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Total antioxidant capacity and superoxide dismutase activity levels in serum and gingival crevicular fluid in post-menopausal women with chronic periodontitis

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Abstract

Objectives: Menopause has been linked with oxidative stress and decreased antioxidant (AO) defence. A connection has been established between menopause and certain periodontal conditions. The objective of this study is to compare serum and gingival crevicular fluid (GCF) total antioxidant capacity (TAOC) and superoxide dismutase (SOD) concentrations in post-menopausal patients with chronic periodontitis (PMCP) with those of pre-menopausal chronic periodontitis patients (CP).

Material and Methods: Thirty-two PMCP patients, 31 CP patients, 25 post-menopausal periodontally healthy controls (PMPH) and 26 pre-menopausal controls (PH) were studied. After clinical measurements and samplings, serum and GCF TAOC and SOD concentrations were established in turn using an automated TAOC assay and spectrophotometric end point measurement. The results were analysed statistically.

Results: Serum and GCF TAOC and SOD concentrations were significantly lower in menopause and periodontitis ($p<0.05$). The lowest values were in the PMCP group, whereas the highest values were in the PH group. While the effect of menopause was more evident in serum antioxidant analysis, the effect of periodontitis was observed to be more apparent in GCF.

Conclusions: A decrease in systemic and local AO defence was observed owing to both menopause and periodontitis. The lowest AO values in the PMCP group suggest that menopause may be a risk factor for periodontitis.

Key words: antioxidants; chronic periodontitis; gingival crevicular fluid; menopause; superoxide dismutase

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Aerobic life is characterized by a steady formation of reactive oxygen species (ROS) (Sies 1991). These species include oxygen-derived free radicals: superoxide ($O_2^{\bullet -}$), hydroxyl ($^{\bullet}OH$), and nitric oxide ($NO^{\bullet}$), and non-radical derivatives of oxygen: hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCL) (Chapple 1997, Halliwell 2000, Waddington et al. 2000). In addition to providing an important function in normal metabolic reactions, they are highly toxic and destructive in nature (Chapple 1997, Halliwell 2000, Waddington et al. 2000). The generation of ROS during the respiratory burst represents an important pathogenic mechanism for tissue damage and diseases associated with phagocytic infiltration (McCord 1993, Chapple 1996, 1997, Halliwell 1996). Phagocytic cells, predominantly polymorphonuclear leucocytes (PMNLs), when appropriately stimulated can release ROS. Fibroblasts, vascular endothelial cells and osteoclasts also pro-

duce ROS (Meier et al. 1990, McCord 1993, Steinbeck et al. 1994, Chapple 1996, 1997, Halliwell 1996).

The removal of ROS by antioxidant (AO) defence systems is essential for healthy aerobic life (Halliwell 1996). AOs are classified as chain breaking AOs, preventative AOs and enzymes like superoxide dismutase (SOD), catalase and glutathione peroxidase that catalyse the breakdown of ROS (Maxwell 1995). Within mammalian tissues the most significant AO is perhaps SOD, which catalyses the dismutation of $O_2^{\bullet -}$ to H_2O_2 and O_2 and together with other AO enzymes provides protection (Curnutte & Babior 1987, Halliwell & Gutteridge 1990, McCord 1993, Waddington et al. 2000). The SOD family includes cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD and extracellular Cu, Zn-SOD (EC-SOD) and shows some sequence homology to the cytosolic Cu, Zn-SOD but is designed for function in the extracellular spaces and has a glycosylated structure (Halliwell 1996, Fridovich 1997). However, activities of EC-SOD within extracellular fluids are extremely low and of little biological relevance (Halliwell & Gutteridge 1990, Waddington et al. 2000). In health, the balance is maintained among oxidants and AOs. Under pathological conditions, a disturbance in favour of ROS production results in oxidative stress (Curnutte & Babior 1987, Sies 1991, Chapple 1996, 1997, Halliwell 1996). Excess production of ROS has been implicated in the pathogenesis of many human diseases and, more recently, periodontitis (Seymour et al. 1986, Curnutte & Babior 1987, Whyte et al. 1989, Sies 1991, Kimura et al. 1993, Shapira et al. 1994, Halliwell 1996, Chapple 1997, Asman 1998).

