Total Antioxidant Capacity and Superoxide Dismutase Activity Levels in Serum and Gingival Crevicular Fluid in Pregnant Women With Chronic Periodontitis

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Background: There is evidence of reduced antioxidant (AO) defense in periodontitis and pregnancy and adverse interactions between periodontitis and pregnancy.

Methods: In this study, serum and gingival crevicular fluid (GCF) total AO capacity (TAOC) and superoxide dismutase (SOD) enzyme concentrations in pregnant patients with chronic periodontitis (CP) were compared to those in non-pregnant patients. Periodontal examinations were performed and GCF/serum samples were obtained from 33 pregnant patients with CP (PCP), 18 pregnant patients with gingivitis (PG), and 21 periodontally healthy pregnant controls (P-controls), monitored in the first and third trimesters; 27 non-pregnant women with CP; and 25 non-pregnant control women. The concentrations of TAOC (automated measurement method) and SOD (spectrophotometric method) were determined.

Results: Periodontal parameters were higher in pregnant patients versus non-pregnant patients and in the CP group compared to controls, whereas TAOC and SOD concentrations were lower ($P<0.05$). All parameters, except plaque index, increased in pregnant subjects in the third trimester compared to the first trimester, whereas TAOC and SOD levels decreased ($P<0.05$). Periodontal parameters were highest and TAOC and SOD levels were lowest in the PCP group in the third trimester ($P<0.05$).

Conclusions: Systemic and local GCF AO levels decreased in pregnancy and periodontitis, and AO defense reached the lowest levels in the last phase of pregnancy, whereas periodontal status deteriorated. These results suggest that reduced AO capacity may be associated with adverse periodontitis–pregnancy interactions, and each situation can be a provocative risk factor for the other. *J Periodontol 2009;80:457-467.*

KEY WORDS

Antioxidants; chronic periodontitis; gingival crevicular fluid; pregnancy; superoxide dismutase.

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Tissue-damaging reactive oxygen species (ROS) are produced continuously by most tissues as metabolic by-products. They are also essential for normal function and metabolism in mammalian cells.[1] These species include the oxygen-derived free radicals superoxide ($O_2^{\cdot-}$), hydroxyl ($\cdot OH$), and nitric oxide ($NO\cdot$), and non-radical derivatives of oxygen, such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCL).[2-4] ROS are capable of initiating lipid peroxidation and damaging DNA and cell membranes.[5,6] All mammalian cells contain antioxidants (AOs) that prevent or limit oxidative tissue injury.[1] AOs are classified as chain-breaking AOs, preventative AOs, and enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), which catalyze the breakdown of ROS.[7] SOD, found in all cells, transforms $O_2^{\cdot-}$ to H_2O_2.[6] The SOD family includes cytosolic Cu, Zn-SOD; mitochondrial Mn-SOD; and extracellular Cu, Zn-SOD (EC-SOD), which shows some sequence homology to the cytosolic Cu, Zn-SOD but functions in the extracellular spaces and possesses attached carbohydrates.[8,9]

Overproduction and/or decreased clearance of ROS by scavenging

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mechanisms may result in oxidative stress and damage.[1,10] Excessive production of ROS has been implicated in the pathogenesis of many human diseases, including periodontitis.[1-5,9,11-13] Higher levels of ROS released by peripheral blood neutrophils,[1-4,11,12] reduced AO capacity,[1-4,14-18] and increased oxidative stress biomarkers[16,19,20] have been reported in periodontitis. Although it is unclear whether increased ROS production is a cause or effect of periodontitis, a role for ROS in the tissue destruction of periodontitis has been suggested.[21]

Pregnancy is a state of oxidative stress, with increased oxygen requirements, placental mitochondrial activity, and production of ROS[10,22] associated with increased lipid peroxides and isoprostanes and decreased expression and activity of AOs.[8,10] Elevated levels of ROS may occur at certain windows in placental development and in pathologic pregnancies, such as those complicated by preeclampsia, miscarriage, early pregnancy loss, defective embryo development, and preterm premature rupture of membranes.[6,10,22-25]

Susceptibility to periodontal infection increases during early gestation because of immune system alterations and hormonal changes,[26] which may be responsible for the periodontal pathologic conditions observed in pregnancy, such as pregnancy gingivitis, granuloma, and periodontitis.[27] Increased synthesis of prostaglandin E_2 in this period may contribute to the pathologic changes.[27,28] Periodontal pathogens, such as *Prevotella intermedia* and *Porphyromonas gingivalis*, increase in the gingival crevicular fluid (GCF) of pregnant women as the severity of pregnancy gingivitis increases.[27] Increases in gingivitis, gingival bleeding, periodontal probing depth (PD), subgingival microflora, and GCF have been reported during pregnancy.[27]

Infections can be major risk factors in preterm births.[28,29] Previous studies[29-31] suggested that periodontal disease may be an independent risk factor for preterm delivery of low birth weight infants. The risk for preterm birth is directly related to the severity of periodontitis,[32] and periodontal disease increases the risk for severe preeclampsia in pregnant women.[25] Other studies[33-35] reported that periodontitis does not increase the risk during pregnancy.

