Received: 2 June 2020 | Revised: 9 October 2020 | Accepted: 3 November 2020

DOI: 10.1002/JPER.20-0406

JOURNAL OF Periodontology

ORIGINAL ARTICLE

Salivary and serum oxidative stress biomarkers and advanced glycation end products in periodontitis patients with or without diabetes: A cross-sectional study

Sema Merve Altıngöz[1] | **Şivge Kurgan**[2] | **Canan Önder**[2] | **Muhittin A. Serdar**[3] | **Uğur Ünlütürk**[4] | **Metin Uyanık**[5] | **Nilgün Başkal**[4] | **Dimitris N. Tatakis**[6] | **Meral Günhan**[2]

[1] Department of Periodontology, Faculty of Dentistry, Lokman Hekim University, Ankara, Turkey

[2] Department of Periodontoloy, Faculty of Dentistry, Ankara University, Ankara, Turkey

[3] Department of Medical Biochemistry, School of Medicine, Acıbadem University, Ankara, Turkey

[4] Department of Endocrinology, Faculty of Medicine, Ankara University, Ankara, Turkey

[5] Department of Medical Biochemistry, School of Medicine, Namık Kemal University, Tekirdağ, Turkey

[6] Division of Periodontology, College of Dentistry, The Ohio State University, Columbus, Ohio, USA

Correspondence
Dr. Sema Merve Altıngöz, Department of Periodontology, School of Dentistry, Lokman Hekim University, 06510-Cankaya, Ankara, Turkey.

Source of Funding
This study was supported by the Ankara University Scientific Research Projects Office, Ankara, Turkey (12B3334002).

Abstract

Background: Non-invasive methods for periodontitis diagnosis would be a clinically important tool. This cross-sectional study aimed to investigate the association between oxidative stress, glycation, and inflammation markers and periodontal clinical parameters in periodontitis and periodontally healthy patients with type 2 diabetes and corresponding systemically healthy controls.

Methods: Sixty-seven periodontally healthy (DM-H, n = 32) and periodontitis (DM-P, n = 35) patients with type 2 diabetes, and 54 systemically healthy periodontitis (H-P, n = 26) and periodontally healthy (H-H, n = 28) controls were included. Clinical periodontal parameters, body mass index, fasting glucose, hemoglobin A1c (HbA1c), along with saliva and serum 8-hydroxy-2′-deoxyguanosine (8-OHdG), malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), advanced glycation end products (AGE), AGE receptor (RAGE) and high sensitivity C-reactive protein (hsCRP) levels were recorded and analyzed.

Results: Salivary 8-OHdG levels were significantly higher in periodontitis compared to periodontally healthy patients, regardless of systemic status ($P < 0.001$). Salivary MDA levels were significantly higher in all disease groups compared to H-H group ($P \leq 0.004$). Serum AGE levels were significantly higher in diabetic groups than systemically healthy groups ($P < 0.001$) and in H-*P* compared to H-H ($P < 0.001$). Bleeding on probing (BOP) and clinical attachment level (CAL) strongly correlated with salivary 8-OHdG and serum hsCRP ($P < 0.001$). In systemically healthy patients, salivary 8-OHdG was the most accurate marker to differentiate periodontitis from controls (AUC = 0.84). In diabetics salivary 4-HNE and RAGE were the most accurate (AUC = 0.85 for both).

Conclusion: Salivary 8-OHdG alone or in combination with 4-HNE, AGE and RAGE for diabetics, and salivary 8-OHdG alone or in combination with MDA and hsCRP for systemically healthy persons, could potentially serve as non-invasive screening marker(s) of periodontitis.

JOURNAL OF Periodontology

KEYWORDS
4-hydroxy-2-nonenal; 8-hydroxy-2-deoxyguanosine; diabetes mellitus, type 2; oxidative stress; periodontitis

1 | INTRODUCTION

Type 2 diabetes mellitus (DM) is a complex systemic disease with strong and bi-directional oral health associations; diabetic patients are at increased risk for periodontitis while infections originating in the oral cavity could represent a risk for aggravation of the systemic disease.[1] The high prevalence of periodontitis in poorly controlled diabetics has led to the characterization of periodontitis as the sixth complication of diabetes.[2] Simultaneously, periodontitis has been recognized as possible risk factor for DM.[1] Growing evidence implicates oxidative stress mechanisms in the pathobiology of both periodontitis and DM.[3] A pro-oxidant state in the periodontium could lead to decrease in insulin sensitivity and contribute to a significant systemic impact, with ensuing damage to organ systems distant from the inflammation focus.[4]

The oral microbial biofilm induces a host inflammatory response that includes release of reactive oxygen species (ROS), which may result in periodontal tissue destruction.[5] ROS can lead to peroxidation of polyunsaturated fatty acids in cell membranes, forming carbon centered radicals (PUFA radicals) or lipid peroxide radicals, and thus to loss of membrane function.[6] Lipid peroxidation degradation products such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE) are detectable in biological fluids and indicate a pro-oxidant state.[6] Increase in ROS levels cause oxidative stress.[7] Many salivary oxidative stress biomarkers can potentially be useful for screening of periodontal disease.[8] Oxidative stress can also affect DNA, resulting in production of 8-hydroxy-2′-deoxyguanosine (8-OHdG), commonly used to evaluate oxidative DNA damage in several chronic inflammatory diseases, such as diabetes mellitus,[9] atherosclerotic cardiovascular disease,[10] rheumatoid arthritis[11] and periodontitis.[12]

During chronic hyperglycemia, as it occurs in poorly controlled DM, proteins and lipids are subject to irreversible non-enzymatic glycation, which leads to formation of advanced glycation end products (AGEs).[13] AGEs and the receptor for AGEs (RAGE) may play significant roles in the pathogenesis of DM-related diseases,[14,15] including DM-related periodontitis.[16,17] When AGEs bind to RAGE, a signaling receptor present on endothelial cells and monocytes, cellular phenotype and function are critically altered, resulting in inflammation and oxidative stress.[18,19] These findings suggest that both ROS and AGE-RAGE interactions might be relevant for the exaggerated inflammatory response and periodontal tissue destruction in DM.

C-reactive protein (CRP) is an extremely sensitive and nonspecific acute-phase marker for inflammation that presents associations with smoking, obesity, coffee consumption, triglycerides, diabetes, and periodontal disease.[20,21] CRP is the best indicator of an individual's systemic inflammatory status.[22] Serum high sensitivity CRP (hsCRP) levels have been strongly associated with clinical periodontal parameters, and particularly with bleeding on probing, a marker of periodontal inflammation.[23–25]

Therefore, we hypothesized that serum and saliva levels of markers of oxidative stress (8-OHdG, MDA, 4-HNE), non-enzymatic glycosylation (AGE, RAGE) and inflammation (hsCRP) might serve as screening biomarkers for periodontitis patients who are systemically healthy or diabetic. The purpose of the present study was to evaluate salivary and serum levels of 8-OHdG, MDA, 4-HNE, AGE, RAGE and hsCRP in generalized periodontitis patients with and without DM and corresponding periodontally healthy controls, as well as the possible associations between periodontal clinical parameters and biomarker levels.

