation of the products of oxidative damage to lipids, proteins and DNA (Pryor 1993, Prior & Cao 1999, Tarpey et al. 2004). Measurement of the products of oxidative modification provides the most direct assessment of oxidative stress. However, as the measurement of different oxidant molecules separately is not practical, and their oxidant effects are additive, measuring the total oxidant status (TOS) of a sample can provide a new and practical approach (Erel 2005).

In this study, levels of MDA, which is an LPO product, and TOS were investigated in serum, saliva and gingival crevicular fluid (GCF) in chronic periodontitis (CP). The data obtained were compared with those from healthy control subjects, and also the relationship between these parameters and clinical periodontal status was investigated.

## Materials and Methods

### Clinical studies

#### *Study groups*

A total of 64 individuals were included in the study, 36 being CP patients (19 males and 17 females, ages between 32 and 55, with an average age of $40.66 \pm 5.31$) and 28 being periodontally healthy controls (13 males and 15 females, ages between 29 and 51, with an average age of $38.5 \pm 6.10$). The subjects were chosen among individuals who referred to Karadeniz Technical University Faculty of Dentistry, Department of Periodontology, due to periodontal problems or for routine periodontal controls. The patients were clinically and radiographically evaluated for CP according to the valid criteria accepted by the American Academy of Periodontology in 1999 (Armitage 1999). It was ensured that the patients had teeth with 30% periodontal bone loss and with $\geqslant 5$ mm deep pockets. The gingivae of the patients were bleeding on probing and had the characteristics of chronic inflammation. The patients had poor oral hygiene, and the amount of accumulated plaque was commensurate with the amount of clinical attachment loss. No evidence of rapid and aggressive periodontal breakdown was observed either from the clinical and radiographic examinations (severe generalized/localized bone loss or tooth mobility) or from the histories of these patients (rapid progression or early tooth loss in the family or themselves due to periodontal reasons). The control group consisted of individuals with no periodontal disease history, with a probing depth (PD) of $\leqslant 3$ mm and a clinical attachment level (CAL) of $\leqslant 1$ mm; with no clinical sign of gingival inflammation who also maintained good oral hygiene (Alpagot et al. 1996).

Among the 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 6 months, being never-smokers, not being alcohol or AO 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 (Black Sea coast region in the north-east of Turkey), were of a lower-middle-class socio-economic status and had similar traditional nutrition habits, primarily fish, vegetables and fruit, which are the most widely cultivated and consumed products in the region due to the natural conditions of the area.

The participants were informed about the study and their consent was obtained. The study project was examined by the Karadeniz Technical University Faculty of Medicine Ethics Committee, and was approved.

#### *Clinical measurements*

The periodontal status of all individuals was detected by measurements of PD, CAL, gingival index (GI) (Löe & Silness 1963), gingival bleeding index (GBI) (Muhlemann & Son 1971) and plaque index (PI) (Silness & Löe 1964). Full-mouth periapical radiographs were taken to determine the level of periodontal bone loss of the patients. PD and CAL were measured on six sites of teeth (mesial, median and distal points at buccal and palatal aspects) (Williams periodontal probe, Hu-Friedy, Chicago, IL, USA). All clinical measurements and radiographic examinations were performed by a single investigator (PhD, E. B.). It was ensured that the total number of teeth in the mouth was $\geqslant 20$.

#### *Collection of samples*

All the samples were collected 48 h following the clinical measurements in the morning following an overnight fast. The participants were told not to eat or drink anything or chew gum that morning. The subjects were questioned on whether they followed these regulations or not, before collection of the samples.

Unstimulated whole saliva samples were used in this study. Saliva samples were obtained in the morning, over 5 min. periods with the patients seated, with instructions to allow saliva to pool in the bottom of the mouth and drain to a collection tube when necessary. Subjects were asked not to swallow any saliva for the duration of the collection to allow the calculation of salivary flow rates. 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 min. at 4°C. The supernatant fraction was then aliquotted into storage vials and kept in liquid nitrogen until analysis (Sculley & Langley-Evans 2002, 2003).