This study investigated the relationship between systemic and local (periodontal) AO defense levels and periodontal status in pregnant women with chronic periodontitis (CP). Serum and GCF total antioxidant capacity (TAOC), SOD concentrations, and periodontal clinical parameters were measured in pregnant women at the beginning and the end of pregnancy and compared to values in women who were not pregnant.

MATERIALS AND METHODS

Clinical Assessments

Study groups. The inclusive enrollment dates of this study were from March 2005 to January 2008. One hundred twenty-four women, consisting of 33 pregnant patients with CP (PCP) in the first trimester (PCP1), 18 pregnant patients with gingivitis (PG) in the first trimester (PG1), 21 pregnant women with periodontal health (pregnant controls; P-controls) in the first trimester (P-control1), 27 non-pregnant patients with chronic periodontitis (CP), and 25 non-pregnant women as controls, enrolled in this study (Table 1). Pregnant women in the first trimester who applied to Karadeniz Technical University's Gynecology and Obstetrics Clinic for pregnancy monitoring and non-pregnant women who applied to the Periodontology Department, Faculty of Dentistry, Karadeniz Technical University were classified as CP and gingivitis cases and periodontal-health cases.[28,29,36,37] Periodontal clinical measures in pregnant women were repeated in their third trimester (PCP3, PG3, and P-control3).

The clinical/periodontal criteria for the diagnosis of the groups were as follows. The CP group had ≥30% periodontal bone loss, with the teeth having periodontal pockets ≥5 mm.[36] Radiographs were not obtained for the pregnant patients. PD and clinical attachment level (CAL) were used as the criteria for periodontal diagnosis.[28,29] The PCP group had four or more sites with PD ≥5 mm and CAL ≥4 mm.[28,29] The PG group had PD <3 mm, no attachment loss, and gingival index (GI) ≥1.[37] The control groups (pregnant and non-pregnant) consisted of periodontally healthy women with PD <3 mm, CAL <2 mm, and low levels of gingival inflammation or bleeding.[29]

All subjects were never-smokers; did not consume alcohol; had no history of systemic diseases or periodontal treatment; had not taken antibiotics, anti-inflammatory drugs, or other drugs during the 6 months prior to enrolling in the study; and had ≥20 teeth. An exception was pregnant subjects who had taken folic acid in the first trimester in connection with pregnancy monitoring.

Subjects in the CP and control groups were selected from individuals who were not in their menstrual cycle (at the time of sampling), had not received hormonal treatment, did not use oral contraceptives, had not entered menopause, and were not lactating. The study data collected for all of the participants included their medical and dental histories, sociodemographic data, and information about lifestyle and habits.

This study was performed with patients from the Gynecology and Obstetrics Clinic. The gestational age was calculated from the number of days since the last menstruation and ultrasonographs. The number of regular visits over the course of pregnancy was

Table 1.

Sociodemographic Data of the Study Subjects

	CP (n = 27)	Control (n = 25)	PCP (n = 33)	PG (n = 18)	P-control (n = 21)
Age (years; mean ± SD)	29.3 ± 3.94	29.73 ± 3.71	29.57 ± 3.56	29.94 ± 3.20	29.0 ± 3.82
Range	24 to 39	26 to 37	25 to 38	27 to 36	22 to 38
Education level (n [%])					
Primary	19 (70%)	7 (28%)	22 (67%)	10 (56%)	7 (33%)
Secondary	8 (30%)	18 (72%)	11 (33%)	8 (44%)	13 (62%)
Higher	0	0	0	0	1 (5%)
Socioeconomic status					
Average	20 (74%)	5 (20%)	18 (55%)	10 (55%)	3 (14%)
Slightly lower than average	7 (26%)	20 (80%)	15 (45%)	8 (45%)	18 (86%)
Previous pregnancy (n [%])	–	–	26 (79%)	13 (72%)	15 (71%)
Previous PLBW (<2,700 g; n [%])	–	–	6 (18%)	–	–

– = no available data; PLBW = preterm low birth weight.

eight. All subjects gave their written informed consent to participate in the study. The study protocol was approved by the Karadeniz Technical University Faculty of Medicine Ethics Committee.

Clinical measurements. PD and CAL (measured using a Michigan "0" probe with Williams markings),‖ GI,[38] gingival bleeding index (GBI),[39] and plaque index (PI)[40] were determined. PD and CAL were measured at six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-palatal, mid-palatal, and disto-palatal), and GI, GBI, and PI were measured at four sites (mid-buccal, mesial, mid-palatal, and distal) of the sampled teeth.

Clinical indices and samples were obtained from the pregnant subjects in the first and third trimesters. All clinical examinations and radiographs (radiographs were taken only from non-pregnant patients) were carried out by a single examiner (EB).

