Oral Diseases (2008) 14, 754–760. doi:10.1111/j.1601-0825.2008.01464.x

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ORIGINAL ARTICLE

Salivary malondialdehyde levels in clinically healthy and periodontal diseased individuals

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BACKGROUND: Lipid peroxidation (LPO) has been implicated in the pathogenesis of several pathologic disorders, including periodontal disease. Malondialdehyde (MDA) is one of many low molecular weight end products of LPO.
OBJECTIVE: This study was conducted to evaluate salivary MDA levels in generalized chronic periodontitis (GCP) subjects.
MATERIALS AND METHODS: The MDA levels were measured in the saliva of 104 subjects, aged 18–65 years. Three groups with different degrees of severity of GCP were established: 30 early (group 1), 30 moderate (group 2) and 14 severe (group 3). Thirty individuals (aged 25–29 years) with clinically healthy periodontium were served as control. Unstimulated whole saliva samples from study subjects were collected, centrifuged at 3000 *g* for 15 min and were then stored at −70°C until analysed. The MDA level was determined with 2-thiobarbituric acid by a colorimetric method at 532 nm.
RESULTS: A significant increase in the MDA level existed in the samples obtained from the three groups of patients compared to the control subjects.
CONCLUSION: Increased MDA levels are with closely associated with the severity and patients status of periodontal disease that has not been previously reported. The detection of salivary MDA level may provide additional advantages in elucidating the pathogenesis of periodontal disease.
Oral Diseases (2008) **14**, 754–760

Keywords: generalised chronic periodontitis; unstimulated whole saliva; malondialdehyde level

Introduction

Periodontal disease affects between 10 and 15% of the world's population, representing the greatest cause of tooth loss (Baelum and Lopez, 2004). Although, mild and moderate forms of chronic periodontitis are rather common (Sheiham, 1997), severe form of periodontitis with advanced tissue destruction are rare worldwide (Albandar *et al*, 1999), including European populations (Morris *et al*, 2001). There is strong evidence that periodontal disease affects a specific, predisposed group of the population that exhibit an exacerbated inflammatory/immune response to the periodontopathogenic bacteria that accumulate on the teeth and around the gingival tissues, which in turn may lead to tissue damage (Battino *et al*, 2003; Baelum and Lopez, 2004). It has been widely reported (Chapple *et al*, 1997; Battino *et al*, 2002) that free radicals (FRs) are often essential for biological processes and tissue damage can easily occur when antioxidant defence systems do not efficiently counteract their action. Malondialdehyde (MDA) is one of many low molecular weight end-products of lipid peroxidation (LPO) and is the most often measured as an index of peroxidation (de Zwart *et al*, 1999). Marnett (1999) reported that MDA has important pathophysiologic effects. The most commonly used test for measurement of MDA is thiobarbituric acid reactive substances (TBARS). A variety of spectrophotometer assays are used to determine the quality of TBARS in biological samples. Whole saliva is an important physiologic fluid that contains a highly complex mixture of substances. Variable amounts of blood, serum, serum products, gingival crevicular fluid (GCF), electrolytes, epithelial and immune cells, microorganisms, bronchial products and other foreign substances also are present in whole saliva (Schenkels *et al*, 1995). Saliva is used to aid in the diagnosis of many systemic diseases (Streckfus and Bigler, 2002), oral diseases (Beloklitskaia, 1992; Bardow *et al*, 2001) and assessment of the severity of some illnesses (Sato, 2002). In addition, saliva has been used as a diagnostic aid for periodontal diseases (Ozmeric, 2004). In this study, for the first time, we determined the MDA level by analysing the status of LPO as the level of TBARS in the unstimulated whole saliva of patients with early, moderate and severe generalized chronic periodontitis (GCP) and healthy control subjects.