2 | MATERIALS AND METHODS

2.1 | Study population and clinical assessments

Periodontally healthy DM patients (DM-H group), DM patients with periodontitis (DM-P group), periodontally and systemically healthy individuals (H-H group), and systemically healthy individuals with periodontitis (H-P group) were recruited from patients presenting to the Department of Periodontology, School of Dentistry, and the Department of Endocrinology, School of Medicine, at Ankara University. Periodontally healthy groups consisted of individuals with negative periodontal disease history; probing depths ≤3 mm and clinical attachment levels ≤1 mm; without clinical signs of gingival inflammation and with good oral hygiene. Chronic periodontitis (CP) was diagnosed according to the 1999 criteria of

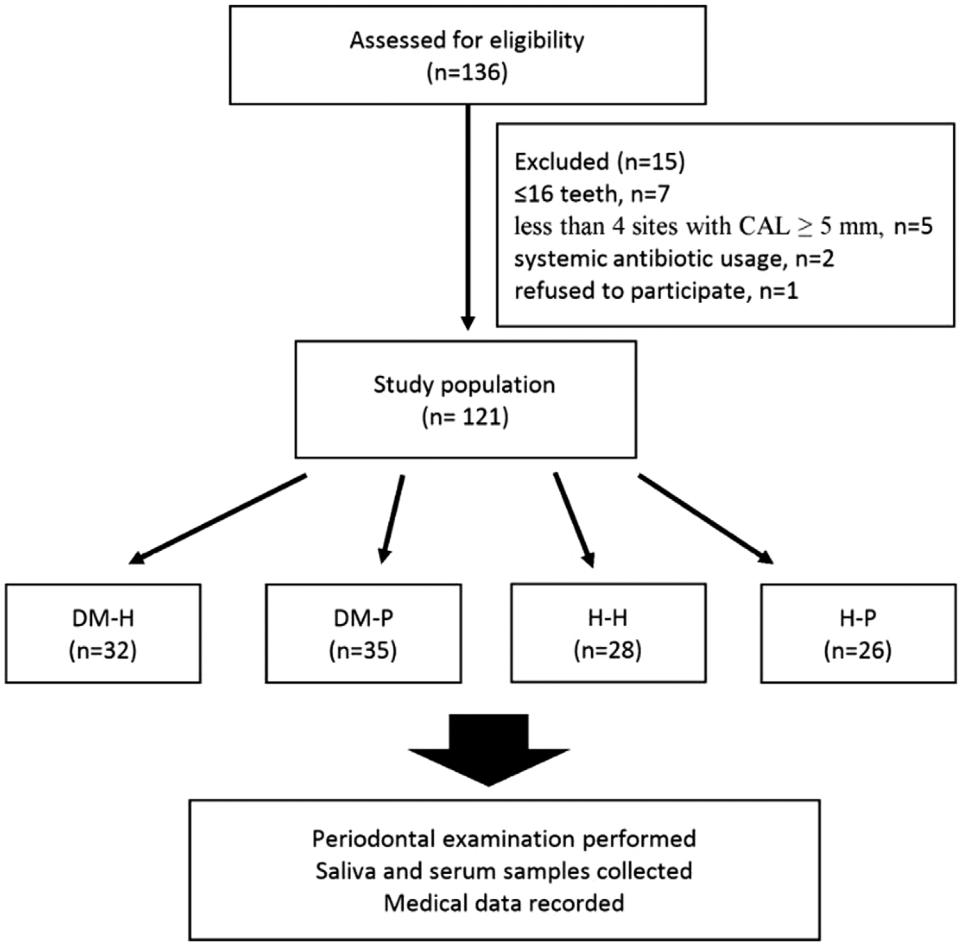

FIGURE 1 Flowchart of the study

the American Academy of Periodontology regarding to extend and severity of disease.[26] The study was reviewed and approved by the Ethics Committee for the use of human subjects in research, Ankara University Faculty of Dentistry (No: 9/1, on 08.01.2011). Patient recruitment took place between 03/2011 and 03/2013. The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2008. The flow chart (Figure 1) describes the distribution of 121 individuals (DM-H group; five males and 27 females, DM-P group; nine males and 26 females, H-H group; 10 males and 18 females and H-P group; eight males and 18 females). 136 individuals, meeting the criteria, were selected for the study. 15 patients were excluded for different reasons (having ≤16 teeth for seven patients, less than four sites with CAL ≥5 mm for five patients, systemic antibiotic usage in the previous 3 months for two patients, refused to participate in the study for one patient). The study was completed with 121 patients. All study participants provided signed informed consent.

Inclusion criteria for CP patients were as follows: (1) aged 35 to 65 years; (2) ≥16 teeth present; (3) ≥8 sites with probing pocket depths (PPD) ≥6 mm; and (4) less than equal to four sites with clinical attachment level (CAL) ≥5 mm, distributed in at least two different quadrants.[27] Based on interdental CAL at site of greatest loss and considering that patients do not need complex rehabilitation, all periodontitis patients in the present study would be diagnosed with Stage III periodontitis, according to the new classification of periodontal diseases.[28] Inclusion criteria for DM patients were: (1) aged 35 to 65 years; (2) type 2 DM, diagnosed by physicians (NB and UÜ), for at least the past 5 years; (3) 6.5%≤ HbA1c <12%. Exclusion criteria for all groups were: uncontrolled diabetes (HbA1c ≥12%); systemic antibiotic or anti-inflammatory medication use in the previous 3 months; non-surgical periodontal therapy in the previous 6 months; surgical periodontal therapy in the previous 12 months; use of calcium channel blockers, phenytoin, or cyclosporine; and pregnancy. Smokers were defined as individuals who smoked ≥10 cigarettes daily for at least the last 5 years.[29] Non-smokers were defined as those who never smoked or reported having quit smoking at least 2 years prior to study entry.

2.2 | Evaluation of periodontal status

All study participants were evaluated clinically at their first visit in the Departments of Periodontology and Endocrinology by one trained and calibrated examiner (SMA). All periodontal variables were assessed at six different sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) per tooth at all teeth present, except third molars. Clinical parameters recorded were plaque index (PI),[30] gingival index (GI),[31] periodontal pocket depth (PPD), CAL, and percentage of bleeding on probing sites (BOP%). Parameters were assessed using a Williams probe and PPD recordings were made to the nearest mm; observations close to 0.5 mm were rounded to the lower whole mm.