Collection of GCF and serum samples. All samples were collected in the morning after individuals had fasted overnight.[14] The subjects were instructed not to eat or drink anything in the morning. Individuals were questioned about their protocol adherence before sample collection.

To avoid irritation, samples were obtained 2 days after the clinical measurements and between 8:00 and 10:00 in the morning. Ten GCF samples were collected from each subject. The samples were collected from mesio-buccal and disto-palatal sites on maxillary premolars and incisors (at least five teeth). In each group, the sampling teeth exhibited the clinical/periodontal criteria for the classification of the group (CP, gingivitis, or periodontal health). The area was isolated with cotton rolls and slightly air-dried; saliva contamination was eliminated, and plaque was gently removed. The samples were obtained in 30 seconds, using standardized paper strips¶ and the orifice method of Rudin et al.[41] The volume was measured using a precalibrated electronic device.# The 10 paper strips that absorbed GCF for each subject were pooled in glass tubes containing 1 ml 20 mM Tris-HCl buffer (pH 6.5). Samples were eluted for 30 minutes at room temperature, and the paper strips were removed and stored in liquid nitrogen until analysis.[2,14,15]

Venous blood for serum was collected in plain tubes that were initially kept at room temperature for 30 minutes. After the blood was collected, the tubes were kept at 4°C for 30 minutes before centrifugation at 1,500 × g for 10 minutes at room temperature. Serum samples were aliquoted into cryogenic vials and stored in liquid nitrogen.[33]

The pregnant women were referred from the Gynecology and Obstetrics Clinic to the Periodontology Department, with a diagnosis of pregnancy in the first trimester. Following clinical measurements and samplings, oral hygiene instructions and plaque-control training were given, and supragingival scaling (without anesthesia) and polishing were conducted; this protocol was repeated at the beginning of the second and third trimesters.

Laboratory Assessments

TAOC assay. TAOC in serum and in GCF samples was measured using an automated measurement method developed by Erel.[42] This method can be used with many complex biologic fluids, including serum, plasma, and urine, as well as beverages and fruit juices. The method is capable of measuring endogenous

‖ Hu-Friedy, Chicago, IL.
¶ Periopaper, Oraflow, Amityville, NY.
# Periotron 8000, Oraflow.

and exogenous antioxidants, such as uric acid, Trolox (a water-soluble vitamin E analog),** vitamin C, and glutathione.

Principle of the 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 colorless *o*-dianisidine molecules to yellow-brown–colored dianisidyl radicals at low pH (1.8). The oxidation reactions progress among dianisidyl radicals, and further oxidation reactions develop. The color formation is increased with further oxidation reactions. Antioxidants in the sample suppress the oxidation reactions and color formation. This reaction can be monitored spectrophotometrically.††

Assay protocol. Briefly, 200 μl reagent 1 (*o*-dianisidine [10 mM], ferrous ion [45 μM] in Clark and Lubs solution [75 mM, pH 1.8])[42] was mixed with 5 μl samples (serum and GCF), and the absorbance of each sample was read spectrophotometrically at 444 nm as a sample blank. Then, 10 μl reagent 2 (H_2O_2 [7.5 mM] in Clark and Lubs solution) was added to the mix, and the last absorbance was read ~3 to 4 minutes after mixing at 444 nm. Trolox was used as a standard, and the results were calculated in Trolox equivalents (mM). The analytical sensitivity of this method was found to be 0.07 mM Trolox equivalents (n = 7) for GCF and 0.04 mM Trolox equivalents (n = 8) for serum. The range of standards used for the TAOC assay was 0 to 1 mM (0, 0.2, 0.4, 0.6, 0.8, and 1) Trolox equivalents.

SOD activity assay. Serum SOD activity was measured by reduction of nitroblue tetrazolium (NBT) by the xanthine-xanthine oxidase system.[43] Briefly, 0.5 ml serum was treated with an ethanol-chloroform (5:3) mixture and subjected to vigorous vortex-mixing for 1 minute. Treated samples were centrifuged (18,000 × g for 60 minutes), and the supernatant was used for the assay. Supernatant (0.250 ml) was mixed with 1.25 ml SOD assay reagent (consisting of 40 ml 0.3 mmol/l xanthine, 20 ml 0.6 mmol/μl EDTA, 20 ml 150 μmol/l NBT, 12 ml 400 mmol/l Na_2CO_3, and 6 ml 0.1% bovine serum albumin). Xanthine oxidase solution (25 μl; 167 U/l) was added, and the tubes were incubated for 20 minutes at 25°C. The reaction was terminated by adding 0.5 ml 0.8 mmol/l $CuCl_2$. Formazan formation was assessed spectrophotometrically at 560 nm. Enzyme activity leading to 50% inhibition was taken as one unit, and bovine erythrocytes (SOD) were used as an external standard. The SOD activity of GCF was measured by the same method, with the exception of the ethanol-chloroform treatment. The concentration results were expressed in U/ml for serum and GCF. The range of standards used for the SOD assay was 0 to 10 U/ml (0, 0.5, 1, 1.5, 2, 4, 6, 8, and 10).