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Received 11 February 2008; revised 22 May 2008; accepted 04 June 2008

Materials and methods

Subject population

This study was carried out between March 2006 and November 2007 in the Periodontal Unit of the Therapeutic Stomatology Department of the Institute of Stomatology at the National Medical Academy of Postgraduate Education (NMAPE) in Kiev, Ukraine. One hundred and four subjects of both genders were recruited. The subjects ranged in age from 18 to 65 years and were categorized into four groups as follows: group 1 included eight males and 22 females with early GCP, group 2 included 11 males and 19 females with moderate GCP, and group 3 included three males and 11 females with severe GCP. Individuals with clinically healthy periodontium served as the control group and included 10 males and 20 females, ranging in age from 22–29 years with no history of periodontal disease. All subjects had at least 20 or more teeth and were systemically healthy. Exclusion criteria included use of non-steroidal anti-inflammatory or antimicrobial drugs, or use of mouthwash or vitamin supplements within a 3-month period before the study commenced, periodontal therapy in the previous 6 months and pregnancy. Inclusion required subjects to have a negative smoking and recreational drug-use history and no special dietary requirements. Informed consent to participate was initially obtained, followed by completion of a medical questionnaire. Only those subjects who fulfilled all inclusion and exclusion criteria were formally enrolled into the study. This study was in agreement with the ethical principles of the World Medical Association Declaration of Helsinki (2002) and approval was granted by the NMAPE Local Research Ethics Committee [12.100-23/KE 6(30) 2005].

Saliva sampling and processing

The method of saliva collection is very important. For each participant, testing sessions were held between 7:45 and 10:15 in the morning, following an overnight fast of at least 8 h and were asked not to drink, except water, or chew gum for the same period. The orientation session was held to familiarize subjects with the collection procedures to reduce any variability in flow rates caused by practice effects and anxiety-related xerostomia. Prior to the collection procedure, the subjects began by rinsing their mouths thoroughly several times with water and then resting quietly for 3 min. Unstimulated whole saliva (~3 ml) was collected by means of the 'spitting-method', according to the directions given by Navazesh (1993). This method is recommended for unstimulated whole saliva collection on the basis of a comparative study (Navazesh and Christensen, 1982). The collection trial started with the instruction to rid the mouth of saliva by swallowing. Subsequently, saliva was allowed to accumulate in the floor of the mouth, without stimulation of saliva secretion by means of oro-facial movements. The participant then spit the accumulated saliva into an ice-chilled sterilized test tube every 60 s. Saliva was collected for 4 min. Prior to analysis, collected saliva was centrifuged at 3000 *g* for 15 min at 4°C. The supernatant fraction was then aliquoted into storage vials and kept at −70°C until required for analysis.

Clinical procedures

Clinical measurement was performed immediately after unstimulated whole saliva collection. To determine the periodontal status of the study subjects, the following assessments were recorded: bleeding on probing (BOP) of the marginal gingival tissues was performed by running a probe along the soft tissue wall at the orifice of the pocket (Mühlemann and Son, 1971); the pocket depth (PD) was the distance in mm from the gingival margin to the base of the probeable crevice; and the clinical attachment loss (CAL) was the distance in mm from the cementoenamel junction to the base of the probeable crevice. Full mouth periapical and vertical bitewing radiographs were obtained to determine the periodontal bone loss at the baseline. All clinical periodontal examinations were performed by the same periodontist. A classification and diagnosis of early, moderate and severe GCP patients was based on the criteria of the American Academy of Periodontology (Wiebe and Putnins, 2000). In brief, on the basis of CAL, the severity of diseased sites was classified and designated as early (1–2 mm), moderate (3–4 mm) and severe (≥5 mm). In addition, the extent of diseased sites was sub-classified and named as generalized when more than 30% of the sites were involved. All clinical measurements were performed on six sites per tooth. The healthy subjects had a PD ≤2 mm, CAL ≤1 mm and a gingival index equal to zero, were not BOP and did not show any signs of bone loss. All clinical parameters were measured by a Williams periodontal probe (tapered tine with a tip diameter of 0.5 mm; Hu-Friedy, Liemen, Germany) with a proper applied probing force (~22 g = ~0.75 N). The control subjects were required to meet the criteria of 'healthy' at all sites. The values were then pooled to give a single mean value for each subject.

Chemicals

Tris HCl–KCl, 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA) and 1,1,3,3 tetraethoxypropane (TMP) were purchased from Sinbias (Donetsk, Ukraine).