ROS are shown to be produced secondarily to the various environmental agents as well as by the endogenous oxygen metabolism, and have recently been considered to be responsible for the aging process, menopause and osteoporosis (Basu et al. 2001, Vural et al. 2005). Menopause has been reported to be associated with an increase in oxidative stress and a decrease in some AOs (Ha 2004, Vural et al. 2005). A significantly lower total AO capacity in post-menopausal women compared than in healthy controls and a decrease of oxidative stress after hormone replacement therapy (HRT) have been demonstrated (Bednarek-Tupikowska et al. 2004).

During menopause there is a decrease in the levels of oestrogen and progesterone circulating in the bloodstream. Oestrogen deficiency is the dominant pathogenic factor for osteoporosis in women. A number of studies have indicated that osteoporosis is associated with biochemical markers of oxidative stress (Basu et al. 2001, Polidori et al. 2001, Maggio et al. 2003). On the other hand, oestrogen is a phenolic compound that shares similarities with the structure of well-known lipophilic AOs such as α-tocopherol. This enables the molecule to detoxify accumulated ROS and act as an antioxidant (Behl 2003). Oestrogen deficiency that is seen after menopause has been shown to lead to increased osteoclastic function stimulated by ROS and, subsequently, to represent the major pathological determinant responsible for post-menopausal bone loss (Manolagas & Jilka 1995, Behl 2003).

Several studies have linked menopause with some periodontal conditions. Hormones, including androgens, oestrogens and progesterone, are localized in periodontal tissues (Gornstein et al. 1999). In post-menopausal women the lowered levels of hormones and the consequent hormonal imbalances have been shown to significantly affect periodontium (Mascarenhas et al. 2003).

Osteoporosis is one of the most critical age-related disorders for post-menopausal women that is characterized by a loss of bone mass and density (Basu et al. 2001) and has been considered a risk factor for periodontal disease (Streckfus et al. 1997, Krejci & Bissada 2000). Other authors have failed to show consistent relationships between osteoporosis and periodontitis (Kribbs 1990, Elders et al. 1992). The number of studies about the effect of ROS or AO activity in periodontal tissues is limited. To our knowledge, there is no study about the menopausal AO status in periodontitis. The aim of this study was to evaluate the total AO capacity and SOD activity in serum and gingival crevicular fluid (GCF) in post-menopausal patients with chronic periodontitis.

Material and Methods

Clinical studies

Study groups

According to the Comité des Nomenclatures de la Fédération Internationale de Gynécologie et d'Obstétrique, the climacteric is the phase of the aging process during which a woman passes from the reproductive to the non-reproductive stage. The menopause is the final menstruation, which occurs during the climacteric. Post-menopause refers to the phase of life that comes after the menopause (Judd 1991).

The current study included a total of 114 women, consisting of 32 post-menopausal subjects with chronic periodontitis (PMCP) (mean age 48.15 ± 4.6 with an age range of 39–55), 31 pre-menopausal subjects with chronic periodontitis (CP) (mean age 37.4 ± 5.4 with an age range of 31–47), 25 post-menopausal periodontally healthy controls (PMPH) (mean age 49.1 ± 4.1 with an age range of 43–55) and 26 pre-menopausal periodontally healthy controls (PH) (mean age 37.1 ± 4.2, with an age range of 30–44). The post-menopausal subjects were selected from among the patients who had applied to the Karadeniz Technical University Faculty of Medicine's Gynaecological and Obstetrics Clinic with menopausal problems, and the pre-menopausal subjects who had applied to the Karadeniz Technical University Faculty of Dentistry's Periodontology department with periodontal problems or for routine controls. The patients with periodontal problems were clinically and radiographically evaluated for chronic periodontitis according to the criteria of the American Academy of Periodontology in 1999 (Armitage 1999). Attention was paid to ensure that the patients in these groups had at least 30% periodontal bone loss, and that the teeth to be sampled had periodontal pockets of ⩾5 mm. The two control groups presented no gingival inflammation and had good oral hygiene.

