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Salivary Oxidative Stress and Dental Caries in Adolescents With Type 1 Diabetes Mellitus: A Cross-Sectional Study

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Abstract

Introduction: Adolescents with type 1 diabetes mellitus (T1DM) may experience alterations in salivary function and oral health, potentially accompanied by increased oxidative stress. This study evaluated salivary oxidative stress biomarkers and their relationship with dental caries in adolescents with T1DM.

Materials and methods: This hospital-based observational cross-sectional study included 56 adolescents aged 12-18 years, comprising 28 participants with T1DM and 28 age- and sex-matched healthy controls. Unstimulated whole saliva was collected and analyzed for malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels, which were normalized to total salivary protein. Salivary flow rate, pH, plaque index, gingival index, and dental caries experience were also assessed. Between-group comparisons, correlation analyses, multivariate linear regression, and binary logistic regression were performed. Correlations were compared between groups, and an MDA × T1DM interaction was tested in the logistic model.

Results: Adolescents with T1DM had significantly higher salivary MDA and 8-OHdG concentrations than controls (both $p < 0.001$). Salivary flow rate and pH were significantly lower in the T1DM group ($p < 0.001$ and $p = 0.007$, respectively). Active caries were more prevalent among adolescents with T1DM ($p = 0.003$), whereas the difference in overall decayed, missing, and filled teeth (DMFT) scores was not statistically significant ($p = 0.087$). MDA was positively correlated with DMFT in the T1DM group ($\rho = 0.659$, $p < 0.001$), but not in controls ($\rho = 0.117$, $p = 0.554$); the difference between correlations was significant ($p = 0.025$). In adjusted logistic regression, each 1 nmol/mg increase in salivary MDA was associated with higher odds of high caries experience (DMFT > 2; adjusted OR = 5.00, 95% CI: 1.68-14.87; $p = 0.004$). A significant MDA × T1DM interaction was observed (OR = 44.81, 95% CI: 1.50-1337.40; $p = 0.028$), indicating that the association between MDA and high caries experience differed according to diabetes status. The MDA association was not significant in controls (OR = 1.04, 95% CI: 0.21-5.26; $p = 0.962$). Addition of the interaction term significantly improved model fit (likelihood-ratio $\chi^2 = 7.06$, $p = 0.008$). The wide CI indicated substantial imprecision in the interaction estimate. 8-OHdG was not significantly correlated with DMFT.

Conclusion: Adolescents with T1DM demonstrated increased salivary oxidative stress, impaired salivary parameters, and a greater active caries burden. Salivary MDA was associated with caries experience only among adolescents with T1DM, suggesting a diabetes-specific link that requires confirmation in larger prospective studies.

Categories: Dentistry
Keywords: adolescent, dental caries, diabetes mellitus, oxidative stress, saliva, type 1 diabetes mellitus (t1d)

Introduction

Type 1 diabetes mellitus (T1DM) is a chronic metabolic disorder characterized by autoimmune destruction of pancreatic β-cells and lifelong dependence on insulin therapy [1]. Adolescence represents a particularly important period in the management of T1DM because of the substantial physiological, behavioral, and hormonal changes that may influence glycemic control and oral health [2]. In addition to its systemic consequences, T1DM may affect the oral environment through alterations in salivary flow, composition, pH, and susceptibility to inflammatory and infectious conditions [3,4]. These changes may contribute to an increased risk of dental caries and other oral complications in affected individuals.

Oxidative stress has emerged as an important biological mechanism associated with chronic hyperglycemia

[5]. Persistent metabolic disturbances may promote the generation of reactive oxygen species and impair antioxidant defenses, resulting in oxidative damage to cellular lipids and DNA [6]. Salivary biomarkers provide a non-invasive approach for evaluating such changes in the oral environment [7]. Elevated oxidative stress markers, including lipid peroxidation products and markers of DNA damage, have been reported in individuals with diabetes and under conditions of chronic stress [8,9]. Therefore, assessment of these biomarkers in saliva may provide insight into the relationship between T1DM and oral oxidative stress.