Biochemical assay

The level of MDA was assayed in the saliva of study subjects, as previously described (Stalnaya and Garishvili, 1977). Briefly, 0.3 ml of collected saliva was mixed with 3 ml of 0.025 M Tris–HCL and 0.175 M KCl buffer (pH 7.4). Then, 2.5 ml of diluted saliva was mixed with 1 ml of 17% (w/v) TCA and centrifuged at 4000 *g* for 10 min. The precipitate was pelleted by centrifugation and the supernatant was reacted with 1 ml of 0.8% (w/v) TBA in a boiling water bath for 10 min. After cooling to room temperature, the absorption of the supernatant was recorded at 532 nm using a spectrophotometer (Zeiss MCS 621 UV-VISl Carl Zeiss, Jena, Germany). The arbitrary values obtained were

compared with a series of standard solutions (1,1,3,3 TMP). The results are expressed as micromoles per millilitre (μmol ml^{-1}).

Statistical analysis

Unless otherwise specified, values were expressed as mean ± s.d. To compare MDA level, BOP, PD and CAL among the study groups, one-way analysis of variance (ANOVA) was used. To determine the significance of differences among the study groups, the *P*-values were adjusted by the Bonferroni *post hoc* analysis method for multiple comparisons. The association between the MDA levels and the clinical parameters were calculated using Pearson's correlation (two-tailed) and expressed by Pearson's correlation coefficient. A *P*-value of <0.05 was considered as the criterion of statistical significance. The data were statistically analysed using SPSS statistical package (SPSS, Chicago, IL, USA). The extent of disease sites was expressed as the percentage to the respective healthy sites, according to the following formula: [(healthy sites value–diseased sites value)/healthy sites value] × 100 and compared.

Table 1 Preliminary study of patients with periodontitis

	Group 1 (n = *30*)	*Group 2* (n = *30*)	*Group 3* (n = *14*)
Mean ± s.d.			
BOP	1.17 ± 0.06[a]	1.85 ± 0.11[a]	2.93 ± 0.15[a]
PD	3.20 ± 0.18[c]	4.65 ± 0.23[b]	4.70 ± 0.25[b]
CAL	1.69 ± 0.31[a]	3.37 ± 0.26[a]	5.95 ± 0.27[a]

Generalized chronic periodontitis was classified as early (group 1), moderate (group 2), and severe (group 3).
[a]Significant differences among groups 1, 2 and 3 ($P < 0.05$).
[b]No significant differences among groups 2 and 3 ($P > 0.05$).
[c]Significant differences when compared with group 2 and 3 ($P < 0.05$).
BOP, bleeding on probing; PD, probing pocket depth; CAL, clinical attachment loss.

Results

The characteristics of the clinical parameters in the preliminary study are shown in Table 1. The comparisons of various clinical parameters among the three groups of patients are shown in Table 2. The differences in the values of BOP and CAL among the three groups of patients were statistically significant ($P < 0.05$). The differences in the values of PD in the group 1 were statistically significant in comparison with the groups 2 and 3 ($P < 0.05$). No significant differences in the value of PD among groups 2 and 3 existed ($P > 0.05$). The observed values for the MDA levels in the healthy control subjects and the three groups of patients were 5.16 ± 0.03, 28.08 ± 1.56, 39.01 ± 1.59 and 65.20 ± 2.00 μmol ml^{-1}, respectively. The concentration of LPO product (MDA level) in the saliva of study subjects is shown in Figure 2. The comparisons of the salivary MDA levels among the three groups of patients and the healthy control subjects are shown in Table 3. Significant differences in the MDA levels of patients with early, moderate and severe GCP in comparison with those of healthy subjects were noted ($P < 0.05$). The differences in the levels of MDA among the three groups of patients were statistically significant ($P < 0.05$). The correlation between the salivary MDA levels and the clinical parameters among the three groups of patients are shown in Table 4. The salivary MDA levels were positively correlated with the three clinical parameters ($P < 0.05$). The extent of

Table 2 Comparisons of various clinical parameters between patients with early (group 1), moderate (group 2), severe (group 3) generalized chronic periodontits and healthy control subjects

