Original Article

Salivary Oxidative Stress Biomarkers in Thai Adolescents and Young Adults with Type 1 Diabetes Mellitus: A Cross-Sectional Study

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ABSTRACT

Aims and Objectives: The primary objectives of this study were to compare salivary oxidative stress (OS) biomarker levels in patients with type 1 diabetes mellitus (T1DM) and without T1DM (non-T1DM) and evaluate the relationships between diabetes, periodontal status, and OS biomarker levels. **Materials and Methods:** Twenty patients with T1DM and 20 age-matched patients without T1DM were enrolled. All participants were 15–23 years of age and had permanent dentition. Unstimulated whole saliva was collected in a sterile test tube before examination of clinical periodontal parameters, including bleeding on probing (BOP). Salivary levels of OS biomarkers—malondialdehyde, protein carbonyl, total oxidant status (TOS), and total antioxidant capacity—were determined using oxidative and antioxidative assays followed by spectrophotometric measurement at 375–532 nm. The relationships between diabetes, periodontal status, and OS biomarkers were analyzed using multiple linear regression. **Results:** TOS was significantly lower in the T1DM group compared with the non-T1DM group (5.06 ± 0.39 vs. 6.44 ± 0.51 µmol H_2O_2 Eq/l, $P = 0.035$). After adjusting for confounding factors (age, gender, BMI, clinical periodontal parameters, BOP, or diabetes status accordingly), the multiple linear regression showed that T1DM was significantly associated with a reduction of TOS level ($P = 0.008$). The BOP > 30% group showed a significant correlation with increased TOS levels compared with the BOP ≤ 30% group ($P = 0.002$). No relationship was found between OS biomarkers and HbA1c levels. **Conclusion:** Salivary TOS levels were related to both diabetes status and the extent of gingival inflammation. Further studies to elucidate the role of OS in relation of periodontal disease and T1DM are required.

Keywords: *Bleeding on probing, Oxidative stress, Saliva, Type 1 diabetes mellitus*

Introduction

Diabetes mellitus is a chronic disease characterized by abnormally high blood glucose levels. The majority of Thai children and adolescents with diabetes had type 1 diabetes mellitus (T1DM).[1] Most patients do not meet the recommended glycemic target and face high risks of complications such as diabetic nephropathy, diabetic retinopathy, and cardiovascular diseases.[2]

Several studies suggested a strong bidirectional relationship between periodontitis and type 2 diabetes mellitus (T2DM).[3-5] Studies showed a higher prevalence

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of periodontal disease in patients with T1DM than in healthy individuals,[6,7] but there was less impact of periodontal treatment on glycemic control in T1DM.[7] However, Peruzzo *et al.*[8] reported that periodontal treatment was effective for glycemic control in patients with T1DM. During the early stage of periodontal disease development, polymorphonuclear neutrophils are activated to release reactive oxygen species (ROS) as part of the first defense mechanism. However, in long-term chronic inflammatory stimulation during the development of periodontitis, ROS produced by the immune cells surpasses the antioxidative defense mechanisms. Excessive ROS induces oxidative stress (OS), leading to irreversible damage to periodontal tissue.[9]

High-glucose concentration leads to increased production of ROS.[10,11] Overproduction of ROS can cause OS resulting in structural and function modifications in proteins, nucleic acids, and lipids.[11] Moreover, the hyperglycemia-induced OS may impair insulin signaling, inducing insulin resistance and promoting the progression and development of diabetes complications.[11]

OS levels are measured by biomarkers as byproducts of the reaction between ROS and biomolecules. Malondialdehyde (MDA) and protein carbonyl (PC) are commonly used as biomarkers in plasma.[12-16] Some studies reported that plasma levels of MDA or PC in the T1DM group were higher than in the control group,[12-14] while others found no differences between the groups.[15,16] Total oxidant status (TOS), developed in 2005,[17] has been used in studies of cancer patients,[18] periodontal disease,[19] and T2DM.[20] In T1DM, Aral *et al.*[21] found that salivary TOS was higher in patients with T1DM at diagnosis compared with a healthy group; however, no significant difference was recorded between patients with T1DM and controls with gingivitis. At a 3-month reevaluation after metabolic control and periodontal treatment, the salivary TOS level in patients with T1DM decreased compared with the baseline, but the difference was not significant. Total antioxidant capacity (TAOC) is one of the most commonly studied biomarkers. A decrease in TAOC plasma levels was found in patients with T1DM,[22,23] while serum and salivary total antioxidant status (TAS) levels were higher in T1DM patients than in the healthy group.[23] By contrast, differences in TAOC between T1DM patients and healthy controls were not found in other studies.[21,24] Another indicator, the OS index (OSI) represents the ratio of total oxidant and antioxidant capacities of a biological sample.[25] OSI in gingival crevicular fluid, saliva, and serum samples of children diagnosed with T1DM were higher than in the healthy controls but decreased after metabolic control of diabetes and sufficient periodontal therapy.[21] The periodontal disease showed conflicting results regarding the relationship between OS biomarkers and clinical periodontal parameters. Some studies found a correlation between OS biomarkers and clinical periodontal parameters,[26,27] while another reported no significant correlation between TOS and TAOC with clinical periodontal parameters, except for a negative correlation between TAOC and clinical attachment level (CAL).[28]