2.3 | Evaluation of diabetes mellitus

Body mass index, fasting glucose (mg/dL)[*] and HbA1c (%)[†] were determined for all participants. Waist and hip circumferences were measured, and waist-hip ratio was calculated once at the beginning of the study.

2.4 | Saliva and serum sampling

Saliva samples were obtained in the morning following overnight fast. Patients were asked to rinse with distilled water. Unstimulated saliva was collected using standard techniques.[32] Approximately, 2 mL of whole saliva was collected in disposable polypropylene tubes and immediately centrifuged (10,000 × g × 10 minutes) to remove cell debris. Blood samples were collected by venipuncture (antecubital vein) in vacutainer tubes without separator, allowed to stand for 30 minutes (room temperature) and then serum was prepared by centrifugation (4000 × g for 10 minutes). Saliva and serum samples were stored at –80°C until analysis.

2.5 | Determination of biochemical parameters

All parameters were determined in both serum and saliva samples by commercially available ELISA kits. Oxidative stress markers (8-OHdG, MDA, and 4-HNE) and AGE were determined using ELISA kits[‡]. RAGE[§] and hsCRP[**] concentrations were determined by ELISA. Assays were performed according to manufacturer's instructions. Color change was measured with a microplate reader[††] at 450 nm. Concentrations were determined based on the respective assay standard curve. All samples were run in duplicate and values were averaged.

2.6 | Statistical analyses

Sample size was determined a priori, using PPD level differences between the groups. For sample size analysis specific software[‡‡] was used. The large effect size was chosen for ANOVA test (0.4). The α-error was selected as 0.05, the power value was 90%. The total sample size was found as 96. However, considering the possibility of confounders and incomplete data, the study was designed to have >120 patients.

All analyses were performed using commercial statistical software.[§§] Data normality was tested by Kolmogorov-Smirnov prior to further analysis. Data showed normal distribution. Descriptive statistics are presented as mean ± SD. Intergroup comparisons of biochemical and clinical parameters were assessed using the ANOVA test with a Bonferroni correction. Correlations between 8-OHdG, MDA, 4-HNE, AGE, RAGE, hsCRP and periodontal clinical parameters were performed using Pearson's correlation and multiple logistic regression analysis. Receiver operating characteristics (ROC) and corresponding area under the curve (AUC) analyses for salivary biomarkers were performed for differentiation of periodontitis from healthy patients. A value of $P < 0.05$ was considered to be significant.

3 | RESULTS

3.1 | Study population

Thirty two periodontally healthy DM patients (DM-H group; mean ± SD age = 51 ± 6.8 years; 27 females), 35 DM patients with periodontitis (DM-P group; aged 51.3 ± 5.4 years; 26 females), 28 periodontally and systemically

[*] hexokinase method DXC, Beckman Coulter
[†] HPLC kit, Immuchrome GmbH, Germany

[‡] Oxiselect™ ELISA kits, Cell Biolabs, Inc. San Diego, CA
[§] Human RAGE ELISA, Ray Biotech, Inc., Norcross, GA.
[**] hsCRP Human ELISA, RAP002; Biovendor Laboratorni Medicina a.s., Česká Republika
[††] SynergyTM HT Microplate Reader, Bio-Tek Instruments, Winooski, WT.
[‡‡] 3.1.9.2. G*Power; https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower.html
[§§] SPSS for Windows, Version 14.0, SPSS Inc., Chicago, IL.

healthy individuals (H-H group; aged 44.8 ± 11.5 years; 18 females), and 26 systemically health individuals with periodontitis (H-P group; aged 46.1 ± 5.3 years; 18 females) were recruited and completed all study procedures.

The results of the demographic, anthropometric and systemic health assessments for all groups are presented in Table 1. Among DM patients, 56 were using oral antidiabetic drugs and 23 were on insulin. Body-mass index (BMI), fasting glucose and HbA1c levels were significantly higher in DM groups compared to systemically healthy groups ($P \leq 0.03$), regardless of periodontal health status. There were no significant differences between groups regarding waist-hip ratio or smoking status ($P > 0.05$). Within each systemic health group, there were no significant differences between periodontally healthy and periodontitis groups, for any of these parameters.

3.2 | Periodontal clinical parameters

As presented in Table 2, PI, GI, PPD, BOP, and CAL were significantly higher in H-P and DM-P groups compared to H-H and DM-H groups ($P < 0.001$).

3.3 | Biochemical parameters

Salivary 8-OHdG levels were significantly higher in DM-P and H-P groups compared to DM-H and H-H groups ($P < 0.001$). In contrast, serum 8-OHdG levels were significantly higher in H-H and H-P groups compared to DM-P and DM-H groups ($P < 0.001$) (see Figure S1 in online *Journal of Periodontology*).

Salivary MDA levels were significantly higher in DM-P, DM-H, and H-P groups than H-H group ($P = 0.002$, $P = 0.002$, $P = 0.004$, respectively) (see Figure S1 in online *Journal of Periodontology*). There were no differences in serum MDA levels among groups (see Figure S1 in online *Journal of Periodontology*).

Salivary 4-HNE levels were significantly higher in DM-P group than the DM-H, H-P and H-H groups ($P = 0.002$, $P = 0.049$, $P = 0.017$, respectively). Diabetic patients had the lowest serum 4-HNE levels ($P < 0.05$) (see Figure S1 in online *Journal of Periodontology*), regardless of periodontal health status.

Salivary AGE levels were significantly higher in DM-H group than DM-P and H-P groups ($P < 0.001$, $P = 0.018$, respectively). Serum AGE levels were significantly higher in diabetic (DM-P and DM-H) than systemically healthy groups ($P < 0.001$), and in H-P group compared to H-H group ($P < 0.001$) (see Figure S2 in online *Journal of Periodontology*).

TABLE 1 Demographic, characteristic and anthropometric parameters

	Diabetes mellitus (DM)		Systemically healthy (H)		DM-H/H-H	DM-H/H-P	DM-P/H-H	DM-P/H-P
	Healthy (H; n = 32)	Periodontitis (P; n = 35)	Healthy (H; n = 28)	Periodontitis (P; n = 26)	*P*-value	*P*-value	*P*-value	*P*-value
Sex (female/male)	(27/5)	(26/9)	(18/10)	(18/8)	0.001	0.001	0.001	0.001
Age (y)	51 ± 6.9	51.3 ± 5.4	44.8 ± 6.5	46.1 ± 5.3	0.012	0.001	0.026	0.001
Smoking status (−/+)	(28/4)	(29/6)	(23/5)	(20/6)	0.074	0.137	0.07	0.073
Body mass index*	30.8 ± 5.2	31.6 ± 5.3	26.1 ± 3.3	26.7 ± 3.7	0.001	0.007	<0.001	<0.001
Waist-hip ratio	0.89 ± 0.07	0.88 ± 0.10	0.86 ± 0.08	0.88 ± 0.07	1	1	1	1
Fasting glucose (mg/dL)*	134.0 ± 49.4	145.0 ± 52.4	94.9 ± 6.7	97.2 ± 11.7	0.001	0.003	<0.001	<0.001
HbA1c (%)*	7.3 ± 1.9	7.7 ± 1.9	4.7 ± 0.2	4.8 ± 0.3	<0.001	<0.001	<0.001	<0.001
Duration of disease (y)	8 ± 6	6 ± 5	–	–	–	–	–	–
Metformin (−/+)	4/28	7/28	–	–	–	–	–	–
Insulin (−/+)	23/9	21/14	–	–	–	–	–	–

HbA1c, hemoglobin A1c. ANOVA test with a Bonferroni correction.
*Differences between DM-H and DM-P or between H-H and H-P groups were not significant ($P > 0.05$).