Care was taken that all subjects fit the following criteria: were never-smokers, had no history of systemic disease, had received no periodontal treatment and had not taken antibiotics, anti-inflammatory drugs or any other drugs over the past 6 months and were not alcohol or antioxidant vitamin consumers. All subjects lived within the same geographical area who shared similar traditional dietary habits, high in fish oils. All the post-menopausal subjects were selected from among patients who had experienced their last menstrual period at least 6 months, but not more than 12 months, ago, had entered a natural menopause, had applied to a gynaecology and obstetrics clinic with menopausal complaints for the first time and had not received HRT. The CP and PH (pre-menopausal healthy) groups' subjects were selected

from individuals who were in the preclimacteric phase but not in the menstruation period, had not received hormonal treatment, did not use oral contraceptives and were not pregnant or lactating. 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 approved (Approval no: 2004/78).

Clinical measurements

The periodontal status, pocket depth (PD) and clinical attachment level (CAL), gingival index (GI) (Löe & Silness 1963), gingival bleeding index (GBI) (Muhlemann & Son 1971) and plaque index (PI) (Silness & Löe 1964) values of the participants were identified. Full-mouth periapical radiographs were taken to determine the periodontal bone loss of the patients. All clinical and radiographical examinations were carried out by a single examiner (E. B.) from the periodontology department. Care was taken that the total number of teeth in the mouth was $\geqslant 20$.

Collection of samples

All samples were collected the following morning after the individuals had fasted overnight (Brock et al. 2004). The individuals were instructed not to eat or drink anything in the morning. Before sample collection, the individuals were checked for protocol adherence.

The GCF samples were obtained from the patients at areas with $\geqslant 5$ mm PD and $\geqslant 30\%$ bone loss. To avoid irritation, the samples were obtained 1 week after the clinical measurements and between 08:00 and 10:00 hours in the morning. The area was isolated with cotton rolls, saliva contamination elimination was ensured and it was slightly air-dried. The samples were obtained in 30 s with standardized periopaper strips (Ora Flow Inc., Amityville, NY, USA) by using the orifice method developed by Rüdin et al. (1970), and volume was measured on a pre-calibrated Periotron 8000 with distilled water. Ten periopaper strips were used for each patient. The same method was used to obtain GCF samples from the control group. The 10 Periopaper strips that absorbed GCF for each subject were pooled in glass tubes and treated with 1 ml 20 mM Tris-HCl buffer (pH 6.5). Samples were eluted for 30 min. at room temperature before removing the periopaper strips (Chapple et al. 1997, 2002, Brock et al. 2004) and storage of the elute in liquid nitrogen until analysis (Chapple et al. 1997, 2002, Brock et al. 2004).

Venous blood was collected in plain tubes (for serum). Plain tubes were initially kept at room temperature for 30 min. The tubes were kept at 4°C for a further 30 min. before centrifugation at 1500 *g* for 10 min (room temperature). Serum samples were aliquoted into cryogenic vials and stored in liquid nitrogen (Brock et al. 2004). Each patient was used as the unit of analysis.

Laboratory studies

Total antioxidant capacity assay

The total antioxidant capacity of serum and GCF samples was measured using a novel automated measurement method developed by Erel (2004). The novel measurement method can be performed on a wide range of complex biological fluids, including serum, plasma, semen, cerebrospinal, pleural and ascites fluids, urine, simple and heterogeneous solutions of pure antioxidants, beverages and fruit juices. This method is capable of measuring endogenous and exogenous antioxidants such as uric acid, Trolox, Vitamin C, glutathione (GSH), bilirubin and (±)-catechin.

Assay principle of the novel method. A standardized solution of Fe^{2+}–*o*-dianisidine complex reacts with a standardized solution of H_2O_2 by a Fenton-type reaction, producing OH. These potent ROS oxidize the reduced colourless *o*-dianisidine molecules to yellow–brown coloured dianisidyl radicals at low pH. The oxidation reactions progress among dianisidyl radicals and further oxidation reactions develop. The colour formation is increased with further oxidation reactions. Antioxidants in the sample suppress the oxidation reactions and colour formation. This reaction can be monitored by spectrophotometry.