						95% Confidence interval	
	Group	*Groups*	*Mean difference*	*Standard error*	*Sig.*	*Lower bound*	*Upper bound*
BOP	Group 1	Group 2	−.67567*	0.01495	.000	−.7123	−.6390
		Group 3	−1.75452*	0.01875	.000	−1.8005	−1.7086
	Group 2	Group 1	.67567*	0.01495	.000	.6390	.7123
		Group 3	−1.07886*	0.01875	.000	−1.1248	−1.0329
	Group 3	Group 1	1.75452*	0.01875	.000	1.7086	1.8005
		Group 2	1.07886*	0.01875	.000	1.0329	1.1248
PD	Group 1	Group 2	−1.44133*	0.03230	.000	−.7123	−.6390
		Group 3	−1.49638*	0.04049	.000	−1.8005	−1.7086
	Group 2	Group 1	1.44133*	0.03230	.000	.6390	.7123
		Group 3	−.05505	0.04049	0.535	−1.1248	−1.0329
	Group 3	Group 1	1.49638*	0.04049	.000	1.7086	1.8005
		Group 2	.05505	0.04049	0.535	1.0329	1.1248
CAL	Group 1	Group 2	−1.68167*	0.04339	.000	−.7123	−.6390
		Group 3	−4.26276*	0.05439	.000	1.8005	1.7086
	Group 2	Group 1	1.68167*	0.04339	.000	.6390	.7123
		Group 3	−2.58110*	0.05439	.000	−1.1248	−1.0329
	Group 3	Group 1	4.26276*	0.05439	.000	1.7086	1.8005
		Group 2	2.58110*	0.05439	.000	1.0329	1.1248

*The mean difference is significant at the 0.05 level.
BOP, bleeding on probing; PD, probing pocket depth; CAL, clinical attachment loss.

Table 3 Comparison of concentration of the lipid peroxidation product, malondialdehyde, in saliva between patients with early (group 1), moderate (group 2), severe (group 3) generalized chronic periodontitis and healthy control subjects

Group	*Groups*	*Mean difference*	*Standard error*	*Sig.*	*95% Confidence interval*	
					Lower bound	*Upper bound*
Control group	Group 1	−22.91933*	0.21765	.000	−23.5052	−22.3335
	Group 2	−33.84867*	0.21765	.000	−34.4345	−33.2628
	Group 3	−60.03990*	0.27284	.000	−60.7743	−59.3055
Group 1	Control	22.91933*	0.21765	.000	22.3335	23.5052
	Group 2	−10.92933*	0.21765	.000	−11.5152	−10.3435
	Group 3	−37.12057*	0.27284	.000	−37.8550	−36.3862
Group 2	Control	33.84867*	0.21765	.000	33.2628	34.4345
	Group 1	10.92933*	0.21765	.000	10.3435	11.5152
	Group 3	−26.19124*	0.27284	.000	−269257	−25.4568
Group 3	Control	60.03990*	0.27284	.000	59.3055	60.7743
	Group 1	37.12057*	0.27284	.000	36.3862	37.8550
	Group 2	26.19124*	0.27284	.000	25.4568	26.9257

*The mean difference is significant at the 0.05 level.

Table 4 Person's correlation coefficients between clinical parameters and MDA level

	Group 1 MDA	*Group 2 MDA*	*Group 3 MDA*
BOP	0.312*	0.329*	0.391*
PD	0.321*	0.459*	0.462*
CAL	0.622*	0.704*	0.711*

*Correlation is significant at the 0.01 level (two-tailed).
MDA, malondialdehyde; BOP, bleeding on probing; PD, probing pocket depth; CAL, clinical attachment loss.