TABLE 2 Periodontal clinical parameters

Clinical Parameters	Diabetes mellitus (DM)		Systemically healthy (H)		DM-H/H-H	DM-P/H-P
	Healthy (H; n = 32)	Periodontitis (P; n = 35)	Healthy (H; n = 28)	Periodontitis (P; n = 26)	*P*-value	*P*-value
PI	1.50 ± 0.46	2.23 ± 0.57*	0.70 ± 0.47	1.89 ± 0.39*	<0.001	0.041
GI	1.01 ± 0.24	1.41 ± 0.31*	0.86 ± 0.29	1.97 ± 1.65*	1	0.046
PPD (mm)	1.74 ± 0.28	3.56 ± 0.79*	1.50 ± 0.27	3.93 ± 0.61*	0.553	0.061
BOP (%)	15 ± 9	61 ± 21*	9 ± 5	63 ± 10*	0.589	1
CAL (mm)	1.91 ± 0.42	4.47 ± 1.44*	1.55 ± 0.27	4.27 ± 0.60*	0.664	1

Independent samples *t* test.
BOP, bleeding on probing; CAL, clinical attachment level; GI, gingival index; PI, plaque index; PPD, probing pocket depth.
*$P < 0.001$ from corresponding periodontally healthy group.

Salivary RAGE levels were significantly higher in DM-H and H-H groups than DM-P group ($P < 0.001$). DM-P, DM-H, and H-P groups had significantly lower serum RAGE levels c

ompared to H-H group ($P < 0.05$) (see Figure S2 in online *Journal of Periodontology*).

Levels of salivary hsCRP were significantly higher in both periodontitis groups compared to the H-H group (respectively $P = 0.026$, $P = 0.045$). Levels of serum hsCRP were significantly higher in the DM-P group compared to the H-H group ($P = 0.023$) (see Figure S2 in online *Journal of Periodontology*).

3.4 | Correlations between biochemical and clinical periodontal parameters

These analyses, reported in Table S1, were based on data from all 121 patients. The strongest correlations among biochemical parameters were found between serum AGE and three other serum markers: 4-HNE, 8-OHdG and HbA1c (r = −0.742, r = −0.572, and r = 0.558, respectively; all $P < 0.001$).

Among clinical periodontal parameters, BOP and CAL had the strongest correlations with biochemical parameters. BOP strongly correlated with salivary 8-OHdG (r = 0.481) and serum hsCRP (r = 0.471), both $P < 0.001$. CAL also strongly correlated with salivary 8-OHdG (r = 0.490) and serum hsCRP (r = 0.480), both $P < 0.001$.

The results of multiple regression analysis (Nagelkerker $R^2 = 0.772$; $P < 0.001$), presented in Table S2, indicate that all salivary biochemical markers and diabetes were statistically significantly independent determinants of periodontitis.

ROC analyses for salivary biomarkers are shown in Figure 2 including cut-off points, sensitivity, specificity, and corresponding area under the curve (AUC). In DM patients, salivary 4-HNE and RAGE were the most accurate biomarkers in differentiating between periodontitis and control patients (4-HNE: sensitivity = 0.829, specificity = 0.812, cutoff value 0.064 μg/mL; RAGE: sensitivity = 0.800, specificity = 0.844; cutoff value 5.61 pg/mL; AUC = 0.85 for both). In non-DM patients, salivary 8-OHdG was the most accurate marker (sensitivity = 0.808, specificity = 0.821; cutoff value 2.37 ng/m; AUC = 0.84) to differentiate periodontitis from controls.

Furthermore, combinations of biomarkers may improve the predictive value for periodontitis compared to single biomarkers. Salivary 8-OHdG, 4-HNE, AGE, and RAGE in diabetic subjects and salivary 8-OHdG, MDA and hsCRP in healthy subjects were selected for combinations. The combination of 8-OHdG/4-HNE/