Assay protocol of the novel method. - Briefly, 200 µl reagent 1[*o*-dianisidine (10 mM), ferrous ion (45 µM) in the Clark and Lubs solution (75 mM, pH 1.8)] was mixed with 5 µl of samples (serum and GCF) and absorbance of each sample was read spectrophotometrically at 444 nm as sample blank. After that, 10 µl reagent 2 [H_2O_2 (7.5 mM) in the Clark and Lubs solution] was added to the mixture, and about 3–4 min. after the mixing the last absorbance was read at 444 nm. Trolox, a water soluble vitamin E analogue, was used as the standard and the results were calculated in mM Trolox equivalents (mmol Trolox equivalents/l). The analytical sensitivity of this method has been found to be 0.07 mM Trolox equivalents ($n = 7$) for GCF, and 0.04 mM Trolox equivalents ($n = 8$) for serum in our study. The range of standards that were used for TAOC assay in this study was 0–1 mM Trolox equivalents (0, 0.2, 0.4, 0.6, 0.8, 1 Trolox equivalent).

SOD activity assay

Serum SOD activity was measured by the reduction of nitroblue tetrazolium (NBT) by xanthine–xanthine oxidase system (Sun et al. 1988). Briefly, 0.5 ml serum was treated with ethanol–chloroform (5:3) mixture and vigorously vortex-mixed for 1 min. Treated samples were centrifuged at 18,000 *g* for 60 min. and the supernatant was used for the assay. 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/µl 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 xanthine oxidase solution (167 U/l) was added and tubes were incubated for 20 min. 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 for 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, 10 U/ml).

Statistical analysis

The normality of the data's distribution was examined using the Shapiro–Wilk test. The difference between the four groups of the variables of the normally distributed serum TAOC concentration, GCF TAOC concentration and GCF TAOC/30 s was assessed using one-way

ANOVA and Tukey's multiple comparison tests. Other variables' non-normal distribution (clinical parameters, serum SOD concentration, GCF SOD concentration, GCF SOD/30 s) was examined with the Kruskal–Wallis test and the Mann–Whitney *U*-test employing the Bonferroni correction. The differences in serum and GCF TAOC concentrations as well as the serum and GCF SOD concentrations within the groups were measured using the paired –*t*-test for groups with normal distribution and the Wilcoxon signed-ranks test for groups that did not have normal distribution. A post hoc power calculation demonstrated a power of >0.8 at the $p<0.05$ level for TAOC and SOD outcomes.

Results

Clinical findings

The mean ± standard deviations of clinical parameters are shown in Table 1. While all clinical parameters were significantly higher for the PMCP and CP groups than the control groups (post-menopausal and pre-menopausal; $p<0.05$), there was no significant difference between the PMCP and CP groups ($p>0.05$). The differences between the control groups (PMPH and PH) were also not statistically significant ($p>0.05$) (Table 1).

The mean ± standard deviations of GCF volumes in the groups were as follows: in the PMCP group $4.83 \pm 0.92\,\mu$l, in the CP group $4.84 \pm 0.97\,\mu$l, in the PMPH group $4.01 \pm 0.60\,\mu$l and in the PH group $4.29 \pm 0.83\,\mu$l. These values represent the mean total ($n = 10$ strips) volumes per subject.

Table 1. Comparison of clinical parameters in post- menopausal chronic periodontitis (PMCP), chronic periodontitis (CP), post-menopausal periodontally healthy control (PMPH) and pre-menopausal control (PH) groups