Dental caries is a multifactorial disease influenced by dietary habits, oral hygiene, salivary characteristics, and host factors. Altered salivary flow and pH in individuals with T1DM may modify the oral environment and potentially increase caries susceptibility [3,10]. However, oxidative stress may represent an additional pathway linking systemic metabolic alterations and oral diseases. Evaluating oxidative stress biomarkers alongside caries experience and salivary parameters may help clarify these relationships while accounting for potential confounding factors, such as plaque accumulation, gingival inflammation, sugar intake, and salivary flow.

Therefore, the present hospital-based study aimed to investigate salivary oxidative stress and dental caries among adolescents with T1DM compared with systemically healthy controls. The primary objectives were to compare salivary malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) concentrations between the two groups and to evaluate the independent association of T1DM status with these biomarkers using multivariable regression analysis. The secondary objectives were to compare salivary flow rate, pH, and dental caries outcomes between the groups; to examine the associations of MDA and 8-OHdG with decayed, missing, and filled teeth (DMFT) scores and compare these correlations between groups; to assess the associations of these biomarkers with HbA1c and diabetes duration among adolescents with T1DM; and to investigate the association between MDA and high caries experience, including whether this association differed according to T1DM status.

Materials And Methods

Study design and ethics

This hospital-based observational cross-sectional comparative study was conducted from April 2025 to March 2026 at the Department of Conservative Dentistry and Endodontics, RKDF Dental College and Research Centre, Bhopal, India. Adolescents with T1DM and systemically healthy controls were recruited from the dental outpatient department of the same institution after screening against the predefined inclusion and exclusion criteria. Controls were individually matched to participants with T1DM on sex and age (±1 year). All participants were screened using a standardized eligibility checklist before enrollment. The institutional ethics committee approved the protocol before recruitment (RKDF/DC/2025/SS-045, dated March 28, 2025). Written informed consent was obtained from the parents or legal guardians and assent from the participants.

Participants

Adolescents aged 12-18 years with at least 20 natural teeth were eligible. Cases had T1DM diagnosed by the American Diabetes Association criteria for at least one year and were receiving insulin [11]. Controls were systemically healthy and had normal fasting blood glucose levels. Controls were considered normoglycemic if their fasting plasma glucose level was below 100 mg/dL (5.6 mmol/L). Matching was performed sequentially as cases were recruited; the next eligible systemically healthy adolescent of the same sex and within ±1 year of age was selected from the outpatient pool. Exclusion criteria in both groups were other systemic, autoimmune, or salivary gland diseases; antibiotic, antioxidant, or vitamin supplement, corticosteroid, or sialagogue use in the previous three months; current or past tobacco or alcohol use; active orthodontic treatment; acute oral infection or periodontitis; fluoride varnish or chlorhexidine use in the previous three months; and inability to cooperate with saliva collection.

Sample size

The sample size was calculated using G*Power statistical software (Ver. 3.1 Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) for the difference between two independent means, with salivary MDA as the primary outcome. The reference study stated a very high effect size (9.04) for salivary MDA in group controls and patients with diabetes, which were 0.26 ± 0.05 μmol/l and 0.81 ± 0.07 μmol/l [12]. With Cohen's d = 0.81, two-sided α = 0.05, and 80% power, 25 participants per group were required. After allowing for approximately 10% unusable samples, 28 participants per group were recruited (N = 56). A Cohen's d of 0.81 was selected as a conventional large effect size because no prior data on salivary MDA in this exact adolescent T1DM population were available; this assumption is acknowledged as a limitation.

Clinical and questionnaire data

Age and sex were recorded for all participants. For cases, diabetes duration, the most recent glycated hemoglobin (HbA1c) within three months, and the insulin regimen were taken from medical records. Glycemic control was classified according to the International Society for Pediatric and Adolescent Diabetes (ISPAD) 2022 Clinical Practice Consensus Guidelines as good (HbA1c < 7.0%) or suboptimal (⩾ 7.0%) [13];

HbA1c and duration were used in the correlation analyses. Parents, assisted by the participant, recorded tooth brushing frequency, fluoride toothpaste use, daily sugar exposure, and dental visits in the previous 12 months.