Unique saliva-based biomarker profiles have been correlated to certain diseases, providing critical information about patients' current physiologic stage,[29,30] but studies in saliva are much less frequent than those in blood. Saliva collection is a simple, inexpensive, and noninvasive technique achieving greater patient cooperation, especially in children and adolescents. Information about salivary OS biomarkers and T1DM is scarce and inconclusive. Therefore, the primary objectives of this study were to compare levels of salivary MDA, PC, TOS, and TAOC in patients with T1DM against non-T1DM patients and evaluate the relationships between diabetes status (T1DM and non-T1DM) and OS biomarker levels and between periodontal status and OS biomarker levels. The relationships between OS biomarker levels and HbA1c levels were studied as well. We hypothesized that hyperglycemia led to increased salivary OS biomarkers, and the salivary biomarker levels were related to diabetes and periodontal status. The null hypothesis is that there is no significant difference in salivary OS biomarker levels, HbA1 level, and periodontal status between patients with T1DM and non-T1DM.

Materials and Methods

Study Population

This cross-sectional case–control study involved 40 participants. All participants were 15–23 years of age and had permanent dentition. Twenty patients diagnosed with T1DM for at least 1 year who attended the Division of Pediatric Endocrinology and Metabolism, Department of Pediatrics, Siriraj Hospital during 2018–2019 were recruited using a convenient sampling method, while 20 age-matched non-T1DM individuals were recruited from the Faculty of Dentistry, Mahidol University. They were participants from a previous study.[31] Individuals were excluded from the study if they were smokers, pregnant, had undergone orthodontic treatment, had taken antibiotics within 2 months, or had periodontal treatment during the

previous 6 months. The study protocol was approved by the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University Institutional Review Board (MU-DT/PY-IRB 2018/045.1810).

The sample size in each group was determined using the MDA level following.

At least 15 participants were required in each group to yield a significance level of 0.05 and a power of 0.8.[16]

Data collection

General and medical information

Age, gender, weight, and height data were collected. In the T1DM group, diabetes status, including age at diagnosis, disease duration, treatment regimens, and average HbA1c values within 1 year, were sourced from medical records. In this study, an average HbA1c level of less than 8% was considered well-controlled T1DM, while HbA1c levels of 8% or more were considered poorly controlled T1DM.

Saliva collection and storage

Whole unstimulated saliva samples were collected using the Navazesh technique.[32] One hour before saliva collection, all participants were refrained from eating, drinking, and teeth brushing. Participants were instructed to rinse their mouths with water before 15–20 mL of whole unstimulated saliva was collected in a sterile test tube and placed on ice. All saliva samples were centrifuged (Allegra™ X-22R Centrifuge, Beckman Coulter™) at 10,000 × *g* for 10 min at 4°C to remove debris and cells. The supernatant was stored at -80°C until used.

Clinical periodontal examinations

Clinical periodontal parameters were measured on fully erupted permanent teeth except for third molars by one examiner (R.C.) with >90% intraexaminer reproducibility (within ± 1.0 mm). Probing depth (PD), CAL, and bleeding on probing (BOP) were recorded at six sites per tooth using a periodontal probe UNC-15 (Hu-Friedy, Chicago, IL, USA).[31] PD was measured to the nearest mm as the distance between the gingival margin and the bottom of the sulcus. CAL was measured as the distance between the cementoenamel junction and the bottom of the sulcus. BOP was registered within 10 s after gentle probing as either presence (BOP+) or absence (BOP-).[33] Plaque index (PI)[34] was recorded on six index teeth (maxillary right first molar, maxillary right lateral incisor, maxillary left first bicuspid, mandibular left first molar, mandibular left lateral incisor, and mandibular right first bicuspid). Each of the four surfaces of the index teeth (buccal, lingual, mesial, and distal) was given a score from 0-3 according to the Silness and Loe PI.