The total TAOC and SOD levels were measured in the total amount of GCF that was collected in 30 seconds. TAOC and SOD concentrations were calculated by dividing the total TAOC and SOD by the volume of GCF.

Statistical Analysis

The normality of the data distribution was examined by the Shapiro-Wilk test. For normally distributed data (serum SOD concentration), comparisons among the five groups (CP and control groups with pregnant women in the first and third trimesters) were performed by one-way analysis of variance and the Tukey multiple comparison tests. Comparisons among the five groups for non-normally distributed data (clinical parameters, serum and GCF TAOC concentrations, GCF TAOC/30 seconds, GCF SOD concentration, and GCF SOD/30 seconds) were made with the Kruskal-Wallis test. Multiple comparisons were made with the Bonferroni-corrected (α/# of pairwise comparisons = 0.05/10 = 0.005) Mann-Whitney U test. The within-group differences between the first and third trimesters of pregnancy were checked with the paired-samples *t* test for the normally distributed data and with the Wilcoxon signed-rank test for other variables. The relationships between clinical parameters and serum and GCF TAOC and SOD levels were evaluated using the Spearman correlation coefficient.

Each subject was used as the unit of analysis. No power analysis was performed because this was a pilot study using a convenience sample. All statistical analyses were performed with a software program.‡‡

RESULTS

All subjects were age-matched with similar lifestyles and socioeconomic situations (slightly below average). One of the subjects had a higher education (degree), whereas the others were primary or secondary school graduates. All women lived in the same geographic region; were married (those who were pregnant); had stable family lives; did not smoke or consume alcohol or narcotic drugs; had traditionally similar nutritional habits; and primarily consumed foods rich in AOs (fish, fruit, and vegetables), as is typical in the region. There were no recorded cases of serious pregnancy complications, infections, or systemic drug use in any of the participants (self-reported data). Most of the pregnant subjects had previous pregnancies. During the study, preeclampsia was detected throughout the pregnancy of one patient (at week 33). One patient diagnosed with aggressive periodontitis had a miscarriage in the second month, and one patient with severe CP had a preterm delivery.

** Sigma-Aldrich Chemical, St. Louis, IL.
†† Beckman DU 530, Beckman, Fullerton, CA.
‡‡ SPSS 12.0 for Windows, SPSS, Chicago, IL.

These three women were excluded from the study. Thus, the study included subjects with uneventful pregnancies, without any serious complications. Based on the obtained information, six preterm low birth weights (<2,700 g) were recorded among the previous deliveries of the pregnant subjects (Table 1).

Clinical Findings

All clinical parameters were significantly higher in PCP, PG, and CP groups than in the control group (P <0.05). Although none of the clinical parameters differed significantly in the PCP1 group versus the CP group (P >0.05), all clinical parameters, with the exception of PI, were significantly higher in the PCP3 group than in the CP group (P<0.05). Although PD and CAL were significantly lower in the PG1 group than in the CP group (P <0.05), GBI was considerably higher in the PG3 group versus the CP group (P <0.05). In the P-control group, although PD and CAL were significantly lower than in the CP group in both trimesters (P <0.05), the differences in GI and GBI in the third trimester were not statistically significant (P>0.05). Although the P-control group did not show a significant difference from the controls in the first trimester (with the exception of CAL), the differences (with the exception of PI) were significant in the third trimester (P <0.05). PD and CAL were significantly higher in the PCP group than in PG and P-control groups (P <0.05). In the pregnant groups, all clinical parameters except PI increased significantly in the third trimester compared to the first trimester (P <0.05; Table 2).

The mean ± SD of GCF volumes are given in Table 2. These values represent the mean total volume (n = 10 strips) per subject. GCF volume increased for PCP and PG groups from the first to the third trimester; however, the P-control group did not show a significant increase in GCF volume.

Laboratory Findings

TAOC. Generally, average TAOC values were lower in the pregnant groups than in the periodontally matched, non-pregnant groups and were lower in the periodontitis groups than in the control groups. In the pregnant groups, the levels decreased significantly from the first trimester to the third trimester (P<0.05; except GCF/30 seconds in P-control group). Overall, the values were lowest in the PCP3 group and highest in the control group. Comparisons among the groups are shown in Table 3.

Although serum TAOC concentrations (mM Trolox equivalent) in the PCP1 group did not differ significantly compared to PG1 and CP groups, they were significantly lower than the P-control1 group (P <0.05).

The mean GCF TAOC concentrations (mM Trolox equivalent) were significantly higher in the PG1 group (P <0.05) and similar in the PG3 group compared to the CP group. In the P-control group, the concentrations were significantly higher than in the CP group for both trimesters and lower than in the control group in the third trimester (P<0.05). Although GCF TAOC/30 seconds (mM Trolox equivalent) levels were significantly higher in PG and P-control groups in the first trimester versus the CP group (P <0.05), the differences were not statistically significant in the third trimester (P>0.05). The values were significantly lower in PG3 and P-control3 groups than in the control group (P <0.05; Table 3).