The GCF samples were collected between 8 and 10 a.m., from the regions with $\geqslant$5 mm PD and $\geqslant$4 mm CAL and $\geqslant$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 in 30 s with standardized paper strips (Periopaper) (Ora Flow Inc., Amityville, NY, USA), using the orifice method (Rüdin et al. 1970), and volume was measured on a precalibrated Periotron 8000 with serum (Chapple et al. 1999). GCF samples were collected in the same way from the subjects in the control group. Ten periopaper strip samples belonging to each subject were pooled in glass tubes and treated with 500 $\mu$l of PBS (pH 6.5). Samples were eluted for 30 min. at room temperature before removing the Periopaper strips (Chapple et al. 1997, Brock et al. 2004) and storage of the eluate in liquid nitrogen until analysis (Chapple et al. 1997, Brock et al. 2004).

Venous blood was collected in plain tubes (for serum). Plain tubes were maintained at 4°C for a further 30 min. before centrifugation at 1500 *g* for 10 min. (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 (LPO) (MDA) assay*

MDA levels of serum, saliva and GCF were determined by the method of Young & Trimble (1991) with slight modification using the high-performance liquid chromatography (HPLC) method. One of the most popular assays of LPO is measurement of the aldehyde MDA, which is formed by the breakdown of lipid hydroperoxide. In the assay, samples are reacted with thiobarbituric acid (TBA) by heating under acidic conditions. Pre-formed MDA in the sample reacts with TBA to form a TBA–MDA adduct consisting of two molecules of TBA and one of MDA. Briefly, 100 $\mu$l of samples were added to10 $\mu$l of 0.2% butylated hydroxytoluene and mixed with 600 $\mu$l of 0.46 M $H_3PO_4$ and left to stand at room temperature for 10 min. 200 $\mu$l of 0.6% TBA was added to all tubes, vortexed and heated for 30 min. at 90°C. After cooling tubes on ice, 400 $\mu$l of sample was mixed with 720 $\mu$l of methanol and 80 $\mu$l 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 5 min., was applied to an HPLC column (ZORBAX Eclipse XDB-C18; 4.6 $\times$ 150 mm; Agilent Technologies, Agilent 1100 series HPLC systems, Waldbronn, Germany). The column was equlibrated with an eluent consisting of 35:65 (v/v) methanol: 25 mM phosphate buffer, pH 6.4, for 60 min. at 1 ml/min. A fluorescence detector at excitation 536 nm and emission 555 nm was used. MDA standards (1.25–0.035 $\mu$M) were prepared from tetraethoxypropane and were included in parallel with all samples.

*Total oxidant status (TOS) assay*

TOS of serum, saliva and GCF samples was measured using a new measurement method developed by Erel (2005). Briefly, 225 $\mu$l Reagent 1 (xylenol orange 150 $\mu$M, NaCl 140 mM and glycerol 1.35 M in 25 mM $H_2SO_4$ solution, pH 1.75) was mixed with 35 $\mu$l of samples (serum, saliva and GCF) and the absorbance of each sample was read spectrophotometrically at 560 nm as a sample blank. After that, 11 $\mu$l 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 min. after the mixing, the last absorbance was read at 560 nm. The analytical sensitivity of the method was found to be 0.0076 absorbance/amount [AX ($\mu$M)$^1$]. The assay is calibrated with $H_2O_2$ and the results are expressed in terms of micromolar $H_2O_2$ equivalent per litre ($\mu$mol $H_2O_2$ 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 $\mu$mol $H_2O_2$ Equiv/l. The total TOS in GCF was measured in the total amount of GCF that was collected in 30 s. TOS concentration was calculated by dividing the total TOS to volume of GCF.