Oxidative Stress Biomarker Measurement

Malondialdehyde

Salivary MDA levels were determined by the reaction of MDA with thiobarbituric acid (TBA) using the Ohkawa method.[35] Briefly, a mixture containing 0.1 mL of sample, 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid solution, and 1.5 mL of 0.8% TBA aqueous solution was heated at 95°C for 60 min. After cooling with water, 1.0 mL of distilled water and 5.0 mL of a mixture of *n*-butanol and pyridine (15:1, v/v) were added, followed by vortexing. The absorbance was measured at a wavelength of 532 nm using a spectrophotometer (Epoch™ Microplate Spectrophotometer, BioTek®). All experiments were performed in triplicate, and the averages were reported in µmol/l.

Protein carbonyl

Salivary PC levels were analyzed using a Protein Carbonyl Content Assay kit (Sigma-Aldrich, St. Louis, Missouri). Carbonyl content was determined by the derivatization of PC groups with 2,4-dinitrophenylhydrazine, leading to the formation of stable dinitrophenyl hydrazone adducts. These were detected spectrophotometrically at 375 nm, proportional to the carbonyls present. Protein concentration in each sample was determined using a Bicinchoninic Acid Protein Assay kit (Thermo Scientific Pierce BCA Protein Assay kit, Thermo Scientific™, Thermo Fisher Scientific, Waltham, Massachusetts). Measurements were performed according to the manufacturer's instructions. All experiments were duplicated, and the averages were reported in nmol carbonyl/mg protein.

Total oxidant status

Salivary TOS levels were measured by a TOS kit (Rel Assay Diagnostics, Turkey). Briefly, 300 µL of reagent 1 (buffer solution H_2SO_4 25 mM, pH 1.75) was mixed with 45 µL of sample, and the absorbance of each sample was read spectrophotometrically at 530 nm. Following this, 15 µL of reagent 2 (ferrous ion 5 mM and O-dianisidine 10 nM in H_2SO_4 solution 25 mM) was added to the mixture. After 5 min incubation at 37°C, the absorbance was read again at 530 nm. Differences in absorbance before and after adding reagent 2 were calculated. All experiments were performed in duplicate, and the averages were reported in µmol H_2O_2 Eq/L.

Total antioxidant capacity

Salivary TAOC levels were measured using an antioxidant assay kit (Sigma-Aldrich). Antioxidant capacity was determined by the formation of the ferryl myoglobin radical from metmyoglobin and hydrogen peroxide. This oxidized ABTS

(2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) produced a radical cation that was measured spectrophotometrically at 405 nm. Measurements were performed according to the manufacturer's instructions. All experiments were performed in duplicate, and the averages were reported in mmol Trolox Eq/l.

Oxidative stress index

The ratio between TOS and TAOC was interpreted as the OSI using the following equation[25]:

OSI = (TOS [µmol H_2O_2 Eq/l]/TOAC [µmol Trolox Eq/l]) × 100

Statistical analysis

All analyses were performed using a statistical software program (PASW statistics 18, IBM, New York, New York), with the Shapiro–Wilk test used to verify normal distribution. Statistically significant differences between groups were identified using the independent sample *t* test or the Mann–Whitney *U* test. Pearson correlation coefficients or Spearman rank correlation coefficients were calculated to determine the level of correlation between OS biomarkers and HbA1c levels, with the Chi-square test used to determine the association between two categorical variables. Multiple linear regression analyses were designed to assess the association between OS biomarkers and diabetes status and periodontal status in two models. Model 1: the confounding factors, including age, gender, BMI, PD, CAL, and BOP, were adjusted; model 2: the confounding factors, including age, gender, BMI, PD, CAL, and diabetes status, were adjusted. A *P*-value <0.05 was considered statistically significant.