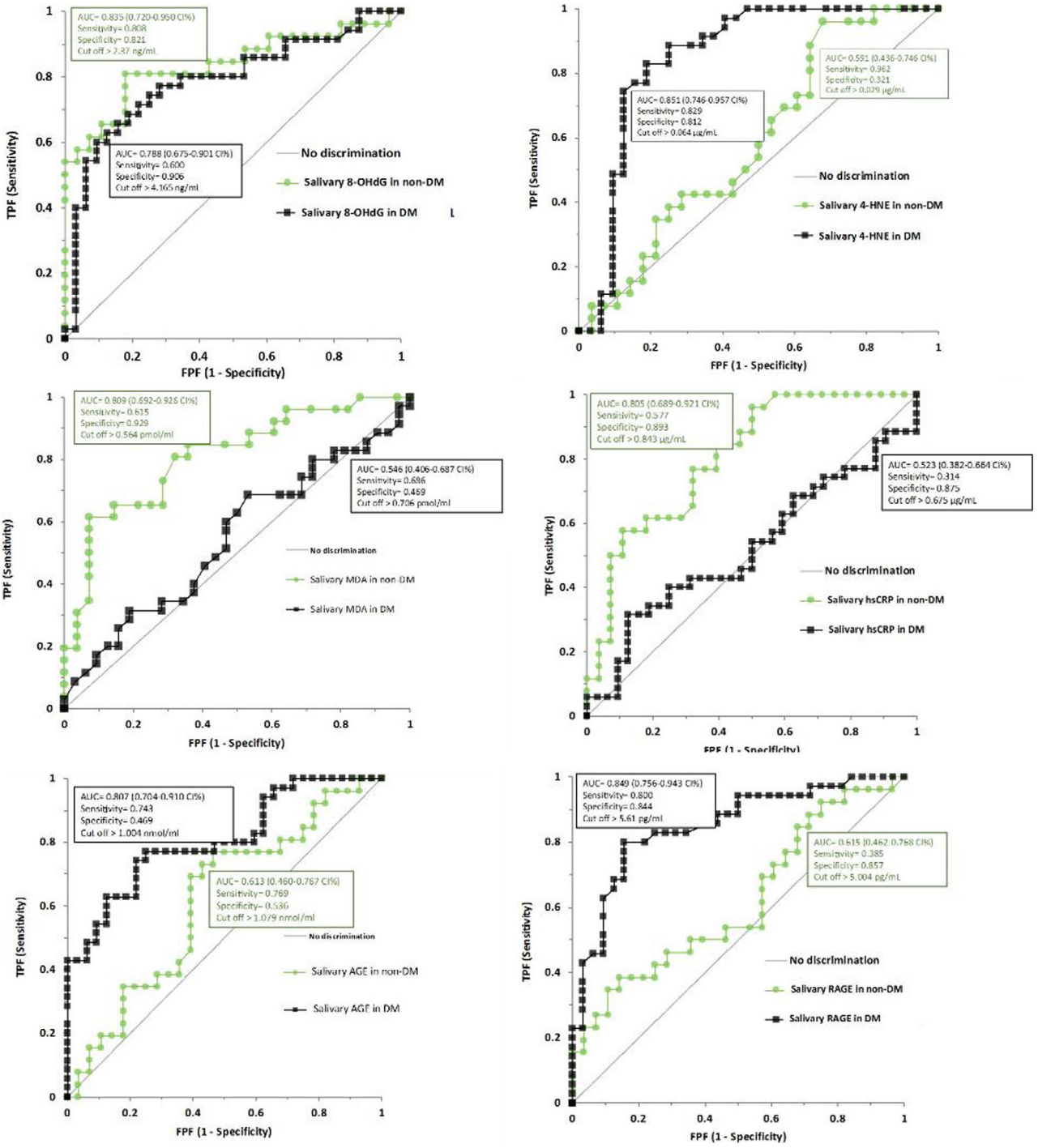

FIGURE 2 Receiver-operating characteristic (ROC) curves of salivary biochemical markers tested for periodontitis screening. Comparison of ROC curve analyses. 4-HNE, 4-hydroxy-2-nonenal; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; AGE, advanced glycation end products; AUC, area under the curve; DM, diabetes mellitus; hsCRP, high sensitivity C-reactive protein; MDA, malondialdehyde; RAGE, receptor for AGE

AGE/RAGE for diabetes patients yielded highest AUCs, ranging from 0.92 to 1.00, and the combinations of 8-OHdG/MDA/hsCRP, 8-OHdG/4-HNE/MDA, and 8-OHdG/4-HNE/MDA/hsCRP for non-diabetes patients yielded highest AUCs, ranging from 0.79 to 0.96 (Table 3, Figure 3).

4 | DISCUSSION

The purpose of the present study was to evaluate salivary and serum levels of oxidative stress (8-OHdG, MDA, 4-HNE), glycation (AGE, RAGE) and inflammation (hsCRP) markers in periodontitis patients with and without DM

TABLE 3 Screening efficacy of individual and combined oxidative stress markers in periodontitis

Diabetes mellitus (+)							Diabetes mellitus (–)						
	Cut off	AUC	AUC (95%)	TP proportion (sensitivity)	TN proportion (specificity)	Odds ratio		Cut off	AUC	AUC (95%)	TP proportion (sensitivity)	TN proportion (specificity)	Odds ratio
Salivary 8-OHdG	**4.17**	**0.79**	**0.67 to 0.88**	0.60	0.91	14.50	**Salivary 8-OHdG**	**2.4**	**0.84**	**0.71 to 0.93**	**0.81**	**0.82**	**19.32**
Salivary MDA	0.71	0.55	0.42 to 0.67	0.69	0.47	1.93	**Salivary MDA**	**0.6**	**0.81**	**0.68 to 0.90**	**0.62**	**0.93**	**20.80**
Salivary 4-HNE	**0.06**	**0.85**	**0.74 to 0.93**	0.83	0.81	20.94	Salivary 4-HNE	0.0	0.59	0.45 to 0.72	0.96	0.32	11.84
Salivary AGE	**1.00**	**0.81**	**0.69 to 0.89**	0.74	0.78	10.32	Salivary AGE	1.1	0.61	0.47 to 0.74	0.77	0.54	3.85
Salivary RAGE	**5.61**	**0.85**	**0.74 to 0.93**	0.80	0.84	21.60	Salivary RAGE	5.0	0.62	0.47 to 0.75	0.38	0.86	3.75
Salivary hs-CRP	0.68	0.52	0.40 to 0.65	0.31	0.88	3.21	**Salivary hs-CRP**	**0.8**	**0.81**	**0.67 to 0.90**	**0.58**	**0.89**	**11.36**
8-OHdG/RAGE		0.89	0.79 to 0.96	0.91	0.78	38.10	8-OHdG/MDA		0.90	0.79 to 0.96	0.92	0.79	44.00
8-OHdG/AGE		0.88	0.78 to 0.95	0.94	0.75	49.50	8-OHdG/4-HNE		0.84	0.71 to 0.92	0.81	0.82	19.32
8-OHdG/4-HNE		0.85	0.74 to 0.93	0.83	0.84	26.10	8-OHdG/CRP		0.84	0.71 to 0.92	0.81	0.82	19.32
AGE/RAGE		0.88	0.78 to 0.95	0.89	0.75	23.25	CRP/MDA		0.81	0.68 to 0.90	0.62	0.93	20.80
4-HNE/RAGE		0.89	0.79 to 0.96	0.91	0.78	38.10	4-HNE/MDA		0.81	0.68 to 0.90	0.62	0.93	20.80
4-HNE/AGE		0.96	0.89 to 0.99	0.91	0.97	330.67	4-HNE/CRP		0.81	0.67 to 0.90	0.58	0.89	11.36
8-OHdG/4-HNE/AGE		0.97	0.90 to 1.00	0.97	0.94	510.00	8-OHdG/4-HNE/hsCRP		0.84	0.71 to 0.92	0.81	0.82	19.32
8-OHdG/4-HNE/RAGE		0.92	0.82 to 0.97	0.91	0.81	46.22	8-OHdG/4-HNE/MDA		0.90	0.79 to 0.96	0.92	0.79	44.00
8-OHdG/AGE/RAGE		0.91	0.82 to 0.97	0.83	0.91	46.72	8-OHdG/hsCRP/MDA		0.90	0.79 to 0.96	0.92	0.79	44.00
4-HNE/AGE/RAGE		0.97	0.90 to 1.00	1.00	0.94	400.00	4-HNE/hsCRP/MDA		0.81	0.68 to 0.90	0.62	0.93	20.80
8-OHdG/4-HNE/AGE/RAGE		0.98	0.92 to 1.00	1.00	0.94	800.00	8-OHdG/4-HNE/MDA/hsCRP		0.90	0.79 to 0.96	0.92	0.79	44.00

AUC, area under the curve; TP, true positives; TN, true negatives; Receiver-operating characteristic curves analysis for salivary biomarkers.