Parameter (sampling area)	Group no. PMCP: 32 CP: 31 PMPH: 25 PH:26	$X \pm$ Standard deviation (sampling area)	Median	Minimum	Maximum	χ^2	p
PD	PMCP	3.41 ± 0.28	3.36	3.02	4.37	85.363	0.001
	CP	3.56 ± 0.45	3.45	3.02	5.02		
	PMPH	1.41 ± 0.24*	1.32	1.15	1.90		
	PH	1.41 ± 0.25‡	1.30	1.14	2.10		
CAL	PMCP	3.78 ± 0.44	3.63	3.23	5.08	85.021	0.001
	CP	3.82 ± 0.54	3.69	3.10	5.75		
	PMPH	1.61 ± 0.24*	1.75	1.30	2.0		
	PH	1.61 ± 0.27‡	1.60	1.25	2.30		
GI	PMCP	1.35 ± 0.61	1.25	0.00	3.00	82.377	0.001
	CP	1.30 ± 0.72	1.25	0.12	3.00		
	PMPH	0.08 ± 0.14*	0.00	0.00	0.50		
	PH	0.04 ± 0.08‡	0.00	0.00	0.30		
GBI	PMCP	1.37 ± 0.78	1.14	0.00	3.00	84.857	0.001
	CP	1.70 ± 0.85	1.50	0.25	3.00		
	PMPH	0.04 ± 0.08*	0.00	0.00	0.30		
	PH	0.03 ± 0.07‡	0.00	0.00	0.30		
PI	PMCP	0.95 ± 0.64	1.00	0.00	2.50	69.033	0.001
	CP	0.95 ± 0.71	0.75	0.00	2.75		
	PMPH	0.06 ± 0.19*	0.00	0.00	1.00		
	PH	0.03 ± 0.08‡	0.00	0.00	0.30		

Kruskal–Wallis test:
*The PMPH group is statistically different from the PMCP and CP groups ($p<0.05$).
‡The PH group is statistically different from the PMCP and CP groups ($p<0.05$).
PD, pocket depth; CAL, clinical attachment level; GI, gingival index; GBI, gingival bleeding index; PI, plaque index.

Laboratory findings

Comparisons between the groups

TAOC. The mean TAOC concentration (mM Trolox equivalent) in serum was 0.38 ± 0.14 in the PMCP patient group, 0.53 ± 0.19 in the CP patient group, 0.46 ± 0.14 in the PMPH group and 0.72 ± 0.21 in the PH group. The lowest values were observed in the PMCP group while the highest values were in the PH group ($p<0.05$). TAOC differences between the four groups were found to be statistically significant ($p<0.05$). The values of the PMCP group were found to be significantly lower than those of the CP group and the PMPH group values were found to be significantly lower than those of the PH and CP groups ($p<0.05$). The results indicated that serum TAOC concentration values were significantly lower in the post-menopausal groups than in the pre-menopausal groups and in the periodontitis groups than in their matched control groups ($p<0.05$) (Fig. 1).

The mean TAOC concentration (mM Trolox equivalent) in GCF was 0.03 ± 0.02 in the PMCP patient group, 0.07 ± 0.03 in the CP patient group, 0.09 ± 0.03 in the PMPH group and 0.12 ± 0.05 in the PH group. Statistically significant differences were observed between all groups. TAOC concentration was observed to be at its lowest in the PMCP group, and highest in the PH group ($p<0.05$). The values were found to be significantly lower in the post-menopausal groups as than in the periodontally matched pre-menopausal groups and in periodontitis groups than were in control groups ($p<0.05$). The GCF TAOC concentration was higher in the post-menopausal control group than in the pre-menopausal CP group ($p<0.05$) (Fig. 1).

The mean GCF TAOC/30 s (mM Trolox equivalent) activity was 0.18 ± 0.11 in the PMCP patient group, 0.36 ± 0.12 in the CP patient group, 0.41 ± 0.11 in the PMPH group and 0.50 ± 0.16 in the PH group. The results showed the highest GCF TAOC/30 s in the PH group, and the lowest in the PMCP group. The differences between all the groups were statistically significant ($p<0.05$).

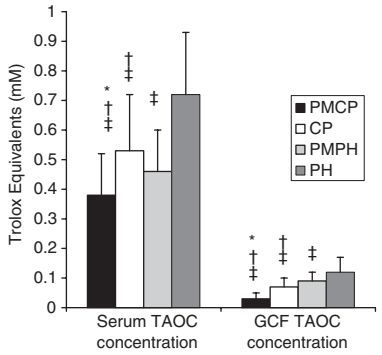

Fig. 1. The comparison of the total antioxidant capacity (TAOC) concentrations in serum and gingival crevicular fluid (GCF) between the groups. *Significant difference as compared with chronic periodontitis (CP) group ($p<0.05$). †Significant difference as compared with post-menopausal periodontally healthy control (PMPH) group ($p<0.05$). ‡Significant difference as compared with pre-menopausal controls (PH) group ($p<0.05$).