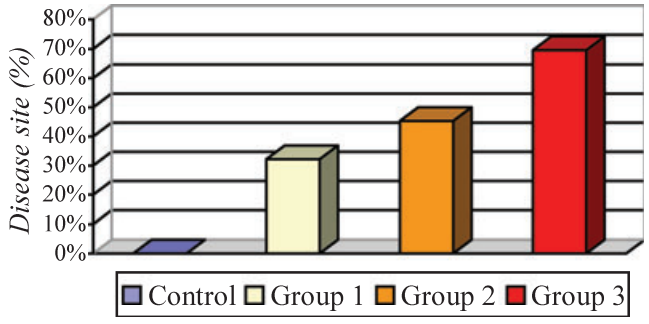

Figure 1 Elevation of the extent in disease site of patients with periodontitis in comparison with healthy control subjects. Generalized chronic periodontitis in patients was classified as early (group 1), moderate (group 2) and severe (group 3). Elevation in the extent of disease sites between groups 1 and 2, groups 2 and 3 and groups 1 and 3, were noticed 41.1, 53.4 and 116.5 times, respectively

diseased sites was as follows: group 1 (32.1%); group 2 (45.3%); and group 3 (69.5%), and it is shown in Figure 1.

Discussion

Saliva acts as a cleansing solution, an ion reservoir, a lubricant and a buffer. In addition to its other host-protective properties, saliva could constitute a first line of defence against FR-mediated oxidative stress

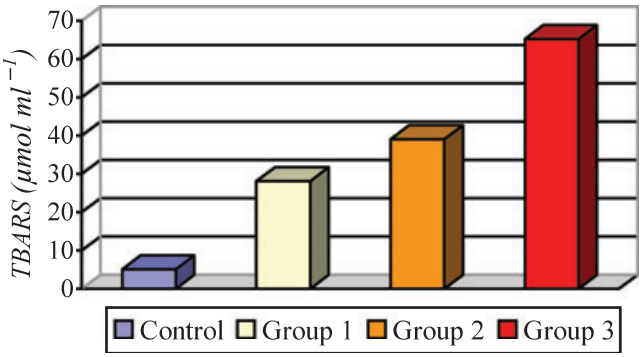

Figure 2 Concentration of the lipid peroxidation product, malondialdehyde, in saliva of patients with periodontitis in comparison with healthy control subjects. Generalized chronic periodontitis in patients was classified as early (group 1), moderate (group 2), and severe (group 3). The differences between the controls and groups 1–3 were significant at ($P < 0.05$)

(Tenovuo *et al*, 1986; Edgar, 1992; Battino *et al*, 2002). The majority of research reports of biomarkers and periodontitis have used GCF as a sample fluid (Taba *et al*, 2005). However, sampling of GCF is time consuming and only reflects gingival inflammation at each specific site sampled. In contrast to GCF, there is an abundance of saliva, and sampling is much easier, less costly and better tolerated by the patient. In addition, as whole saliva represents a pooled sample with contributions from all periodontal sites, analysis of biomarkers in saliva may provide an overall assessment of disease status as opposed to site-specific GCF analysis (Miller *et al*, 2006). FR-induced LPO, and because of high molecules reactivity, has been implicated in the pathogenesis of several pathological disorders including periodontal disease (Waddington *et al*, 2000). In this study, the level of MDA in unstimulated whole saliva was significantly higher in patients with early, moderate and severe GCP than in control healthy subjects (Figure 2). Gutteridge (1995) has shown that measuring the concentration of LPO products could assess the extent of tissue damage. In a study of rats with