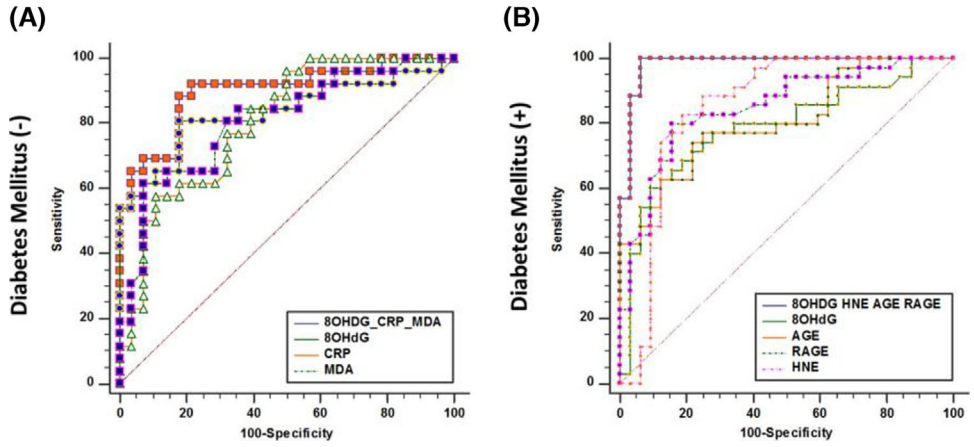

FIGURE 3 Receiver-operating characteristic (ROC) curves analysis of significant salivary markers and their combinations (**A**) for the non-diabetes mellitus group, and (**B**) for the diabetes mellitus group. 8OHdG, 8-hydroxy-2′-deoxyguanosine; AGE, advanced glycation end products; CRP, C-reactive protein; HNE, 4-hydroxy-2-nonenal; MDA, malondialdehyde; RAGE, receptor for AGE

and corresponding periodontally healthy controls, as well as the possible associations between periodontal clinical parameters and biomarker levels. Salivary 8-OHdG was the only biomarker that was significantly elevated in periodontitis, compared to periodontal health, regardless of systemic health status (DM or systemically healthy). The results also show that BOP and CAL, respective clinical markers of periodontal inflammation and tissue destruction, were strongly and positively correlated with both salivary 8-OHdG and serum hsCRP. In addition, ROC analysis indicated that salivary 4-HNE and RAGE in DM patients and salivary 8-OHdG in systemically healthy patients had the greatest accuracy in differentiating between periodontitis and periodontal health. These novel results offer new prospects for the non-invasive screening of periodontitis in both diabetics, a population with high prevalence of the disease, and systemically healthy patients.

8-OHdG is one of >20 oxidative bases and it was first reported by Kasai and Nishimura.[33] It is a sensitive parameter for DNA damage and the most studied of base damage products.[34] In the present study, salivary 8-OHdG levels were higher in periodontitis patients than periodontally healthy controls, regardless of whether these groups were systemically healthy or diabetics, further supporting the concept of increased oxidative stress in periodontitis.[12,35–37] These results, the first to be reported in DM patients, are consistent with salivary 8-OHdG levels in systemically healthy periodontitis patients, as recently analyzed in a systematic review.[15] The association of CAL with salivary 8-OHdG confirms the results of other relevant studies,[35,36] In terms of the ability of salivary 8-OHdG to differentiate between periodontitis and periodontally healthy controls in systemically healthy patients, the performance of this oxidative stress biomarker appears to be better than other individual host salivary markers, such as matrix metalloproteinases[38,39] and pro-inflammatory cytokines.[39] Prospective studies are needed to validate the usefulness of salivary 8-OHdG as a diagnostic screening tool for periodontitis, especially in at risk populations such as diabetics and smokers. Serum 8-OHdG levels were found to be significantly lower in the DM-P group compared to the H-H and H-P groups in our study (see Figure S1 in online *Journal of Periodontology*). It is an expected result that individuals with diabetes have low serum 8-OHdG levels due to the use of antidiabetic drugs.

MDA is an another important marker of tissue injury associated with oxidative stress.[40] In this study, salivary MDA levels were significantly higher in H-P group compared to H-H group, similar to salivary 8-OHdG levels (see Figure S1 in online *Journal of Periodontology*). Also salivary MDA level positively correlates with CAL (see Table S1 in online *Journal of Periodontology*). These results are consistent with previous studies.[41,42] According to the ROC analysis, salivary MDA has a good specifity for periodontitis in systemically healthy individuals (Figure 3). In this context, salivary MDA and 8-OHdG might be related to the local effects of periodontal disease.

4-HNE is considered a biomarker of oxidative stress, is identified as one of the most formidable reactive aldehydes, and is one of the major toxic products generated from lipid peroxides of omega -6-polyunsaturated fatty acids.[43] In this study, salivary 4-HNE levels were positively correlated with BOP and CAL values and significantly higher in DM-P group than in H-P group. According to ROC analysis, salivary 4-HNE has a good sensitivity (83%) and specificity (81%) for discriminating periodontitis in diabetes patients (see Figure 3).

Serum and salivary AGE levels are considered suitable markers for assessing diabetic complications in uncontrolled diabetic patients, and meaningless in

well-controlled diabetic patients.[44] In the present study serum AGE levels were significantly higher in DM groups than in systemically healthy groups, as anticipated (see Figure S2 in online *Journal of Periodontology*). The present study is the first to analyze serum AGE levels in systemically healthy periodontitis patients and controls. The novel finding of significantly elevated serum AGE levels in the H-P group, compared to the H-H group, further strengthens the association between systemic AGE and periodontitis.

Consistent with previous studies, the circulating sRAGE levels were significantly lower in diabetic patients compared to systemically healthy controls, a finding attributed to the excessive binding of sRAGE to circulating AGE ligands.[45,46] Wu et al. found that the plasma levels of sRAGE were significantly lower in the DM group compared to non-DM group.[46] Similar to the present study, Singhal et al. reported that the highest levels of serum sRAGE were seen in systemically and periodontally healthy subjects and the lowest in DM periodontitis patients.[47] AGE-RAGE binding occurs less in healthy individuals.[45] Therefore, higher plasma levels of sRAGE is an expected result. The present study is the first to analyze salivary RAGE levels in periodontitis patients. ROC analysis in the present study indicated that, in DM patients, salivary RAGE levels had great accuracy in differentiating between periodontitis and periodontally healthy patients.