#### Statistical analysis

The normality of the data distribution was examined using the Kolmogorov–Smirnov test. The differences between the CP and control groups were investigated with independent-*t* test for data with normal distribution (PD, CAL, serum, and GCF MDA concentrations, GCF MDA/30 s, saliva and GCF TOS concentrations, GCF TOS/30 s), and with the Mann–Whitney U-test for data that did not display a normal distribution (GI, GBI, PI, saliva MDA concentration, serum TOS concentration). Two-way ANOVA was used to find any differences in terms of groups and gender. Furthermore, differences in TOS and MDA values in serum, saliva and GCF within both groups were investigated by repeated measures ANOVA. The correlations between clinical parameters and MDA and TOS levels were examined using the Pearson's correlation coefficient. A $p < 0.05$ value was accepted as being statistically significant.

## Results

#### Clinical findings

The mean values of the clinical parameters are listed in Table 1. All the clinical parameters were found to be significantly higher in the CP group compared with the control group ($p < 0.05$) (Table 1).

The mean saliva flow rate was measured as 0.34 $\pm$ 0.28 ml/min. in the CP group and 0.31 $\pm$ 0.33 ml/min. in the control group. No significant difference was observed in terms of saliva flow rate between male and female subjects, and between CP and control groups ($p$>0.05).

#### Laboratory findings

*LPO (MDA)*

The mean values of MDA levels in serum, saliva and GCF in the CP and control groups are given in Table 2. While serum

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

| Parameters | Groups | | Test statistics |
|---|---|---|---|
| | CP ($n = 36$) | Control ($n = 28$) | |
| PD | 3.92 ± 0.52 | 1.18 ± 0.38 | $t = 23.163$* |
| CAL | 4.45 ± 0.86 | 0.27 ± 0.25 | $t = 27.538$* |
| GI | 1.46 (0.6–2.5) | 0 (0–0.3) | $Z = 6.948$* |
| GBI | 2.16 (0.83–3) | 0 (0–0.2) | $Z = 6.988$* |
| PI | 1 (0–3) | 0 (0–0) | $Z = 6.382$* |

Normally distributed data are expressed as $\bar{X}$ ± SD and non-normally distributed data are expressed as median (minimum–maximum).
*The difference is significant between the groups ($p < 0.05$).
PD, probing depth; CAL, clinical attachment level; GI, gingival index; GBI, gingival bleeding index; PI, plaque index; CP, chronic periodontitis.

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

| Parameters | Groups | | Test statistics |
|---|---|---|---|
| | CP ($n = 36$) | Control ($n = 28$) | |
| Serum MDA ($\mu$M) | 0.60 ± 0.16 | 0.58 ± 0.16 | $t = 0.453$ |
| Saliva MDA ($\mu$M) | 0.1 (0.07–0.21) | 0.06(0.04–0.14) | $Z = 5.06$* |
| GCF MDA conc ($\mu$M) | 0.88 ± 0.18 | 0.67 ± 0.13 | $t = 4.96$* |
| GCF MDA/30 s ($\mu$M) | 3.52 ± 0.72 | 1.78 ± 0.42 | $t = 12.02$* |
| Serum TOS ($\mu$M $H_2O_2$ Equivalent) | 16.54(11.2–39.8) | 13.93(11.6–15.8) | $Z = 4.85$* |
| Saliva TOS ($\mu$M $H_2O_2$ Equivalent) | 6.03 ± 1.37 | 4.16 ± 0.63 | $t = 7.20$* |
| GCF TOS conc ($\mu$M $H_2O_2$ Equivalent) | 39.19 ± 5.95 | 31.40 ± 5.54 | $t = 5.35$* |
| GCF TOS/30 s ($\mu$M $H_2O_2$ Equivalent) | 156 ± 19.12 | 83.93 ± 22.86 | $t = 13.72$* |
| GCF volume ($\mu$l) | 4.01 ± 0.36 | 2.64 ± 0.36 | $t = 14.910$* |

Normally distributed data are expressed as $\bar{X}$ ± *SD* and non-normally distributed data are expressed as median (min–max).
*The difference is significant between the groups ($p < 0.05$).
GCF, gingival crevicular fluid; MDA, malondialdehyde; TOS, total oxidant status; CP, chronic periodontitis; $H_2O_2$, hydrogen peroxide.