spontaneous and experimental periodontitis, there are high levels of LPO, as measured by MDA, in the blood and periodontal tissues in comparison with control animals (Voskresenskiĭ and Tkachenko, 1991). An increase in the concentration of LPO in the gingival tissues of rats with experimental periodontitis has been reported (Sobaniec *et al*, 1999). The studies of Sobaniec and Sobaniec-Lotowska (2000) indicated that rats with periodontitis have higher blood LPO product concentration than rats with healthy periodontium. LPO caused by oxygen radicals from *Fusobacterium*-stimulated netrophils has been suggested as a possible model for emergence of periodontitis (Cimasoni, 1974). Furthermore, neutrophils-mediated tissue damage in the periodontium was demonstrated by Altman *et al* (1992), where it was demonstrated that human neutrophil could lyses epithelial cell in an *in vitro* model mediated. Until recently, defects associated with neutrophil functions were believed to predisposed individual to infection. However, there is a growing body of evidence suggesting that the neutrophil abnormalities in periodontal disease may be the result of a chronic hyperactivated or 'primed' state of periodontal neutrophil (Van Dyke and Serhan, 2003). Elevated MDA levels of patients with periodontitis in several studies have been reported. Marton *et al* (1993) showed that the MDA content of chronic apical periodontitis tissues was higher than in healthy tissue of the same individuals. Also, gingival biopsies and plasma of patients with chronic periodontitis have been shown to have higher levels of TBARS in comparison with healthy subjects has been reported (Panjamurthy *et al*, 2005). Cimasoni (1974) has suggested that saliva LPO levels might be used as an indicator of periodontal damage. Only a few papers have focused on saliva and have addressed an increased level of MDA in relation to patients with periodontitis in comparison with healthy controls. Akalin *et al* (2007) has shown significantly high levels of MDA in the saliva of patients with periodontitis in comparison with healthy control subjects ($P < 0.05$). Significantly a high concentration of LPO product in saliva between patients with periodontal disease and healthy subjects ($P < 0.005$) has been shown (Tsai *et al*, 2005). Specifically, their study indicated that LPO product concentration was correlated with the gingival index, PD and probing attachment level. Although a different classification was used, our findings were in agreement with their report and indicate that an elevated MDA level is markedly related to the clinical status of patients. In addition, the current results have demonstrated a positive correlation between the MDA level and the values of the BOP, CAL and PD in three groups of patients (Table 4). In this regard, the lack of significant differences in the value of PD between groups 2 and 3 in the this study indicates that it is a normal part of the inflammatory process in periodontal disease and largely related to the severity of tissue destruction or bone loss. We noticed that PD has limited use in the diagnosis of periodontitis. A patient could have severe GCP with almost 70% of bone loss and mobile teeth, but 4 mm PD as a result of recession (data not shown). Also, Balwant *et al* (2006) has shown significantly high levels of MDA in unstimulated whole saliva of patients with periodontitis in comparison with healthy control subjects ($P < 0.05$). Socransky and Haffejje (1991) have reported that chronic periodontitis may affect one or several periodontal sites within the mouth, leading to different levels of tissue destruction. Only one study has investigated MDA levels in unstimulated whole saliva of early, moderate and severe patients with periodontitis (Mashayekhi *et al*, 2005), and enhanced levels of TBARS in the saliva of patients with severe periodontitis in comparison with control subjects has been reported ($P < 0.01$). In contrast, our results showed that the MDA level was incrementally elevated as a function of the progression in disease severity among the three groups of patients in comparison with the healthy control subjects (Figure 2). It is possible, as suggested by Sheikhi *et al* (2001), that the increased salivary MDA level could occur through the mechanism of superoxide anion production during the interaction of periodontopathogenes or their products and neutrophils within periodontal tissues or pockets, and could be associated with increased percentage of GCF in saliva in periodontitis (Cimasoni, 1974). In addition, during gingival inflammation GCF adds more inflammatory products such as reactive oxygen species to saliva, providing a blind loop, which worsens the situation (Chapple, 1997; Battino *et al*, 1999; Zappacosta *et al*, 1999). It is established that LPO increases with the severity of the disease, reflecting the extent of tissue injury (Halliwell and Chirico, 1993). We noticed a 41.1, 53.4 and 116.5 times elevation in the extent of disease sites between groups 1 and 2, groups 2 and 3 and groups 1 and 3, respectively. We suggest, therefore, that the generalized condition of the periodontium may have an effect on the MDA level in whole saliva. In conclusion, the results of our study highlight the possible clinical value of unstimulated whole saliva as a valid and convenient diagnostic biofluid. This novel approach to harness the potential of salivary MDA levels may prove to be useful in identifying patients with GCP and may provide additional advantages in elucidating the pathogenesis of periodontal disease. In addition, increased MDA levels are closely related to the clinical periodontal status of patients and are associated with the severity of disease, which might be a key mechanism for understanding periodontal disease. Further studies on a large series should be performed to clarify the exact role of MDA levels in early, moderate and severe GCP.

Acknowledgements

This work was supported by the Therapeutic Stomatology Department of the National Medical Academy of Postgraduate Education (named after P. L. Shupyk) in Kiev, Ukraine.