Prior to the study, the single calibrated examiner underwent a training and calibration exercise on 10 adolescents (not included in the final sample). Intra-examiner reliability was assessed by re-examining the same 10 participants after a one-week interval under identical conditions. A total of 280 teeth (surfaces) were evaluated. Cohen's kappa (κ) coefficient for DMFT scoring was κ = 0.86 (95% CI 0.78-0.94), and for the binary active-caries outcome κ = 0.84 (95% CI 0.75-0.93), both exceeding the pre-specified threshold of 0.80 and indicating substantial to almost perfect agreement. The examiner was not blinded to T1DM status during clinical examination.

Caries experience was assessed using the DMFT index according to the World Health Organization criteria [14]. Active caries was defined as the presence of at least one cavitated lesion with softened dentin, following the WHO diagnostic criteria [14]. High caries experience for logistic regression was defined a priori as DMFT > 2. The cutoff DMFT > 2 was selected because it represented the upper half of the observed distribution in the sample and provided adequate numbers of events for logistic regression. Plaque and gingival statuses were scored using the plaque index [15] and gingival index [16].

Missing teeth were scored as "M" in the DMFT index only when the participant or parent reported that the tooth had been extracted because of caries or its sequelae (pain, infection, or abscess). Teeth extracted for orthodontic reasons, trauma, or other non-carious causes were excluded from the "M" component. Filled teeth were recorded as "F" when a restoration was present, irrespective of the restorative material. Recurrent (secondary) caries adjacent to restorations was recorded as "D" (decayed) when soft or leathery dentin was detected at the restoration margin according to WHO criteria, and was therefore counted in both the DMFT total and the active-caries outcome.

Saliva collection and processing

Unstimulated whole saliva was collected between 08:00 and 10:00 under identical standardized conditions in both the T1DM and control groups. Participants fasted from food and caloric beverages for a minimum of two hours before collection; plain water was permitted until 30 minutes prior to collection. They avoided tooth brushing, any oral hygiene measures, smoking or tobacco use, alcohol, caffeine-containing drinks, and antioxidant or vitamin supplements for at least 12 hours (consistent with the study exclusion criteria). Immediately before collection, participants rinsed with distilled water and then passively drooled for exactly five minutes into sterile graduated tubes kept on ice while seated upright. In adolescents with T1DM, saliva was collected at least two hours after the morning insulin dose (or the last rapid-acting insulin bolus) and before any mid-morning snack or correction dose. Participants and parents were asked about symptomatic hypoglycemia or marked hyperglycemia in the preceding 12 hours; no severe hypoglycemic episodes requiring assistance were reported on the morning of collection. Hydration status was not formally quantified, but participants were instructed to maintain their usual fluid intake and to avoid dehydration. Tubes were kept on ice. The flow rate (mL/min) was calculated from the five-minute volume, and pH was measured using a calibrated digital pH meter. Samples were centrifuged at 3000 × g for 10 minutes at 4^{o}C. Supernatants were aliquoted and stored at −80^{o}C without repeated freeze-thaw cycles.