SOD. The mean SOD concentrations were also lower in the pregnant groups than in the periodontally matched, non-pregnant groups and were lower in the periodontitis groups than in the control groups. Overall, SOD values also decreased significantly in the pregnant groups from the first trimester to the third trimester (P <0.05; with the exception of P-controls). The values were lowest in the PCP3 group and highest in the control group. Comparisons among the groups are shown in Table 3.

Serum SOD concentrations were significantly lower in the PG3 group than in the CP group (P <0.05), whereas the levels in the PCP group were not significantly different from PG and P-control groups at the same periods (P>0.05).

GCF SOD concentrations were significantly lower in the PG3 group than in the CP group (P<0.05). Although concentrations in the P-controls were significantly higher than in the CP group in the first trimester, the difference in the third trimester was not statistically significant (P >0.05). The mean GCF SOD/30 seconds activities were significantly lower in the PG3 group than in the CP group (P <0.05). Although the level in the P-control group was significantly higher than in the CP group in the first trimester (P<0.05), the difference in the third trimester was not statistically significant (P>0.05; Table 3).

Correlations

Statistically significant, strong negative correlations were observed between the clinical parameters and serum and GCF TAOC and SOD levels in pregnant women in both trimesters and in the non-pregnant groups when the groups were evaluated together (Table 4).

DISCUSSION

To our knowledge, this was the first study to investigate periodontal disease and AO defenses during pregnancy. The results showed that systemic and local/GCF AO defense decreased in pregnancy and periodontitis. The AO values decreased as the pregnancy progressed, reaching the lowest levels during the third trimester of pregnancy, which corresponded with deterioration of periodontal status.

Table 2.

Comparison of Clinical Parameters Among and Within the Groups

Parameter (sampling site)	Group	Mean ± SD	Mean ± SD	Z	P
PD (mm)	CP (n = 27)	3.19 ± 0.16*			
	Control (n = 25)	1.25 ± 0.18			
		Trimester 1	Trimester 3		
	PCP (n = 33)	3.29 ± 0.34*†‡	3.77 ± 0.35*†‡§	4.94	0.001‖
	PG (n = 18)	2.34 ± 0.21*‡§	2.82 ± 0.20*‡§	3.72	0.001‖
	P-control (n = 21)	1.15 ± 0.07§	2.06 ± 0.19*§	4.02	0.001‖
		χ^2 = 106.158	χ^2 = 117.534		
		P = 0.001	*P* = 0.001		
CAL (mm)	CP (n = 27)	3.36 ± 0.25*			
	Control (n = 25)	1.49 ± 0.2			
		Trimester 1	Trimester 3		
	PCP (n = 33)	3.53 ± 0.40*†‡	4.01 ± 0.36*†‡§	5.01	0.001‖
	PG (n = 18)	2.65 ± 0.20*‡§	3.08 ± 0.20*‡§	3.62	0.001‖
	P-control (n = 21)	1.27 ± 0.16*§	2.18 ± 0.24*§	4.01	0.001‖
		χ^2 = 107.441	χ^2 = 115.112		
		P = 0.001	*P* = 0.001		
GI	CP (n = 27)	1.15 ± 0.58*			
	Control (n = 25)	0.08 ± 0.09			
		Trimester 1	Trimester 3		
	PCP (n = 33)	1.26 ± 0.46*‡	1.84 ± 0.56*‡§	4.52	0.001‖
	PG (n = 18)	0.93 ± 0.47*‡	1.39 ± 0.57*	2.76	0.006‖
	P-control (n = 21)	0.10 ± 0.10§	0.97 ± 0.36*	3.83	0.001‖
		χ^2 = 89.107	χ^2 = 79.907		
		P = 0.001	*P* = 0.001		
GBI	CP (n = 27)	1.42 ± 0.70*			
	Control (n = 25)	0.10 ± 0.14			
		Trimester 1	Trimester 3		
	PCP (n = 33)	1.67 ± 0.73*‡	2.31 ± 0.74*‡§	4.58	0.001‖
	PG (n = 18)	1.56 ± 0.48*‡	2.46 ± 0.49*‡§	3.64	0.001‖
	P-control (n = 21)	0.12 ± 0.15§	1.03 ± 0.24*	3.93	0.001‖
		χ^2 = 88.293	χ^2 = 91.443		
		P = 0.001	*P* = 0.001		
PI	CP (n = 27)	0.92 ± 0.69*			
	Control (n = 25)	0.05 ± 0.09			
		Trimester 1	Trimester 3		
	PCP (n = 33)	1.05 ± 0.67*‡	0.83 ± 0.39*‡	2.10	0.036‖
	PG (n = 18)	0.72 ± 0.36*‡	0.83 ± 0.41*‡	0.67	0.503
	P-control (n = 21)	0.08 ± 0.13§	0.11 ± 0.11§	1.16	0.245
		χ^2 = 78.824	χ^2 = 83.161		
		P = 0.001	*P* = 0.001		
GCF volume (μl)	CP (n = 27)	4.50 ± 0.60*			
	Control (n = 25)	3.47 ± 0.54			
		Trimester 1	Trimester 3		
	PCP (n = 33)	4.77 ± 0.66*‡	5.29 ± 0.46*‡§	4.78	0.001‖
	PG (n = 18)	4.03 ± 0.60*	4.50 ± 0.48*‡	3.72	0.001‖
	P-control (n = 21)	3.66 ± 0.46§	3.70 ± 0.32§	0.52	0.600
		χ^2 = 42.502	χ^2 = 70.382		
		P = 0.001	*P* = 0.001		