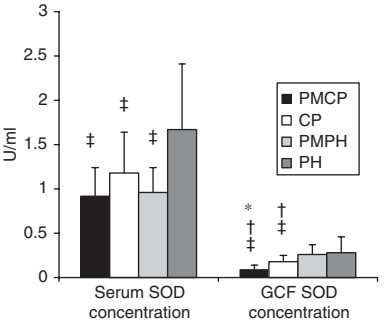

Fig. 2. The comparison of the superoxide dismutase (SOD) concentrations in serum and gingival crevicular fluid (GCF) between the groups. *Significant difference as compared with chronic periodontitis (CP) group ($p<0.05$).

†Significant difference as compared with post-menopausal periodontally healthy control (PMPH) group ($p<0.05$).
‡Significant difference as compared with pre-menopausal controls (PH) group ($p<0.05$).

SOD. The mean serum SOD concentration (U/ml) was 0.92 ± 0.32 in the PMCP patient group, 1.18 ± 0.46 in the CP patient group, 0.96 ± 0.28 in the PMPH group and 1.67 ± 0.74 in the PH group. Serum SOD concentrations in the PMCP, CP and PMPH groups were found to be significantly lower than in the PH group ($p<0.05$). While findings showed the serum SOD levels to be at their lowest in the PMCP group and highest in the PH group, the differences between the PMCP and CP groups, and between the PMCP and PMPH groups, were not statistically significant ($p>0.05$). In addition, serum SOD concentration was higher in the CP group than in the PMPH group; however, the difference was found to be statistically insignificant ($p>0.05$) (Fig. 2). The results showed that serum SOD concentrations were lower in the post-menopausal groups than in the pre-menopausal groups and lower in the periodontitis groups than in their matched control groups. However, while the values for the pre-menopausal control group were found to be significantly highest, the differences among the others did not reach a level of statistical significance.

The mean GCF SOD concentration (U/ml) was 0.09 ± 0.05 in the PMCP patient group, 0.18 ± 0.07 in the CP group, 0.26 ± 0.11 in the PMPH group and 0.28 ± 0.18 in the PH group. While the PH group values were the highest among all groups, the lowest GCF SOD concentration was observed in the PMCP group ($p<0.05$). Both disease groups (pre- and post-menopausal) were found to have significantly lower GCF SOD concentration than the periodontally healthy controls and the PMCP group values were also found to be significantly lower than those of the CP group ($p<0.05$), while in periodontal health no significant differences were observed between pre- and post-menopausal groups ($p>0.05$) (Fig. 2).

The mean GCF SOD/30 s activity levels (U) were 0.43 ± 0.24 in the PMCP patient group, 0.82 ± 0.23 in the CP group, 1.11 ± 0.48 in the PMPH group and 1.26 ± 0.88 in the PH group. The PH group showed the highest values, while the lowest values were observed in the PMCP group. Although the PMCP group values were found to be significantly lower than those in the CP, PMPH and PH groups ($p<0.05$), no statistically significant difference was found between the CP and two control groups ($p>0.05$).

Comparisons within the groups

In comparisons of the TAOC and SOD concentrations between the serum and GCF within each group, the serum concentrations were found to be significantly higher than those of the GCF in all groups ($p<0.05$) (Figs 1 and 2).

Discussion

The findings of the study indicate that serum and GCF TAOC and SOD concentrations are reduced in post-menopausal women and also in women with periodontitis. The values were found to be lowest in the PMCP group and highest in the PH group and significant differences were observed between the groups.