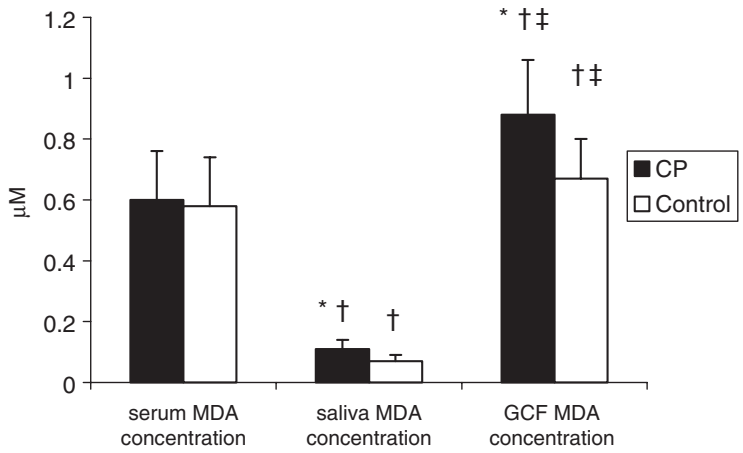


*Fig. 1.* Comparison of malondialdehyde (MDA) concentrations in serum, saliva and GCF within and between the groups. *Significant difference as compared with the control group ($p < 0.05$). †Significant difference as compared with serum ($p < 0.05$). ‡Significant difference as compared with saliva ($p < 0.05$).

MDA concentrations were almost the same in the CP and control groups, the values obtained in saliva and GCF were significantly higher in the CP group compared with the control group ($p < 0.05$) (Fig. 1). The GCF MDA/30 s ($\mu$M) value was also significantly higher in the CP group than the control group ($p < 0.05$).

*TOS*

The mean 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 ($p < 0.05$) (Fig. 2). The GCF TOS/30 s ($\mu$M $H_2O_2$ Equivalent) value was also significantly higher in the CP group when compared with the values obtained for the control group ($p < 0.05$).

Generally, MDA and TOS concentrations appeared to be the lowest in saliva, and yet the highest in GCF ($p < 0.05$) (Figs 1 and 2). There was no significant difference between male and female subjects in terms of MDA and TOS levels in serum, saliva and GCF ($p > 0.05$) (data not given).

### Correlations

Correlations between clinical parameters and MDA and TOS levels in serum, saliva and GCF were investigated together for all individuals. On analysis, while statistically significant, strong and positive correlations were observed between clinical parameters and MDA and TOS levels in saliva and GCF, statistically significant yet weak correlations were observed between clinical parameters and serum TOS values ($p < 0.05$). No significant relation was observed with the serum MDA level ($p > 0.05$). Correlations between the clinical parameters and MDA and TOS GCF/30 s values were found to be stronger than those between the clinical parameters and GCF concentrations (Table 3).

In addition, statistically significant intermediate or strong positive correlations were observed between serum, saliva and GCF MDA and TOS levels ($p < 0.05$) (Table 4).