Biochemical assays

Total protein was measured using a bicinchoninic acid assay (Pierce BCA Protein Assay Kit; Thermo Fisher Scientific, Rockford, IL, USA). Raw total protein concentrations (mg/mL) and raw biomarker concentrations are reported in addition to the protein-normalized values. MDA was quantified as a thiobarbituric acid-reactive substance (TBARS) using a commercial colorimetric kit (TBARS Assay Kit, Item No. 10009055; Cayman Chemical, Ann Arbor, MI, USA) and read at 530-540 nm against an MDA standard curve. Although TBARS assays are commonly used as an index of lipid peroxidation, they are not entirely specific for MDA because thiobarbituric acid can react with other aldehydes; results are therefore expressed as MDA equivalents. According to the manufacturer, the assay has a detection range of approximately 0-50 µM; under the conditions used, the lower limit of detection was 0.5 µM, and the lower limit of quantification was 1.5 µM. Saliva samples were diluted 1:2 with assay buffer when concentrations exceeded the upper end of the standard curve. Spike-recovery experiments performed on saliva matrix yielded recoveries of 92%-108%. All samples were assayed in duplicate within a single batch. The observed intra-assay coefficient of variation was 4.2%, and the inter-assay coefficient of variation was not applicable (single batch).

Statistical analysis

Analyses were performed using IBM SPSS Statistics, version 26 (IBM Corp., Armonk, NY, USA). Normality was assessed using the Shapiro-Wilk test. Normally distributed continuous variables were presented as mean ± standard deviation (SD) and compared using the independent-samples t-test after assessment of variance homogeneity with Levene's test. Non-normally distributed variables (8-OHdG and DMFT) were presented as median (interquartile range (IQR)) and compared using the Mann-Whitney U test. Mean differences with 95% CIs and Cohen's d were calculated for t-tests, whereas rank-biserial correlations were reported for rank-

based comparisons. Categorical variables were compared using Pearson's chi-square test, and effect sizes are expressed as ORs and phi coefficients (ϕ).

The primary analyses were the between-group comparisons of salivary MDA and 8-OHdG concentrations. Secondary analyses included comparisons of salivary flow rate, pH, DMFT, and active caries prevalence. Spearman's rank correlation coefficients (ρ), with 95% CIs calculated using Fisher's z transformation, were used to assess associations of MDA and 8-OHdG with DMFT scores and, among participants with T1DM, with HbA1c levels and diabetes duration. Correlations of MDA and 8-OHdG with DMFT were compared between the T1DM and control groups using the independent-group Fisher z test for the difference in correlation coefficients. The 95% CI for the difference in ρ (Δρ) was obtained by nonparametric bootstrap with 5,000 resamples. Multivariable linear regression models were fitted for MDA and natural log-transformed 8-OHdG concentrations, with T1DM status, plaque index, gingival index, salivary flow rate, age, and sex included as predictors; these models were considered exploratory. Residual normality, Durbin-Watson statistics, and variance inflation factors were assessed. For the log-transformed 8-OHdG model, exponentiated regression coefficients (exp(β)) were interpreted as multiplicative changes in the geometric mean.

Binary logistic regression was used to estimate adjusted ORs for high caries experience, defined as DMFT >2. Three separate models were fitted, with T1DM status, MDA concentration, or 8-OHdG concentration as the primary predictor; each model was adjusted for plaque index, age, sex, frequent sugar intake (≥2 exposures/day), and brushing frequency (≥ twice daily). Model fit was assessed using the likelihood-ratio test and McFadden's pseudo-R^2. For the MDA model, Nagelkerke's pseudo-R^2 and the Hosmer-Lemeshow goodness-of-fit test were also reported. No formal adjustment for multiple comparisons was applied because the primary outcomes were limited and prespecified; however, the possibility of false-positive findings among the secondary and exploratory analyses is acknowledged. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

Results

A total of 56 adolescents were included, comprising 28 patients with T1DM and 28 healthy controls. The groups were comparable in terms of age, sex, plaque index, and gingival index (all $p > 0.05$). However, the unstimulated salivary flow rate and salivary pH were significantly lower in the T1DM group than in controls ($p < 0.001$ and $p = 0.007$, respectively) (Table *1*). Among participants with T1DM, the mean diabetes duration was 7.20 ± 3.55 years, and the mean HbA1c was 8.71 ± 1.45%.