This study is the first to compare the TAOC and SOD levels of pre- and post-menopausal chronic periodontitis patients. Our findings show that there is a reduction of both peripheral and local AO defence in the post-menopausal groups in comparison with periodontally matched pre-menopausal groups, implying that such a difference may be related to hormonal status rather than periodontal status alone. Reduced hormonal levels in the post-menopausal period have been associated with a reduction in AO defence and an increase in oxidative stress relative to pre-menopausal controls (Bednarek-Tupikowska et al. 2004, Ha 2004, Isomura et al. 2004, Vural et al. 2005). Post-menopausal oestrogen deficiency has also been linked with oxidative stress (Wassmann et al. 2001). Oestrogen has been shown to demonstrate a structural resemblance to certain lipophilic AOs, and to be capable of detoxifying accumulated ROS (Behl 2003, Isomura et al. 2004) and to play a protective role against oxidative stress (Bhavnani et al. 2001, Wassmann et al. 2001, Telci et al. 2002). A decrease in SOD activity has also been observed in ovariectomized rats or rats that have entered natural menopause (Ha 2004, Moorthy et al. 2005, Muthusami et al. 2005). The findings of the post-menopausal groups in our study appear to be consistent with these data. Despite our efforts to keep the age difference between pre- and post-menopausal female volunteers to a minimum, the mean difference was still 10 years. We cannot therefore eliminate age-related differences in TAOC as a confounder in our study, and further investigation of age-matched pre- and post-menopausal patients would shed light upon the influence of age upon TAOC in GCF and serum.

Our findings also show that there is a reduction in systemic and local AO defence in periodontitis, confirming the results of Brock et al.'s (2004) study, which utilized a different assay for TAOC. Increased ROS production in phagocytic cells that have become activated in the peripheral blood of periodontitis patients (Whyte et al. 1989, Kimura et al. 1993, Shapira et al. 1994, Asman 1998) may contribute to a decrease in AO defence or an increase in oxidative stress. Panjamurthy et al. (2005) found that, in individuals with periodontitis, some oxidative stress markers increase and some AOs decrease as compared with that in healthy individuals and stated that these changes may be related to the increase in oxidative stress in periodontitis.

The number of studies concerned with AO defence capacity in oral fluids is limited (Guarnieri et al. 1991, Moore et al. 1994, Chapple et al. 1997, Brock et al. 2004, Akalin et al. 2005). Our TAOC findings are consistent with the study carried out by Brock et al. (2004), which shows that TAOC is significantly lower in the plasma and GCF of CP patients than in controls. In contrast, however, TAOC and SOD concentrations were found to be significantly higher in serum than in GCF in our

study. This difference may be due to a number of factors. In the studies by Chapple et al. (1997) and Brock et al. (2004), the patients were age-matched and a different methodology was used. We also calibrated our Periotron with distilled water rather than serum or ''artificial GCF'' (Chapple et al. 1999, Brock et al. 2004) and used a different elution buffer for GCF samples (Tris-HCl). The assay was run at a low pH, which could have resulted in the precipitation of proteins onto the Periopaper sampling strips, leading to a loss of TAOC relative to samples eluted into PBS–BSA in the former studies. This aspect of our GCF assay warrants further investigation. However, even if our GCF TAOC data are artificially low, the same methodology was used for all four subject groups and the differences between groups remain valid differences, worthy of discussion. GCF TAOC/30 s was also lowest in the PMCP group and highest in the PH group, as was the GCF TAOC concentration, and significant differences were observed between all groups. The method of Periotron calibration has, therefore, not significantly impacted upon these data. Furthermore, as in our previous study (Akalin et al. 2005), our present study found that total GCF SOD/30 s was not significantly lower in CP patients than in the controls. However, in that study we also observed that gingival SOD activity increased significantly in CP patients when compared with that of healthy subjects. This was also shown by Panjamurthy et al. (2005) and can be explained by the fact that the AO defence systems in extracellular fluids differ from those of the tissues (Chapple 1996, Brock et al. 2004). SOD is an antioxidant enzyme system up-regulated in cells and tissues by oxidative stress. However, AO enzymes like SOD are of little biological significance in the extracellular fluids (Halliwell & Gutteridge 1990), as the radical scavenging antioxidants are more efficient at radical removal in this environment. Therefore, one would expect levels of scavenging AOs to be sacrificially decreased in extracellular fluids under conditions of oxidative stress, even though tissue levels of SOD might have been up-regulated to combat the oxidative stress.