Author contributions

This manuscript is a part of Khalili's PhD research. Khalili was responsible for conception and design of study, all clinical

and biochemical performances, analysis and interpretation of data, drafting of manuscript, critical revision of the article for important intellectual content, and final approval of the manuscript. Prof. Biloklytska was involved in design of study, and supervised the project. The author and co-author discussed the results and commented on the manuscript.

References

Akalin FA, Baltacioğlu E, Alver A *et al* (2007). Lipid peroxidation levels and total oxidant status in serum, saliva and gingival crevicular fluid in patients with chronic periodontitis. *J Clin Periodontol* **34:** 558–565.

Albandar JM, Brunelle JA, Kingman A (1999). Destructive periodontal disease in adults 30 years of age and older in the United States, 1988–1994. *J Periodontol* **70:** 13–29.

Altman LC, Baker C, Fleckman P *et al* (1992). Neutrophil-mediated damage to human gingival epithelial cells. *J Periodontal Res* **27:** 70–79.

Baelum V, Lopez R (2004). Periodontal epidemiology: ''towards social science or molecular biology?''. *Community Dent Oral Epidemiol* **32:** 239–249.

Balwant R, Simmi K, Rajnish J *et al* (2006). Salivary lipid peroxidation product malonaldehde in various dental diseases. *World J Med Sci* **1:** 100–101.

Bardow A, Nyvad B, Nauntofte B (2001). Relationships between medication intake, complaints of dry mouth, salivary flow rate and composition, and the rate of tooth demineralization in situ. *Arch Oral Biol* **46:** 413–423.

Battino M, Bullon P, Wilson M *et al* (1999). Oxidative injury and inflammatory periodontal diseases: the challenge of anti-oxidants to free radicals and reactive oxygen species. *Crit Rev Oral Biol Med* **10:** 458–476.

Battino M, Ferreiro MS, Gallardo I *et al* (2002). The antioxidant capacity of saliva. *J Clin Periodontol* **29:** 189–194.

Battino M, Ferreiro MS, Quiles JL *et al* (2003). Alterations in the oxidation products, antioxidant markers, antioxidant capacity and lipid patterns in plasma of patients affected by Papillon–Lefevre syndrome. *Free Radic Res* **37:** 603–609.

Beloklitskaia GF (1992). Indices characterizing the expression of hyperesthesia of the hard dental tissues in patients with periodontitis. *Stomatologiia (Mosk)* **1:** 29–31 (article in Russian).

Chapple IL (1997). Reactive oxygen species and antioxidants in inflammatory diseases. *J Clin Periodontol* **24:** 287–296.

Chapple IL, Mason GI, Garner I *et al* (1997). Enhanced chemiluminescent assay for measuring the total antioxidant capacity of serum, saliva and crevicular fluid. *Ann Clin Biochem* **34:** 412–421.

Cimasoni G (1974). The crevicular fluid. *Monogr Oral Sci* **3:** 1–122.

Edgar WM (1992). Saliva: its secretion, composition and functions. *Br Dent J* **172:** 305–312.

Gutteridge JMC (1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clin Chem* **41:** 1819–1828.

Halliwell B, Chirico S (1993). Lipid peroxidation: its mechanism, measurement, and significance. *Am J Clin Nutr* **57:** 715S–725S.

Marnett LJ (1999). Lipid peroxidation-DNA damage by malondialdehyde. *Mutat Res* **424:** 83–95.

Marton IJ, Balla G, Hegedus C *et al* (1993). The role of reactive oxygen intermediates in the pathogenesis of chronic apical periodontitis. *Oral Microbiol Immunol* **8:** 254–257.

Mashayekhi F, Aghahoseini F, Rezaie A *et al* (2005). Alteration of cyclic nucleotides levels and oxidative stress in saliva of human subjects with periodontitis. *J Contemp Dent Pract* **15:** 46–53.

Miller CS, King CP Jr, Langub MC *et al* (2006). Salivary biomarkers of existing periodontal disease: a cross-sectional study. *J Am Dent Assoc* **137:** 322–329.

Morris AJ, Steele J, White DA (2001). The oral cleanliness and periodontal health of UK adults in 1998. *Br Dent J* **191:** 186–192.

Mühlemann HR, Son S (1971). Gingival sulcus bleeding–a leading symptom in initial gingivitis. *Helv Odontol Acta* **15:** 107–113.