Results

Demographic and descriptive data

Twenty patients with T1DM and 20 age-matched non-T1DM individuals were included in this study. The average ages were 18.39 and 18.35 years in the T1DM and non-T1DM groups, respectively. The average HbA1c level in the T1DM group was 8.1% (range 6.8%–11.8%), with 4.8% in the non-T1DM group. The average duration of T1DM was 10 years (range 3–19 years).[31]

Average PD, CAL, and BOP were significantly higher in the T1DM group compared with the non-T1DM group, while PI was not significantly different between groups. Details are described in a study by Chairatnathrongporn *et al.*[31]

The participants were classified according to the extent of BOP as presenting with localized (BOP ≤ 30%) and generalized (BOP > 30%) gingival inflammation.[36] A significant association was found between BOP and diabetes status ($P < 0.001$). Eighteen out of 20 participants in the T1DM group had BOP > 30%. The BOP > 30% group showed significantly higher PD and CAL, while scores of PI were not significantly different [Table 1].

Salivary OS biomarkers

Comparison of the OS biomarkers between the T1DM and non-T1DM groups showed that the mean salivary TOS level was significantly lower in the former than in the latter (5.06 ± 1.73 vs. 6.45 ± 2.26, $P = 0.035$). No significant differences in levels of salivary MDA, PC, TAOC, and OSI were observed between the two groups [Table 2].

Table 1: Demographic and descriptive data

Variable	All participants (*n* = 40)	BOP ≤ 30% (*n* = 17)	BOP > 30% (*n* = 23)	*P*-value
Age (years)	18.20 ± 1.90	18.00 ± 1.58	18.35 ± 2.12	0.570
Gender, *n* (%)				
Male	15 (38%)	3 (18%)	12 (52%)	**0.026***
Female	25 (62%)	14 (82%)	11 (48%)	
Diabetes status, *n* (%)				
T1DM	20 (50%)	2 (12%)	18 (78%)	**<0.001***
Non-T1DM	20 (50%)	15 (88%)	5 (22%)	
Periodontal status				
PI	1.00 ± 0.36	0.91 ± 0.38	1.07 ± 0.33	0.145
PD (mm)	2.52 ± 0.22	2.34 ± 0.18	2.65 ± 0.14	**<0.001****
CAL (mm)	1.42 (0.25; 2.20)	1.23 (0.25; 1.91)	1.79 (0.60; 2.20)	**0.003*****

T1DM = type 1 diabetes mellitus, PI = plaque index, PD = probing depth, CAL = clinical attachment level, BOP = bleeding on probing

* Chi-square test showed statistical significance at $P < 0.05$

** Independent *t*-test showed statistical significance at $P < 0.05$

*** Mann–Whitney *U* test showed statistical significance at $P < 0.05$

Table 2: Salivary levels of oxidative stress biomarkers in the T1DM and non-T1DM groups (mean ± SD or median (minimum; maximum))

Variable	T1DM (n = 20)	Non-T1DM (n = 20)	P-value
MDA (µmol/L)	3.11 ± 1.92	3.88 ± 1.88	0.209
PC (nmol carbonyl/mg protein)	1.34 (0.33; 4.28)	1.00 (0.33; 5.39)	0.144
TOS (µmol H_2O_2 Eq/L)	5.06 ± 1.73	6.45 ± 2.26	**0.035***
TAOC (mmol Trolox Eq/L)	0.09 ± 0.07	0.12 ± 0.06	0.171
OSI	7.18 (1.09; 147.85)	5.62 (1.21; 43.53)	0.626

T1DM = type 1 diabetes mellitus, MDA = malondialdehyde, PC = protein carbonyl, TOS = total oxidant status, TAOC = total antioxidant capacity, OSI = oxidative stress index

* Independent t-test showed statistical significance at $P < 0.05$

Table 3: Salivary levels of oxidative stress biomarkers in well-controlled and poorly-controlled T1DM subgroups (mean ± SD or median (minimum; maximum))

Variable	Well-controlled T1DM (n = 10)	Poorly-controlled T1DM (n = 10)	P-value
MDA (µmol/L)	3.19 ± 2.24	3.04 ± 1.67	0.865
PC (nmol carbonyl/mg protein)	1.98 ± 1.32	1.73 ± 1.30	0.674
TOS (µmol H_2O_2 Eq/L)	4.66 ± 1.12	5.46 ± 2.16	0.319
TAOC (mmol Trolox Eq/L)	0.09 (0.00; 0.18)	0.06 (0.03; 0.26)	0.940
OSI	6.74 (2.06; 147.85)	7.18 (1.09; 27.17)	0.762

T1DM = type 1 diabetes mellitus, MDA = malondialdehyde, PC = protein carbonyl, TOS = total oxidant status, TAOC = total antioxidant capacity, OSI = oxidative stress index

Patients with T1DM were divided according to diabetic control into well (HbA1c < 8%) and poorly (HbA1c ≥ 8%) controlled groups, with no differences in levels of salivary OS biomarkers observed between the two subgroups [Table 3].