* Significant difference compared to the control group ($P < 0.005$).
† Significant difference compared to the PG group in the same trimester ($P < 0.005$).
‡ Significant difference compared to the P-control group in the same trimester ($P < 0.005$).
§ Significant difference compared to the CP group ($P < 0.005$).
‖ Significant difference between the first and third trimesters ($P < 0.05$).

Table 3.

Comparison of TAOC and SOD Values Among and Within the Groups

Parameter	Group	Mean ± SD	Mean ± SD	Z	*P*
Serum TAOC concentration	CP (n = 27)	0.59 ± 0.20*			
	Control (n = 25)	0.75 ± 0.21			
		Trimester 1	Trimester 3		
	PCP (n = 33)	0.51 ± 0.14*†	0.34 ± 0.10*†‡§	4.93	0.001‖
	PG (n = 18)	0.60 ± 0.11	0.48 ± 0.12*	3.73	0.001‖
	P-control (n = 21)	0.65 ± 0.11	0.55 ± 0.12*	3.09	0.002‖
		χ^2 = 23.382	χ^2 = 60.284		
		P = 0.001	*P* = 0.001		
GCF TAOC concentration	CP (n = 27)	0.09 ± 0.03*			
	Control (n = 25)	0.17 ± 0.04			
		Trimester 1	Trimester 3		
	PCP (n = 33)	0.07 ± 0.02*†‡	0.04 ± 0.01*†‡§	5.01	0.001‖
	PG (n = 18)	0.12 ± 0.03*§	0.09 ± 0.02*†	3.72	0.001‖
	P-control (n = 21)	0.15 ± 0.03§	0.12 ± 0.03*§	3.28	0.001‖
		χ^2 = 80.038	χ^2 = 98.069		
		P = 0.001	*P* = 0.001		
GCF TAOC/30s	CP (n = 27)	0.40 ± 0.11*			
	Control (n = 25)	0.59 ± 0.10			
		Trimester 1	Trimester 3		
	PCP (n = 33)	0.35 ± 0.07*†‡	0.22 ± 0.04*†‡§	5.01	0.001‖
	PG (n = 18)	0.51 ± 0.09§	0.41 ± 0.08*†	3.62	0.001‖
	P-control (n = 21)	0.55 ± 0.08§	0.48 ± 0.07*	2.61	0.009
		χ^2 = 67.724	χ^2 = 87.200		
		P = 0.001	*P* = 0.001		
Serum SOD concentration	CP (n = 27)	1.20 ± 0.41*			
	Control (n = 25)	1.87 ± 0.67			
		Trimester 1	Trimester 3		
	PCP (n = 33)	0.85 ± 0.34*	0.59 ± 0.20*§	4.99	0.001‖
	PG (n = 18)	1.06 ± 0.34*	0.69 ± 0.16*§	4.84	0.001‖
	P-control (n = 21)	1.13 ± 0.36*	0.91 ± 0.19*	2.41	0.026
		F = 19.81	F = 44.764		
		P = 0.001	*P* = 0.001		
GCF SOD concentration	CP (n = 27)	0.19 ± 0.06*			
	Control (n = 25)	0.40 ± 0.26			
		Trimester 1	Trimester 3		
	PCP (n = 33)	0.15 ± 0.09*†‡	0.06 ± 0.02*†‡§	5.01	0.001‖
	PG (n = 18)	0.23 ± 0.06*†	0.14 ± 0.01*†§	3.72	0.001‖
	P-control (n = 21)	0.28 ± 0.06§	0.25 ± 0.11	1.79	0.073
		χ^2 = 57.721	χ^2 = 94.750		
		P = 0.001	*P* = 0.001		
GCF SOD/30s	CP (n = 27)	0.82 ± 0.22*			
	Control (n = 25)	1.41 ± 0.92			
		Trimester 1	Trimester 3		
	PCP (n = 33)	0.66 ± 0.33*†‡	0.34 ± 0.12*†‡§	5.01	0.001‖
	PG (n = 18)	0.93 ± 0.29	0.64 ± 0.09*†§	3.72	0.001‖
	P-control (n = 21)	1.1 ± 0.26§	0.97 ± 0.36	1.28	0.198
		χ^2 = 35.532	χ^2 = 81.906		
		P = 0.001	*P* = 0.001		

30s = 30 seconds.
* Significant difference compared to the control group (*P* <0.005).
† Significant difference compared to the P-control group in the same trimester (*P* <0.005).
‡ Significant difference compared to the PG group in the same trimester (*P* <0.005).
§ Significant difference compared to the CP group (*P* <0.005).
‖ Significant difference between the first and third trimesters (*P* <0.05).