The findings of our study show that systemic and local AO levels reduce in relation to both menopause and periodontitis. The fact that serum TAOC is significantly lower than that in the CP group in the post-menopausal period even in periodontal health suggests that serum TAOC is affected by menopause in addition to the impact of periodontal status. The fact that GCF TAOC is lower in periodontitis groups than in the controls regardless of being pre-menopausal or post-menopausal, whereas when periodontally matched groups are compared, it is again lower in post-menopausal groups, shows that while GCF TAOC is affected locally by periodontal status, it also appears to be affected by menopause. While similar comments could be made for extracellular SOD concentrations, the biological significance of EC-SOD is thought to be minimal. SOD is predominantly an intracellular AO enzyme and only low activities are present in plasma and in other extracellular fluids (Halliwell & Gutteridge 1990).

Aging, menopause and osteoporosis are associated with increased oxidative stress and reduced antioxidant defence mechanisms (Isomura et al. 2004, Moorthy et al. 2005). A correlation between oestrogen deficiency, osteopenia/osteoporosis and the prevalence of tooth loss and alveolar bone loss has also been reported (Mascarenhas et al. 2003). However, while in our study we made efforts to avoid age differences between the groups, the ages of the post-menopausal subjects were naturally greater than those of the pre-menopausal subjects. For this reason, we could only match the periodontitis and control groups in terms of their ages among the pre- and post-menopausal women. In order to reduce the age differences to a minimum, we paid special attention to select women who had newly entered menopause (6–12 months). The post-menopausal subjects consisted of women who had refused and/or had never begun HRT. These 6–12 months also enabled confirmation that the subjects had entered menopause and were the minimum period of time required to enable decisions about HRT to be made. These periodontally matched groups showed no significant differences in any periodontal parameter. Although the comparatively low AO levels in the post-menopausal groups may also be due to aging, our data also support effects of menopause upon TAOC and SOD. The reported reductions in AO levels after ovariectomy and their return to normal or increase after HRT (Bednarek-Tupikowska et al. 2004) demonstrate the effects of hormones on AO defence independent of age. Interestingly, Brock et al. (2004) did not find a significant correlation between serum AO concentration and age with their TAOC assay.

When one compares these two groups that are matched in terms of periodontal status, the significantly lower levels of AO in the post-menopausal groups indicate that oxidative stress may further increase in the later years of the post-menopausal period. It is believed that ovarian deficiency and its associated physiological effects, not aging, are the predominant causes of bone loss during the first 2 decades after menopause (Mascarenhas et al. 2003), rather than aging per se. As the aim of our study was to compare pre-menopausal and post-menopausal groups, male subjects were not included in the study. Longitudinal studies including men of comparable ages or those involving women at further stages of menopause or undergoing HRT and those suitable for risk factor research (Tonetti & Claffey 2005) may provide more enlightening results.

Diabetes and smoking, which are accepted as being true risk factors for periodontitis (Haber et al. 1993, Löe 1993, Tonetti & Claffey 2005) and cardiovascular diseases which in the recent years have been reported to be related to periodontitis (Emingil et al. 2000), are also situations associated with excess ROS production and high levels of oxidative stress. However, it is not clear whether this increased ROS accumulation is the cause or the result of these diseases (Bauer & Bauer 1999). Therefore, the relationship between CP and oxidative stress is an important area of study that may provide some explanations underpinning the mechanistic links between CP and smoking, diabetes and cardiovascular diseases. When we consider these data together with the results of our study, we hypothesize that menopause may be a potential risk factor for the severity and progression and even the initiation of periodontal disease owing to reduced AO capacity and/or increased oxidative stress. However, longitudinal intervention studies directed at exploring oxidative stress and antioxidant status are needed to prove/disprove this hypothesis.

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Clinical Relevance

Scientific rationale: Recent evidence suggests links between menopause and oxidative stress as well as menopause and periodontal destruction. This study compares serum and gingival crevicular fluid antioxidant capacity in periodontally matched pre-menopausal and post-menopausal women with periodontitis or periodontal health.

Principal findings: The lowest AO levels were in the post-menopausal periodontitis group while the highest levels were in pre-menopausal controls.

Practical implications: The results indicate reduced peripheral and local antioxidant capacity in post-menopausal subjects, irrespective of periodontal status. While these data can be explained by age differences between pre- and post-menopausal groups, they also suggest that post-menopausal women may be at increased risk of periodontitis owing to a reduced antioxidant defence capacity and/or increased oxidative stress. Further studies of age-matched pre- and post-menopausal women are needed to help answer this question.