Simple linear regression analysis showed that T1DM was significantly associated with the reduction of TOS (P = 0.035). After adjusting for confounders (age, gender, BMI, PD, CAL, and BOP) in model 1, the multiple linear regression model demonstrated that the T1DM group was associated with a decrease in TOS (P = 0.008) compared with the non-T1DM group. No association between other OS biomarkers and diabetes status was found. In model 2, the BOP > 30% group significantly correlated with increased TOS level (P = 0.002) compared with the BOP ≤ 30% group [Table 4].

Relationship between salivary OS biomarker levels and HbA1c levels in the T1DM group

Correlation analysis showed no association between salivary OS biomarker levels and HbA1c levels in the T1DM group.

Discussion

This study found that no significant differences in levels of salivary MDA, PC, TAOC, and OSI were observed between the two groups; only the mean salivary TOS level in the T1DM group was significantly lower than the non-T1MD group. The null hypothesis related to the relationship between T1DM and salivary OS biomarker level was accepted. However, the null hypothesis related to the relationship between salivary biomarker levels with diabetes and periodontal status was rejected as the adjusted multiple linear regressions showed that T1DM was significantly associated with the reduction of TOS level and the BOP > 30% group showed a significant correlation with increased TOS level compared with the BOP ≤ 30% group. In an animal study, Heidarisasan *et al.*[37] found that MDA and TOS in kidney tissue of untreated T1DM rats were significantly higher than in the controls, but no significant differences between MDA and TOS were found between treated T1DM rats and the controls. Similarly, in a human study, Grabia *et al.*[38] reported that the T1DM group had higher TOS than healthy controls; however, TOS levels tended to decrease in the long duration of T1MD compared with the early onset of T1DM. Moreover, Aral *et al.*[21] investigated salivary TOS in T1DM patients at diagnosis compared with systemically healthy children with gingivitis (G) and without gingivitis (H), salivary TOS was significantly higher in the T1DM group than in the H group, but no significant difference was found between the T1DM and the G group. Salivary TOS in T1DM reduced after 3 months of insulin administration and periodontal therapy, but the reduction was not statistically significant. These study results suggested that metabolic control reduced the level of TOS, which also explained the lower mean salivary TOS level in the T1DM group

Table 4: Multiple linear regression of TOS (R^2 = 0.384)

Variable	Coefficients	SE	*P*-value
Diabetes status			
T1DM	-2.973	1.044	**0.008***
Non-T1DM	0.00		
Age	-0.024	0.014	0.094
Gender			
Male	0.559	0.680	0.417
Female	0.00		
BMI	0.129	0.105	0.230
PD	-2.152	2.266	0.349
CAL	-0.425	0.754	0.577
BOP			
BOP > 30%	3.088	0.894	**0.002***
BOP ≤ 30%	0.00		

T1DM = type 1 diabetes mellitus, BMI = body mass index, PD = probing depth, CAL = clinical attachment level, BOP = bleeding on probing

* Multiple linear regression showed statistical significance at $P < 0.05$

in our study since participants in the T1DM group had undergone treatment for 3–19 years.

The difference in the levels of salivary TAOC between the two groups was not found in this study. Sixty percent of the total volume of unstimulated whole saliva is produced by submandibular glands.[39] However, parotid glands are the main source of antioxidants in saliva.[40] Perestrelo *et al.*[41] analyzed OS and the activity of antioxidants in the salivary gland of streptozotocin-induced diabetic rats. The study reported an increase in MDA and a decrease in TAS in the parotid glands, while the submandibular glands showed an increase in TAS and no difference in the MDA content. Moreover, Zalewska *et al.*[42] found that, in the late stage of streptozotocin-induced diabetic disease, submandibular glands became the main source of antioxidants as an attempt to compensate for antioxidant dysfunction in the parotid glands. The T1DM participants in this study had a long duration of diabetes, the salivary glands may adapt to the diabetes condition, and submandibular glands produced antioxidants in compensation for antioxidant dysfunction in parotid glands. This may explain no difference in TAOC level between the T1DM and non-T1DM groups.