Variable		T1DM (n = 28)	Control (n = 28)	Test value (df)	p-value	Effect size
Age (years)	Mean ± SD	15.60 ± 1.71	14.89 ± 1.44	t (54) = 1.69	0.097	d = 0.45
Sex, n (%)	Male	15 (53.6)	14 (50.0)	χ^2 (1) = 0.07	0.789	φ = 0.04
	Female	13 (46.4)	14 (50.0)			
Plaque index	Mean ± SD	1.11 ± 0.25	0.99 ± 0.34	t (54) = 1.44	0.155	d = 0.39
Gingival index	Mean ± SD	1.03 ± 0.39	1.02 ± 0.36	t (54) = 0.09	0.929	d = 0.02
Salivary flow (mL/min)	Mean ± SD	0.30 ± 0.08	0.44 ± 0.12	t (54) = −4.84	< 0.001*	d = −1.29
Salivary pH	Mean ± SD	6.81 ± 0.30	7.03 ± 0.30	t (54) = −2.80	0.007*	d = −0.75
Brushing, n (%)	≥ twice daily	15 (53.6)	17 (60.7)	χ^2 (1) = 0.29	0.589	φ = 0.07
	< twice daily	13 (46.4)	11 (39.3)			
Fluoride toothpaste use, n (%)	Yes	18 (64.3)	21 (75.0)	χ^2 (1) = 0.76	0.383	φ = 0.12
	No	10 (35.7)	7 (25.0)			
Frequency of sugar intake, n (%)	≥ 2 exposures/day	10 (35.7)	9 (32.1)	χ^2 (1) = 0.08	0.778	φ = 0.04
	< 2 exposures/day	18 (64.3)	19 (67.9)			
Dental visit in past 12 months, n (%)	Yes	20 (71.4)	13 (46.4)	χ^2 (1) = 3.62	0.057	φ = 0.25
	No	8 (28.6)	15 (53.6)			

TABLE 1: Demographic, clinical, diabetes-related, and lifestyle characteristics of the study participants.

Continuous variables are presented as mean ± standard deviation (SD) and were compared using the independent-samples t-test; categorical variables are presented as frequency (percentage) and were compared using Pearson's chi-square test; all expected cell counts were ≥5; df: degree of freedom; T1DM: type 1 diabetes mellitus; *p < 0.05 was considered statistically significant; Effect sizes are presented as Cohen's d or phi (φ).

Salivary MDA concentrations were significantly higher in adolescents with T1DM than in controls (p < 0.001). Similarly, 8-OHdG concentrations were significantly higher in the T1DM group (p < 0.001). Although the median DMFT score was higher in the T1DM group, the difference was not statistically significant (p = 0.087). In contrast, active caries was significantly more prevalent among adolescents with T1DM, affecting 20 (71.4%) participants compared with nine (32.1%) controls (p = 0.003) (Table *2*).

Outcome		T1DM (n = 28)	Control (n = 28)	Difference (95% CI)	Test value (df)	p-value	Effect size
MDA (nmol/mg)	Mean ± SD	3.41 ± 0.91	2.55 ± 0.60	0.86 (0.45, 1.27)	t (54) = 4.17	< 0.001*	d = 1.11
8-OHdG (ng/mg)	Median (IQR)	0.79 (0.44, 0.92)	0.42 (0.29, 0.49)	0.27 (0.12, 0.47)	U = 612.0	< 0.001*	r = 0.56
DMFT	Median (IQR)	3.00 (1.00, 5.00)	2.00 (1.00, 3.00)	1.00 (0.00, 2.00)	U = 495.0	0.087	r = 0.26
Active caries lesions	n (%)	20 (71.4)	9 (32.1)	39.3% (15.2, 63.4)	χ^2 (1) = 8.65	0.003*	OR = 5.28

TABLE 2: Comparison of salivary oxidative stress biomarkers and dental caries outcomes between adolescents with T1DM and controls.