## Discussion

This is the first study where TOS was investigated in serum, saliva and GCFs of CP patients by a novel method. Several approaches are possible to demonstrate the involvement of oxidative stress in the pathophysiologic mechanisms of diseases. One of these approaches is the assay of end products of LPO (Romero et al. 1998). One consequence of radical (ROS) damage could be increased LPO, as lipids are among the most readily oxidizable substrates (Sochaski et al. 2002). LPO gives

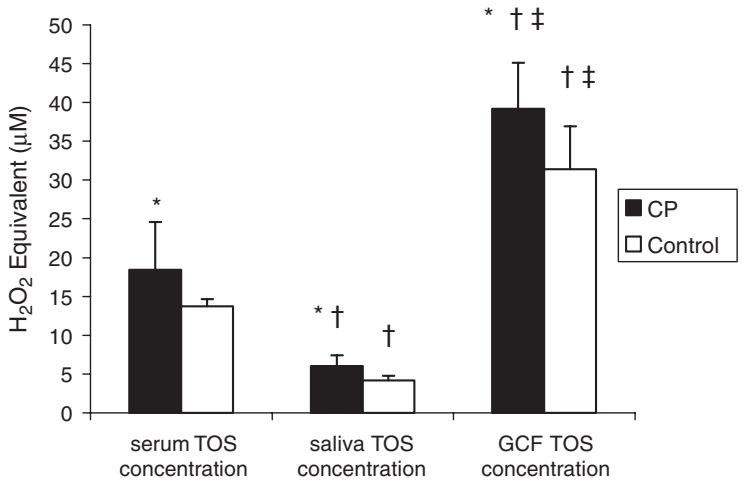


*Fig. 2*. Comparison of total oxidant status concentrations in serum, saliva and gingival crevicular fluid within and between the groups. *Significant difference as compared with the control group ($p<0.05$). †Significant difference as compared with serum ($p<0.05$). ‡Significant difference as compared with saliva ($p<0.05$).

*Table 3*. Correlations between clinical parameters, serum, saliva and GCF MDA and TOS values in all subjects

| | *r* | *p* | | *r* | *p* |
|---|---|---|---|---|---|
| PD-sa MDA | 0.529 | 0.001 | GI-GCF MDA/30 s | 0.769 | 0.001 |
| PD-GCF MDA conc | 0.539 | 0.001 | GI-sTOS | 0.321 | 0.010 |
| PD-GCF MDA/30 s | 0.806 | 0.001 | GI-sa TOS | 0.541 | 0.001 |
| PD-sTOS | 0.385 | 0.002 | GI-GCF TOS conc | 0.474 | 0.001 |
| PD-sa TOS | 0.638 | 0.001 | GI-GCF TOS/30 s | 0.767 | 0.001 |
| PD-GCF TOS conc | 0.521 | 0.001 | GBI-sa MDA | 0.583 | 0.001 |
| PD-GCF TOS/30 s | 0.815 | 0.001 | GBI-GCF MDA conc | 0.444 | 0.001 |
| CAL-sa MDA | 0.527 | 0.001 | GBI-GCF MDA/30 s | 0.723 | 0.001 |
| CAL-GCF MDA conc | 0.567 | 0.001 | GBI-sa TOS | 0.549 | 0.001 |
| CAL-GCF MDA/30 s | 0.827 | 0.001 | GBI-GCF TOS conc | 0.456 | 0.001 |
| CAL-s TOS | 0.402 | 0.001 | GBI-GCF TOS/30 s | 0.757 | 0.001 |
| CAL-sa TOS | 0.634 | 0.001 | PI-sa MDA | 0.401 | 0.001 |
| CAL-GCF TOS conc | 0.513 | 0.001 | PI-GCF MDA/30 s | 0.568 | 0.001 |
| CAL-GCF TOS/30 s | 0.817 | 0.001 | PI-sa TOS | 0.557 | 0.001 |
| GI-sa MDA | 0.535 | 0.001 | PI-GCF TOS conc | 0.429 | 0.001 |
| GI-GCF MDA conc | 0.514 | 0.001 | PI-GCF TOS/30 s | 0.635 | 0.001 |

s, serum; sa, saliva; GCF, gingival crevicular fluid; MDA, malondialdehyde; TOS, total oxidant status; PD, probing depth; CAL, clinical attachment level; GI, gingival index; GBI, gingival bleeding index; 30 s; 30 second; conc, concentration; *r*, Pearson's correlation coefficient.