Salivary hsCRP levels in H-P and DM-P groups were significantly higher compared to the H-H group, without significant differences between H-P and DM-P groups (see Figure S2 in online *Journal of Periodontology*). These findings are consistent with previous studies.[48–49] These results suggest that the impact of periodontitis, due to its inflammatory nature, on salivary hsCRP is far greater than the impact of the metabolic disease. The significantly positive correlations between serum hsCRP levels and BOP or CAL levels are consistent with previous results in the literature.[23,50]

When combinations of salivary 8-OHdG/4-HNE/AGE/RAGE levels in DM groups and salivary 8-OHdG/MDA/hsCRP levels in systemically healthy groups were analyzed, the respective combinations yielded higher AUCs than single biomarkers for discriminating periodontitis from periodontal health (Table 3). This suggests that a potential screening test might be more reliable if it includes more than one biomarker.

The cross-sectional nature of the present study is its main limitation. Due to the study design, randomization could not be performed in patients' selection, which could lead to patient selection bias. Other limitations of the study include a relatively small sample size and no data on potential confounders, such microbiome.

5 | CONCLUSION

Salivary 8-OHdG and MDA levels were significantly higher in periodontitis patients regardless of systemic status. In conclusion, the present study results suggest that salivary 8-OHdG alone or in combination with 4-HNE, AGE and RAGE for diabetics, and salivary 8-OHdG alone or in combination with MDA and hsCRP for systemically healthy persons, could potentially serve as non-invasive screening marker(s) of periodontitis, which might be of great use when there is need to screen at risk patients. Further randomized controlled, interventional, and longitudinal studies involving larger sample sizes are needed to ascertain the present study findings and to establish the utility of salivary oxidative stress markers as a non-invasive diagnostic screening tool for periodontitis.

ACKNOWLEDGMENTS

The study was supported by the Ankara University Department of Periodontology and Department of Endocrinology, and by the Ohio State University Division of Periodontology. The authors report no conflicts of interest related to this study.

AUTHOR CONTRIBUTIONS

SMA contributed to study design, worked at the diabetes clinic, collected the samples, recorded clinical data, and wrote the manuscript with input from other authors. SK contributed to study design, helped to collect samples, and recorded clinical data, helped interpret the results and wrote the manuscript with input from other authors. CÖ analyzed the clinical data and helped interpret the results. MS contributed to study design, performed statistical analysis, and helped interpret the results. MS and MU contributed to biochemical analysis. MG contributed to study design, directed the implementation of the research, helped interpret the results and was the study coordinator. DNT contributed to study design, directed the implementation of the research, and helped with interpretation of results and manuscript revision. NB and UÜ helped with diagnosis and recruitment of diabetic patients to the study. All authors reviewed and approved the submitted final manuscript.

ORCID

Sema Merve Altıngöz https://orcid.org/0000-0002-9709-6226
Şivge Kurgan https://orcid.org/0000-0002-7868-4217
Canan Önder https://orcid.org/0000-0002-8995-6361
Muhittin A. Serdar https://orcid.org/0000-0002-3014-748X
Uğur Ünlütürk https://orcid.org/0000-0002-5054-1396

Metin Uyanık https://orcid.org/0000-0001-7083-0454
Nilgün Başkal https://orcid.org/0000-0001-9157-4816
Dimitris N. Tatakis https://orcid.org/0000-0001-6327-3610
Meral Günhan https://orcid.org/0000-0002-3848-6195

REFERENCES

1. Gurav A, Jadhav V. Periodontitis and risk of diabetes mellitus. *J Diabetes*. 2011;3:21-22.
2. Loe H. Periodontal disease. The sixth complication of diabetes mellitus. *Diabetes Care*. 1993;16:329-334.
3. Chapple ILC, Milward MR, Dietrich T. The prevalence of inflammatory periodontitis is negatively associated with serum antioxidant concentrations. *J Nutr*. 2007;137:657-664.
4. Soory M. Oxidative stress induced mechanisms in the progression of periodontal diseases and cancer: a common approach to redox homeostasis? *Cancer*. 2010;2:670-692.
5. Pendyala G, Thomas B, Kumari S. The challenge of antioxidants to free radicals in periodontitis. *J Indian Soc Periodontol*. 2008;12:79-83.
6. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev*. 2014:360438.
7. Gharbi A, Hamila A, Bouguezzi A, et al. Biochemical parameters and oxidative stress markers in Tunisian patients with periodontal disease. *BMC Oral Health*. 2019;22(1):225.
8. Kc S, Wang XZ, Gallagher JE. Diagnostic sensitivity and specificity of host-derived salivary biomarkers in periodontal disease amongst adults: systematic review. *J Clin Periodontol*. 2020;47(3):289-308.
9. Arana C, Cutando A, Ferrera MJ, et al. Parameters of oxidative stress in saliva from diabetic and parenteral drug addict patients. *J Oral Pathol Med*. 2006;35:554-559.
10. Di Minno A, Turnu L, Porro B, et al. 8-Hydroxy-2-deoxyguanosine levels and cardiovascular disease: a systematic review and meta-analysis of the literature. *Antioxid Redox Signal*. 2016;24:548-555.
11. Rall LC, Roubenoff R, Meydani SN, et al. Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) as a marker of oxidative stress in rheumatoid arthritis and aging: effect of progressive resistance training. *J Nutr Biochem*. 2000;11:581-584.
12. Paredes-Sánchez E, Montiel-Company JM, Iranzo-Cortés JE, et al. Meta-analysis of the use of 8-OHdG in saliva as a marker of periodontal disease. *Dis Markers*. 2018;2018:7916578.
13. Singh VP, Bali A, Singh N, et al. Advanced glycation end products and diabetic complications. *Korean J Physiol Pharmacol*. 2014;18(1):1-14.
14. Rahbar S. The discovery of glycated hemoglobin: a major event in the study of nonenzymatic chemistry in biological systems. *Ann N Y Acad Sci*. 2005;1043:9-19.
15. Amir J, Waite M, Tobler J, et al. The role of hyperglycemia in mechanisms of exacerbated inflammatory responses within the oral cavity. *Cell Immunol*. 2011;272:45-52.
16. Lalla E, Lamster IB, Schmidt AM. Enhanced interaction of advanced glycation end products with their cellular receptor RAGE: implications for the pathogenesis of accelerated periodontal disease in diabetes. *Review Ann Periodontol*. 1998;3:13-19.
17. Taylor JJ, Preshaw PM, Lalla E. A review of the evidence for pathogenic mechanisms that may link periodontitis and diabetes. *J Clin Periodontol*. 2013;40(suppl 14):S113-134.
18. Anderson MM, Requena JR, Crowley JR, et al. The myeloperoxidase system of human phagocytes generates Nepsilon-(carboxymethyl)lysine on proteins: a mechanism for producing advanced glycation end products at sites of inflammation. *J Clin Invest*. 1999;104:103-113.
19. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res*. 2010;107:1058-1070.
20. Saito M, Ishimitsu T, Minami J, et al. Relations of plasma high-sensitivity C-reactive protein to traditional cardiovascular risk factors. *Atherosclerosis*. 2003;167:73-79.
21. Tüter G, Kurtis B, Serdar M. Evaluation of gingival crevicular fluid and serum levels of high-sensitivity C-reactive protein in chronic periodontitis patients with or without coronary artery disease. *J Periodontol*. 2007;78:2319-2324.
22. D'Aiuto F, Parkar M, Andreou G, et al. Periodontitis and atherogenesis: causal association or simple coincidence? *J Clin Periodontol*. 2004;31:402-411.
23. Bokhari SA, Khan AA, Butt AK, et al. Periodontitis in coronary heart disease patients: strong association between bleeding on probing and systemic biomarkers. *J Clin Periodontol*. 2014;41:1048-1054.
24. Bokhari SA, Khan AA, Butt AK, et al. Non-surgical periodontal therapy reduces coronary heart disease risk markers: a randomized controlled trial. *J Clin Periodontol*. 2012;39:1065-1074.
25. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *J Clin Periodontol*. 2008;35:277-290.
26. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol*. 1999;4:1-6.
27. Koromantzos PA, Makrilakis K, Dereka X, et al. Effect of non-surgical periodontal therapy on C reactive protein, oxidative stress, and matrix metalloproteinase (MMP)-9 and MMP-2 levels in patients with Type 2 diabetes: a randomized controlled study. *J Periodontol*. 2012;83:3-10.
28. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: framework and proposal of a new classification and case definition. Review. *J Periodontol*. 2018(89 Suppl 1):S159-S172. Erratum in: J Periodontol. 2018;89:1475.
29. Buduneli N, Kardesler L, Isik H, et al. Effects of smoking and gingival inflammation on salivary antioxidant capacity. *J Clin Periodontol*. 2006;33:159-164.
30. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand*. 1964;22:121-135.
31. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand*. 1963;21:533-551.
32. Caglayan F, Miloglu O, Altun O, et al. Oxidative stress and myeloperoxidase levels in saliva of patients with reccurrent aphthous stomatitis. *Oral Dis*. 2008;12:700-704.
33. Kasai H, Nishimura S. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Res*. 1984;12:2137-2145.