Navazesh M (1993). Methods for collecting saliva. *Ann N Y Acad Sci* **694:** 72–77.

Navazesh M, Christensen CM (1982). A comparison of whole mouth resting and stimulated salivary measurement procedures. *J Dent Res* **61:** 1158–1162.

Ozmeric N (2004). Advances in periodontal disease markers. *Clin Chim Acta* **343:** 1–16.

Panjamurthy K, Manoharan S, Ramachandran CR (2005). Lipid peroxidation and antioxidant status in patients with periodontitis. *Cell Mol Biol Lett* **10:** 255–264.

Sato TP (2002). A pH curve of human resting saliva sampled with a small paper slip and its medical application. *Pathophysiology* **8:** 283–290.

Schenkels LC, Veerman EC, Nieuw Amerongen AV (1995). Biochemical composition of human saliva in relation to other mucosal fluids. *Crit Rev Oral Biol Med* **6:** 161–175.

Sheiham A (1997). Is the chemical prevention of gingivitis necessary to prevent severe periodontitis? *Periodontol 2000* **15:** 15–24.

Sheikhi M, Bouhafs RK, Hammarström KJ *et al* (2001). Lipid peroxidation caused by oxygen radicals from Fusobacterium-stimulated neutrophils as a possible model for the emergence of periodontitis. *Oral Dis* **7:** 41–46.

Sobaniec H, Sobaniec-Lotowska ME (2000). Morphological examinations of hard tissue of periodontium and evaluation of selected processes of lipid peroxidation in blood serum of rats in the course of experimental periodontitis. *Med Sci Monit* **6:** 875–881.

Sobaniec H, Sobaniec-Lotowska ME, Zimnoch L (1999). Correlation between morphological changes in gingival tissues and increased concentration of lipid peroxides in the course of experimental periodontitis in rats. *Med Sci Monit* **5:** 838–844.

Socransky SS, Haffejje AD (1991). Microbial mechanisms in the pathogenesis of destructive periodontal diseases: a critical assessment. *J Periodont Res* **26:** 195–212.

Stalnaya ID, Garishvili TG (1977). The method of determines the malondialdehyde with the aid of thiobarbituric acid. In: Orekhovich VN, ed. *Modern methods in biochemistry*. Meditsina: Moscow, pp. 66–68 (article in Russian).

Streckfus CF, Bigler LR (2002). Saliva as a diagnostic fluid. *Oral Dis* **8:** 69–76.

Taba M Jr, Kinney J, Kim AS *et al* (2005). Diagnostic biomarkers for oral and periodontal diseases. *Dent Clin North Am* **49:** 551–571.

Tenovuo J, Lehtonen OP, Aaltonen AS *et al* (1986). Antimicrobial factors in whole saliva of human infants. *Infect Immun* **51:** 49–53.

Tsai CC, Chen HS, Chen SL *et al* (2005). Lipid peroxidation: a possible role in the induction and progression of chronic periodontitis. *J Periodont Res* **40:** 378–384.

Van Dyke TE, Serhan CN (2003). Resolution of inflammation: a new paradigm for the pathogenesis of periodontal diseases. *J Dent Res* **82:** 82–90.

Voskresenskiĭ ON, Tkachenko EK (1991). The role of lipid peroxidation in the pathogenesis of periodontitis. *Stomatologiia (Mosk)* **4:** 5–10 (article in Russian).

Waddington RJ, Moseley R, Embery G (2000). Reactive oxygen species: a potential role in the pathogenesis of periodontal diseases. *Oral Dis* **6:** 138–151.

Wiebe CB, Putnins EE (2000). The periodontal disease classification system of the American Academy of Periodontology–an update. *J Can Dent Assoc* **66:** 594–597.

Zappacosta B, Persichilli S, De Sole P *et al* (1999). Effect of smoking one cigarette on antioxidant metabolites in the saliva of healthy smokers. *Arch Oral Biol* **44:** 485–488.

de Zwart LL, Meerman JH, Commandeur JN *et al* (1999). Biomarkers of free radical damage applications in experimental animals and in humans. *Free Radic Biol Med* **26:** 202–226.