*Table 4*. Correlations between serum, saliva and GCF MDA and TOS levels in all subjects

| | *r* | *p* | | *r* | *p* |
|---|---|---|---|---|---|
| sa MDA-GCF MDA conc | 0.404 | 0.001 | GCF MDA/30 s-sa TOS | 0.537 | 0.001 |
| sa MDA-GCF MDA/30 s | 0.586 | 0.001 | GCF MDA/30 s-GCF TOS/30 s | 0.741 | 0.001 |
| sa MDA-GCF TOS/30 s | 0.490 | 0.001 | s TOS-GCF TOS/30 s | 0.424 | 0.001 |
| GCF MDA conc-s TOS | 0.489 | 0.001 | sa TOS-GCF TOS conc | 0.438 | 0.001 |
| GCF MDA 30/s-s TOS | 0.532 | 0.001 | sa TOS-GCF TOS/30 s | 0.540 | 0.001 |

s, serum; sa, saliva; GCF, gingival crevicular fluid; MDA, malondialdehyde; TOS, total oxidant status; GBI, gingival bleeding index; 30 s; 30 second; conc, concentration; *r*, Pearson's correlation coefficient.

rise to a number of secondary products, mainly aldehydes, with the ability to exacerbate oxidative damage. MDA is the principal and most studied product of polyunsaturated fatty acid peroxidation (Del Rio et al. 2005). In the present study, while the serum MDA level in the CP group did not differ significantly compared with periodontal health, saliva and GCF values displayed significant increases, and the highest MDA concentration was observed in GCF. Our results are in accordance with the studies demonstrating an increase in LPO levels in serum, saliva, GCF and gingiva in periodontitis (Sobaniec & Sobaniec-Lotowska 2000, Sheikhi et al. 2001, Tüter et al. 2001, Mashayekhi et al. 2005, Panjamurthy et al. 2005, Tsai et al. 2005). Marton et al. (1993) demonstrated that MDA levels in the gingiva around the teeth with chronic apical periodontitis were higher compared with healthy gingiva. On the other hand, our findings also appear to support various earlier studies that revealed that the AO capacity declines in serum, saliva and GCF in periodontitis (Chapple et al. 1997, 2002, Battino et al. 2002, Sculley & Langley-Evans 2002, 2003, Brock et al. 2004, Pavlica et al. 2004, Baltacioğlu et al. 2006). Panjamurthy et al. (2005) reported that the disturbance in AO defence system due to overproduction of LPO products at inflammatory sites could be related to a higher level of oxidative stress in periodontitis patients.

A possible association of significantly higher LPO concentrations with an increased percentage of GCF in the saliva of periodontitis patients was reported (Tsai et al. 2005). Increased GCF flow relates to increased PMN levels, which in turn contribute to overall peroxidase enhancement by myeloperoxidase activity (Battino et al. 2002). Moreover, the increased MDA level in saliva in CP in the present study could partly be due to an increase of LPO in the saliva itself against the increasing amounts of bacteria and their products, and partly due to increased leakage of ROS to saliva from serum and GCF. It is reported that, a disturbance of the balance between ROS and AOs contributes significantly to the development of inflammatory oral diseases (Chapple 1997, Battino et al. 1999, 2002). Together with the previous findings, the increased MDA level in saliva detected in our study suggests an increase in the level of LPO in saliva and oral environment in periodontitis. Local decreases in AO capacity in GCF (Chapple et al. 1997, Battino et al. 2002, Sculley & Langley-Evans 2002, Brock et al. 2004) have been reported to be likely of greater significance in the aetiology of periodontitis and associated damage to periodontium than the

more systemic changes observed in whole saliva (Sculley & Langley-Evans 2003). MDA levels in GCF, which were higher than those in serum and saliva, in our study, show that a local increase in the LPO level is more prominent in the periodontal region/pocket in CP, and is more significant than the systemic increase in terms of periodontal disease pathology.