34. Dizdaroglu M, Jaruga P, Birincioglu M, et al. Free radical damage to DNA: mechanisms and measurement. *Free Radic Biol Med*. 2002;32:1102-1115.
35. Yang X, Li C, Pan Y. The influences of periodontal status and periodontal pathogen quantity on salivary 8-hydroxydeoxyguanosine and interleukin-17 levels. *J Periodontol*. 2016;87:591-600.
36. Villa-Correa YA, Isaza-Guzmán DM, Tobón-Arroyave SI. Prognostic value of 8-hydroxy-2′-deoxyguanosine and human neutrophil elastase/α1-proteinase inhibitor complex as salivary biomarkers of oxidative stress in chronic periodontitis. *J Periodontol*. 2015;86:1260-1267.
37. Önder C, Kurgan Ş, Altıngöz SM, et al. Impact of non-surgical periodontal therapy on saliva and serum levels of markers of oxidative stress. *Clin Oral Investig*. 2017;21:1961-1969.
38. Takane M, Sugano N, Iwasaki H, et al. New biomarker evidence of oxidative DNA damage in whole saliva from clinically healthy and periodontally diseased individuals. *J Periodontol*. 2002;73:551-554.
39. Takane M, Sugano N, Ezawa T, et al. A marker of oxidative stress in saliva: association with periodontally-involved teeth of a hopeless prognosis. *J Oral Sci*. 2005;47:53-57.
40. Gursoy UK, Könönen E, Pradhan-Palikhe P, et al. Salivary MMP-8, TIMP-1, and ICTP as markers of advanced periodontitis. *J Clin Periodontol*. 2010;37:487-493.
41. Baltacioglu E, Yuva P, Aydin G, et al. Lipid peroxidation levels and total oxidant/antioxidant status in serum and saliva from patients with chronic and aggressive periodontitis. Oxidative stress index: a new biomarker for periodontal disease? *J Periodontol*. 2014;85:1432-1441.
42. Wei D, Zhang X-L, Wang Y-Z, et al. Lipid peroxidation levels, total oxidant status and superoxide dismutase in serum, saliva and gingival crevicular fluid in chronic periodontitis patients before and after periodontal therapy. *Australian Dental Journal*. 2010;55:70-78.
43. Akalin FA, Baltacioglu E, Alver A, et al. Lipid peroxidation levels and total oxidant status in serum, saliva and gingival crevicular fluid in patients with chronic periodontitis. *J Clin Periodontol*. 2007;34:558-565.
44. Garay-Sevilla ME, Regalado JC, Malacara JM, et al. Advanced glycosylation end products in skin, serum, saliva and urine and its association with complications of patients with type 2 diabetes mellitus. *J Endocrinol Invest*. 2005;28:223-230.
45. Devangelio E, Santilli F, Formoso G, et al. Soluble RAGE in type 2 diabetes: association with oxidative stress. *Free Radic Biol Med*. 2007;43:511-518.
46. Wu TL, Tsai CC, Wang YY, et al. The association between the RAGE G82S polymorphism, sRAGE and chronic periodontitis in Taiwanese individuals with and without diabetes. *J Periodontal Res*. 2015;50:881-889.
47. Singhal S, Pradeep AR, Kanoriya D, et al. Human soluble receptor for advanced glycation end products and tumor necrosis factor-α as gingival crevicular fluid and serum markers of inflammation in chronic periodontitis and type 2 diabetes. *J Oral Sci*. 2016;58:547-553.
48. Dholey MK, Kole D, Rambabu D, et al. Comparative estimation of salivary and serum C-reactive protein levels in chronic periodontitis with or without Type II diabetes mellitus: a clinico-biochemical study. *SRM J Res Dent Sci*. 2017;8:99-104.
49. Giannobile WV, Beikler T, Kinney JS, et al. Saliva as a diagnostic tool for periodontal disease: current state and future directions. *Periodontol 2000*. 2009;50:52-64.
50. Trivedi S, Lal N, Mahdi AA, et al. Evaluation of antioxidant enzymes activity and malondialdehyde levels in patients with chronic periodontitis and diabetes mellitus. *J Periodontol*. 2014;85:713-720.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Altıngöz SM, Kurgan Ş, Önder C, et al. Salivary and serum oxidative stress biomarkers and advanced glycation end products in periodontitis patients with or without diabetes: A cross-sectional study. *J Periodontol*. 2021;92:1274–1285.
https://doi.org/10.1002/JPER.20-0406