MDA and 8-OHdG concentrations were expressed per milligram of total salivary protein; normally distributed continuous data are presented as mean ± SD and were compared using the independent-samples t-test; non-normally distributed continuous data were presented as median (interquartile range) and were compared using the Mann–Whitney U test; categorical data were presented as frequency (percentage) and were compared using Pearson's chi-square test; and effect sizes were presented as Cohen's d, rank biserial, and odds ratio. Difference in proportions with Wald 95% CI. df: degree of freedom; T1DM: type 1 diabetes mellitus; MDA: malondialdehyde; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; DMFT: Decayed, missing, and filled teeth; *p < 0.05 was considered statistically significant.

Among adolescents with T1DM, salivary MDA was positively correlated with DMFT (ρ = 0.659, p < 0.001), whereas no correlation was found in controls (ρ = 0.117, p = 0.554); the difference between the two correlations was statistically significant (Δρ = 0.54, 95% CI 0.09-0.99; Fisher z = 2.24, p = 0.025) (Table *3*). 8-OHdG was not correlated with DMFT in either group, and the correlations did not differ (p = 0.476). Neither biomarker correlated with HbA1c or diabetes duration.

Variable pair		T1DM (n = 28), ρ (95% CI)	p	Control (n = 28), ρ (95% CI)	p	Δρ (95% CI)	Fisher Z test	p-value
MDA	DMFT	0.659 (0.358, 0.835)	< 0.001*	0.117 (−0.290, 0.488)	0.554	0.542 (0.091, 0.992)	0.24	0.025*
	HbA1c	0.177 (−0.232, 0.533)	0.367	NA	NA	NA	NA	NA
	Diabetes duration	0.374 (−0.022, 0.669)	0.05	NA	NA	NA	NA	NA
8-OHdG	DMFT	0.051 (−0.349, 0.436)	0.795	−0.161 (−0.521, 0.248)	0.413	0.212 (−0.351, 0.732)	0.71	0.476
	HbA1c	0.120 (−0.287, 0.490)	0.543	NA	NA	NA	NA	NA
	Diabetes duration	0.141 (−0.267, 0.506)	0.474	NA	NA	NA	NA	NA

TABLE 3: Correlations of salivary oxidative stress biomarkers with caries experience and diabetes-related variables.

Correlations were assessed using Spearman's rank correlation coefficient; correlation coefficients were presented with 95% CIs calculated using Fisher's z transformation; diabetes-related variables were not applicable to the control group; MDA: malondialdehyde; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; DMFT: decayed, missing, and filled teeth; HbA1c: glycated hemoglobin; T1DM: type 1 diabetes mellitus; and *p < 0.05 was considered statistically significant.

Correlations in the T1DM and control groups were compared using the Fisher z test for independent correlations, with bootstrap 95% CIs (5,000 resamples) for the difference in ρ.

After adjusting for plaque index, gingival index, salivary flow, age, and sex, T1DM remained independently associated with higher salivary MDA concentrations (B = 0.909, p = 0.001) and higher log-transformed 8-OHdG concentrations (B = 0.661, p < 0.001) (Tables *4*, *5*). The gingival index was also independently associated with MDA concentration (p = 0.016). The unadjusted Spearman correlation between gingival

index and salivary MDA was weak and non-significant (ρ = −0.12, p = 0.38). In the multivariable model, the negative coefficient for gingival index (B = −0.701, p = 0.016) emerged after simultaneous adjustment for plaque index and salivary flow rate. Variance inflation factors (VIFs) were low (maximum VIF = 1.62), indicating no substantial multicollinearity. Sensitivity analyses that sequentially removed plaque index or salivary flow attenuated the gingival-index coefficient and rendered it non-significant, suggesting a possible suppression effect rather than a direct inverse relationship. This finding should not be interpreted as evidence that greater gingival inflammation reduces oxidative stress.