Table 4.

Correlations Between the Clinical Parameters and TAOC and SOD Levels Within the Pregnant and Non-Pregnant Groups When the Groups Were Evaluated Together

Group	Parameter	r	*P*	Parameter	r	*P*
Pregnant groups (trimester 1)	sTAOC-PD	–0.398	0.001	GCF TAOC/30s-PD	–0.701	0.001
	sTAOC-CAL	–0.368	0.001	GCF TAOC/30s-CAL	–0.666	0.001
	sSOD-PD	–0.267	0.020	GCF TAOC/30s-GI	–0.564	0.001
	sSOD-CAL	–0.263	0,020	GCF TAOC/30s-PI	–0.526	0.001
	GCF TAOC conc-PD	–0.731	0.001	GCF SOD conc-PD	–0.574	0.001
	GCF TAOC conc-CAL	–0.720	0.001	GCF SOD conc-CAL	–0.561	0.001
	GCF TAOC conc-GI	–0.610	0.001	GCF SOD conc-GI	–0.524	0.001
	GCF TAOC conc-GBI	–0.525	0.001	GCF SOD conc-GBI	–0.523	0.001
	GCF TAOC conc-PI	–0.546	0.001	GCF SOD/30s-PD	–0.506	0.001
Pregnant groups (trimester 3)	sTAOC-PD	–0.600	0.001	GCF TAOC/30s-GI	–0.619	0.001
	sTAOC-CAL	–0.582	0.001	GCF TAOC/30s-PI	–0.607	0.001
	sSOD-PD	–0.501	0.001	GCF SOD conc-PD	–0.525	0.001
	sSOD-CAL	–0.501	0.001	GCF SOD conc-CAL	–0.833	0.001
	GCF TAOC conc-PD	–0.847	0.001	GCF SOD conc-GI	–0.804	0.001
	GCF TAOC conc-CAL	–0.832	0.001	GCF SOD conc-GBI	–0.617	0.001
	GCF TAOC conc-GI	–0.662	0.001	GCF SOD conc-PI	–0.572	0.001
	GCF TAOC conc-GBI	–0.592	0.001	GCF SOD/30s-PD	–0.744	0.001
	GCF TAOC conc-PI	–0.597	0.001	GCF SOD/30s-CAL	–0.727	0.001
	GCF TAOC/30s-PD	–0.738	0.001	GCF SOD/30s-GI	–0.608	0.001
	GCF TAOC/30s-CAL	–0.722	0.001	GCF SOD/30s-GBI	–0.569	0.001
Non-pregnant groups	sTAOC-PD	–0.376	0.004	GCF TAOC/30s-PD	–0.484	0.001
	sSOD-PD	–0.509	0.001	GCF TAOC/30s-CAL	–0.430	0.001
	sSOD-CAL	–0.494	0.001	GCF TAOC/30s-GI	–0.473	0.001
	GCF TAOC conc-PD	–0.734	0.001	GCF TAOC/30s-GBI	–0.446	0.001
	GCF TAOC conc-CAL	–0.723	0.001	GCF SOD conc-PD	–0.662	0.001
	GCF TAOC conc-GI	–0.657	0.001	GCF SOD conc-CAL	–0.655	0.001
	GCF TAOC conc-GBI	–0.660	0.001	GCF SOD conc-GI	–0.574	0.001
				GCF SOD conc-GBI	–0.571	0.001

r = Spearman correlation coefficient; s = serum, 30s = 30 seconds; conc = concentration.

Our serum findings were consistent with recent reports[6,10,22,24] indicating a significant decline in AO defense in pregnancy. ROS are generated during normal embryonic metabolism, and AO enzymes have a protective effect against ROS.[23,44] Pregnancy is a physiologic state accompanied by an increased oxygen requirement. Higher levels of oxidative stress biomarkers have been found in normal pregnancy compared to non-pregnancy.[45]

Changes in AO defense during periodontitis have not been studied extensively. Our findings suggest that systemic AO capacity declines because of CP and are consistent with recent studies showing decreased AO capacity[1,14-18] and increased oxidative stress markers in CP.[16,19,20] Various studies[1-4,11,12] demonstrated increased ROS production in activated polymorphonuclear leukocytes in peripheral blood in periodontitis. Oxidative stress lies at the heart of the periodontal tissue damage that results from host–microbial interactions as a direct result of excess ROS activity/AO deficiency or indirectly by the generation of a proinflammatory state.[1]

Relatively few studies[2,14,15,18] have investigated AO capacity in oral fluids in periodontitis. Our TAOC findings were consistent with those of previous studies[14,17] which showed that TAOC was significantly lower in the plasma and GCF of patients with CP compared to controls. As in our previous study,[17] SOD activity was also lower in the CP group than in controls in the present study. However, the biologic role of EC-SOD is unclear. SOD is predominantly found bound to tissues and has extremely low activities in plasma and other extracellular fluids.[8] Thus, measuring SOD activity alone to assay GCF AO capacity is inadequate. Together with the decrease in TAOC values, our findings suggest that GCF AO levels decline in pregnancy and CP and that GCF AO defense was more influenced by pregnancy than by CP. The reduction of AO defense was higher in pregnancy than in CP.