Serum, saliva and GCF TOS levels, which were significantly higher in the CP group compared with the controls, suggest that both a local and systemic increase occur in oxidant status in CP. While the TOS level in the serum was found to be higher than that in the saliva, the highest levels were observed in GCF. Our results revealed that although TOS was locally higher in the periodontal region in CP, it also displayed a significant systemic increase, and even a higher peripheral TOS occured compared with that in saliva. These findings suggest that LPO displays a higher local increase in CP, while other events related to oxidative stress, such as protein carbonilation (Sculley & Langley-Evans 2003, Dalle-Donne et al. 2006), increase in ROS production and decrease in AO capacity, can be more effective in the systemic increase of oxidant status in CP.

While strong positive correlations were observed between all the clinical parameters and saliva and GCF MDA and TOS levels, statistically significant weak correlations were observed between serum TOS and PD, CAL and GI. GCF/30 s values of both MDA and TOS displayed stronger relations with the peridontal status, compared with concentration. This finding is in accordance with the findings of Tsai et al. (2005), who measured LPO levels in GCF and detected higher correlation co-efficients between total LPO (GCF/30 s) level and periodontal parameters compared with those with LPO concentration. 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.

Whole unstimulated saliva was collected in this study (Sculley & Langley-Evans 2002, 2003, Tsai et al. 2005) as it represents the major intra-oral condition regarding the saliva state and composition. It also contains some elements of GCF and tissue metabolites that may be useful in the determination of tissue degradation (Navazesh 1993, Kaufman & Lamster 2000, Sculley & Langley-Evans 2002, 2003). In addition, stimulating saliva flow has been demonstrated to increase saliva volume and disrupt the concentration (Moore et al. 1994, Sculley & Langley-Evans 2002, 2003). Moreover, the mastication process used in most stimulation techniques may cause the expulsion of relatively high quantities of GCF from the periodontal pocket, and may artificially increase the elements tested with contributions from the plasma (Chapple et al. 1997, Sculley & Langley-Evans 2002, 2003). In our study, no difference was observed between the CP and control groups in terms of saliva flow rate. Thus, we consider that the comparisons we made in saliva concentrations have a certain value.

In conclusion, the results of our study suggest that a significant oxidative stress may occur in periodontitis, LPO being higher in the oral environment, predominantly in the periodontal region, with TOS increasing both locally and peripherally. The findings also suggest that significant relations are present between oxidant status and periodontal status, and that oxidative stress may play an important role in the pathology of periodontitis and the associated tissue damage. It is not clear whether this event is the cause or a result of periodontitis. Oxidative stress plays an important role in the pathologies related to smoking and diabetes mellitus, which are among the risk factors of periodontitis (Haber et al. 1993, Löe 1993, Tonetti & Claffey 2005), and cardiovascular diseases, which were reported to be related to periodontitis (Emingil et al. 2000). Therefore, investigation of the role of oxidative stress in periodontitis will not only be very useful in clarifying the pathogenic mechanisms of these relations, but will also place focus on AO therapeutic approaches in the treatment of periodontitis.

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Address:
*Dr. Ferda Alev Akalın*
*Department of Periodontology*
*Faculty of Dentistry*
*Hacettepe University*
*06100 Sıhhıye*
*Ankara*
*Turkey*
E-mail: akalin@hacettepe.edu.tr

**Clinical Relevance**

*Scientific rationale for the study*: Oxidative stress is significant in the pathology of periodontitis. This study has evaluated LPO and TOS as possible signs of oxidative stress in serum, saliva and GCF in periodontitis patients.

*Principal findings*: LPO was increased locally in the periodontal region, while TOS displayed both systemic and local increases, predominantly in the periodontal region. These parameters were closely related to periodontal status.

*Practical implications*: Systemic increase of oxidant status is particularly important in terms of clarification of the pathogenic mechanisms of the relations between periodontitis and systemic diseases/conditions and AO-treatment approaches.