Our study results indicated that the BOP > 30% group was significantly associated with increased salivary TOS levels, as shown by the adjusted regression model; in addition, the BOP > 30% group had significantly higher mean PD and CAL than the BOP ≤ 30% group. BOP is the most reliable and validated clinical diagnostic tool for assessing gingival inflammation.[43] Roberts *et al.*[44] investigated the impact of inflammation induced by experimental gingivitis on the function of neutrophils. The results showed that the ROS production was significantly elevated at day 21 of plaque accumulation. In late-onset periodontitis patients, neutrophils have produced more ROS causing tissue damage.[45] Further, gingival inflammation is associated with progression to periodontitis.[46] Therefore, the 30% limited BOP sites could be used as the treatment goal for controlling gingival conditions to maintain the oxidant-antioxidant balance at a normal physiologic level regardless of diabetes status.

According to our results, there was no relationship between OS biomarkers and HbA1c levels in patients with T1DM, concurring with previous studies.[47,48] The HbA1c level is the average blood glucose level over the previous 2–3 months but does not reflect intermittently increased glucose levels daily. However, glycemic swings have been shown to produce more OS than constantly elevated levels.[49] This may explain the lack of association between OS biomarkers and HbA1c levels in our study. On the other hand, some studies showed conflicting results. Cheprasova *et al.*[50] found a strong positive correlation between glucose concentration and the levels of OS markers and a negative correlation between the activity of antioxidant enzymes and glucose concentration, while Valente *et al.*[51] reported that the correlations between glycemic variability and OS markers were heterogeneous. The difference in patients' characteristics or medications might have caused inconsistent results among these studies.

Chairatnathrongporn *et al.*[31] found that this T1DM group had significantly higher mean CAL, PD, and BOP than the non-T1DM group. Further analysis performed in the present study found that BOP was associated with diabetes status, and the BOP > 30% group had higher CAL and PD than the BOP ≤ 30%

group [Table 1]; besides, the BOP > 30% group was associated with increased TOS levels [Table 4]. In other words, patients with T1DM were associated with more gingival inflammation (BOP), periodontal damage (higher CAL and PD), and increased TOS levels. However, the present study was a cross-sectional case–control study with a limited number of participants; the casual relationship of OS biomarkers, BOP and T1DM cannot be concluded. Whether the OS biomarkers play roles in the link between periodontal condition and T1DM warrants further investigation. We found more periodontal damage in patients with T1DM with gingival inflammation, in line with the reports of a higher prevalence of periodontal disease in patients with T1DM than in healthy patients.[6,52] We suggest that periodic oral prophylaxis should be implemented into the treatment of patients with T1DM to control periodontal conditions. Further studies to elucidate the pathogenesis and relationship of these two diseases would help to offer proper management in terms of prevention and early diagnosis of complications. Prospective interventional studies of OS biomarkers in saliva and plasma samples should be conducted with a larger sample size of T1DM patients and various degrees of periodontal inflammation to better understand the relationship between T1DM (systemic) and periodontal disease (local) oxidative damage. Moreover, our results showed no differences in salivary TAOC, while previous study found increased OS biomarkers accompanying decreased antioxidant biomarkers.[26] Individual antioxidants and calculated salivary flow rates should be considered in future studies.

Conclusions

Within the study limits, no significant differences in levels of salivary MDA, PC, and TAOC were found between T1DM patients and the healthy controls, except for lower TOS in patients with T1DM. No correlation was found between salivary OS biomarker levels and HbA1c levels. Though the T1DM group was associated with a decrease in TOS compared with non-T1DM group, patients with T1DM were associated with high gingival inflammation (>30%), higher PDs, CALs, and TOS levels. Salivary TOS levels were related to both diabetes status and the extent of gingival inflammation. Further studies to elucidate the role of OS in relation to periodontal disease and T1DM are required.

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Conflicts of interest

There are no conflicts of interest.

Authors contributions

Thanwarat Aroonrangsee: Conceptualization, Methodology, Formal analysis, Data interpretation, Writing-Original Draft. Rachanin Chairatnathrongporn: Formal analysis, Data collection. Rudee Surarit: Conceptualization, Methodology. Kallapat Tansriratanawong: Data collection. Jeerunda Santiprabhob: Data collection. Chatkoew Boriboonhirunsarn: Data collection. Ananya Promsudthi: Conceptualization, Methodology, Data collection, Writing-Original Draft. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Ethical policy and institutional review board statement

The study protocol was approved by the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University Institutional Review Board (MU-DT/PY-IRB 2018/045.1810).

Patient declaration of consent

Informed consent was obtained from all individuals who participated in this study.

Data availability statement

The dataset used in this study is available on request from Dr.Thanwarat Aroonrangsee (E-mail: thanwarat_a@hotmail.com).

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