In the advanced stages of pregnancy, derangement of oxidative balance can lead to improper activation of inflammatory changes, triggering premature labor and other complications.[6,10,24,44] Decreased expression of SOD and GPx, increased levels of lipid peroxidation, and abnormally high $O_2^{\cdot-}$ synthesis rates have been noted in women with preeclampsia or those experiencing early pregnancy loss.[10,24,25] Inflammation and reduced AO activity lead to increased lipid peroxidation and cytokine synthesis and a widespread increase in oxidative stress in the mother. In the third trimester, the mother's AO capacity is no longer able to compensate; preeclampsia and premature delivery may occur.[46] Similar mechanisms may also exist in the pregnancy–periodontitis relationship. Periodontal disease is a polymicrobial infection that may lead to bacteremia and induce pregnancy complications.[28,29] The emergence of periodontitis during pregnancy may create an additional oxidative load for the mother, leading to a cycle in which cytokine synthesis and inflammation exacerbate each other. In this cycle, pregnancy exacerbates the reduction in AO defense. Thus, a decreased AO defense may be an important mechanism in the adverse interactions between pregnancy and periodontitis. In the present study, the deterioration of periodontal condition as AO levels decreased and reached the lowest levels in the pregnant groups in the third trimester supports this idea.

In PCP and PG groups, clinical periodontal parameters and GCF volume increased significantly from the first to the third trimester, whereas in the P-control group, no significant increase occurred in GCF volume, although periodontal parameters increased. This suggests that in P-control subjects, the changes of periodontal parameters in the clinical level are independent from the subclinical level and/or from the GCF flow rate. If the periodontium is healthy at the beginning of pregnancy, its deterioration at the end of pregnancy might be explained by hormonal influences rather than by bacteria-related inflammatory changes.

In some women, gingival and periodontal problems emerge and/or increase and periodontal health is adversely affected during pregnancy.[27] Although these changes are temporary and frequently disappear after pregnancy, they can persist in some patients. We believe that because of a decreased AO defense and the mechanisms outlined above, pregnancy may be a risk factor that initiates and/or exacerbates periodontal disease. Periodontal diseases are more widespread among women[27] because of events in their lives, such as pregnancy and menopause. These events alter hormonal and metabolic balances, decrease AO levels,[17,27,47] and may have a significant role in periodontal destruction. This underscores the importance of good plaque control and periodontal monitoring in pregnant women. Therefore, obstetricians and periodontists should consider the benefits of AO supplements for pregnant patients to decrease the risk for periodontal breakdown.

There were a number of limitations to our study. In accordance with pregnancy monitoring, pregnant women took folic acid in the first trimester. We did not interfere with this for ethical and health reasons, although this may have affected the results in the pregnant women. However, when the pregnancy is considered in its normal balance and stability, the results are typical for pregnant women in the study and are valuable in terms of comparisons between and within the groups. We did not take radiographs of pregnant women for ethical reasons. We referred to studies on pregnant women for the diagnosis of CP and gingivitis, without radiographic evaluations. Baseline clinical parameters of the PCP and P-control groups were similar to those of periodontally matched, non-pregnant groups. The similar PD and CAL values in PCP1 and CP groups implied similar periodontal bone levels, with no significant difference between the groups.

The present results may be considered preliminary findings of a pilot study. We believe that further longitudinal studies of the changes in oxidant status in pregnant women that conform to risk factor research criteria[50] could help to clarify whether these changes contribute to periodontitis progression or other adverse systemic effects during pregnancy. This will be exceptionally beneficial for mothers and their babies.

CONCLUSIONS

Peripheral and GCF AO defenses decrease in pregnancy and CP and decline to their lowest levels in the last phase of pregnancy. The lowest AO levels in the pregnant women with periodontitis suggest that AO defense is affected more when pregnancy and periodontitis coexist. The strong correlations between AO levels and periodontal status, particularly in pregnant women in the third trimester, suggest that pregnancy may be a risk factor for periodontitis. Pregnancy and periodontitis reduce AO capacity, which may be an important pathogenic mechanism leading to disease exacerbation and pregnancy complications. Smoking and diabetes, which are accepted risk factors for periodontitis[48-50] and cardiovascular disease that have been reported to be related with periodontitis,[51] are also conditions in which ROS production and oxidative stress are high.[13] However, it is unclear whether oxidative stress is causative for or is a result of these conditions.[13] Thus, clarification of the relationship between periodontitis and oxidative stress is particularly important.

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