Predictor	B	95% CI	t value	p-value
Constant	3.513	1.302 to 5.723	3.19	0.002*
T1DM (vs. control)	0.909	0.395 to 1.422	3.56	0.001*
Plaque index	0.167	−0.547 to 0.881	0.47	0.640
Gingival index	−0.701	−1.267 to −0.136	−2.49	0.016*
Salivary flow, mL/min	0.275	−1.828 to 2.379	0.26	0.794
Age, years	−0.033	−0.168 to 0.102	−0.49	0.626
Male sex	−0.087	−0.516 to 0.342	−0.41	0.686

TABLE 4: Multivariable linear regression analysis of factors associated with salivary malondialdehyde concentration.

Multivariable linear regression was performed with salivary MDA concentration as the dependent variable; model fit: $R^2 = 0.346$, adjusted $R^2 = 0.266$, $F(6,49) = 4.33$, $p = 0.001$; residual normality: Shapiro–Wilk W = 0.990, p = 0.914; Durbin–Watson statistic = 1.85; maximum variance inflation factor (VIF) = 1.62; B indicates unstandardized regression coefficient; MDA: malondialdehyde; T1DM: type 1 diabetes mellitus; T1DM was coded relative to the control group, and sex was coded as male relative to female; *p < 0.05 was considered statistically significant.

Predictor	B	95% CI	t value	p-value	exp(B) (95% CI)
Constant	−1.961	−3.303 to −0.620	−2.94	0.005*	0.14 (0.04 to 0.54)
T1DM (vs. control)	0.661	0.350 to 0.973	4.26	< 0.001*	1.94 (1.42 to 2.65)
Plaque index	−0.006	−0.439 to 0.427	−0.03	0.979	0.99 (0.64 to 1.53)
Gingival index	−0.039	−0.382 to 0.304	−0.23	0.821	0.96 (0.68 to 1.36)
Salivary flow, mL/min	1.273	−0.003 to 2.550	2.00	0.051	3.57 (1.00 to 12.80)
Age, years	0.033	−0.048 to 0.115	0.82	0.415	1.03 (0.95 to 1.12)
Male sex	0.044	−0.217 to 0.304	0.34	0.738	1.04 (0.81 to 1.36)

TABLE 5: Multivariable linear regression analysis of factors associated with log-transformed salivary 8-OHdG concentration

Multivariable linear regression was performed using the natural logarithm of salivary 8-OHdG concentration as the dependent variable because of its non-normal distribution; model fit: $R^2 = 0.322$, adjusted $R^2 = 0.238$, $F(6,49) = 3.87$, $p = 0.003$; residual normality: Shapiro–Wilk W = 0.979, p = 0.423; Durbin–Watson statistic = 1.80; B indicates unstandardized regression coefficient; exp(B): multiplicative change or ratio of geometric means in 8-OHdG concentration per one-unit increase in the predictor; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; T1DM: type 1 diabetes mellitus; T1DM was coded relative to the control group, and sex was coded as male relative to female; *p < 0.05 was considered statistically significant.

In the logistic regression analysis, model fit varied across the sequential models. Model 0, which included T1DM status alone, was not statistically significant (likelihood-ratio test, p = 0.401; Nagelkerke $R^2 = 0.140$). Models 1 and 2, which included MDA, demonstrated significant overall model fit (likelihood-ratio p = 0.005 and p = 0.010, respectively). In Model 2, T1DM status was not independently associated with high caries experience after adjustment for MDA (OR = 0.98, 95% CI: 0.24-4.02; p = 0.975). Model 3, which additionally included the MDA × T1DM interaction term, demonstrated the highest explanatory power (Nagelkerke $R^2 = 0.491$; Akaike Information Criterion = 69.9).

The interaction term was statistically significant (OR = 44.81, 95% CI: 1.50-1337.40; Wald p = 0.028). The likelihood-ratio comparison also indicated improved model fit after adding the interaction term (LR χ^2 = 7.06, df = 1, p = 0.008). The interaction OR represents the ratio of the conditional MDA odds ratios between the two groups. The conditional MDA OR was 1.04 (95% CI: 0.21-5.26) among controls and 46.6 (95% CI: 2.7-815.6) among adolescents with T1DM. However, the large interaction estimate and its extremely wide CI indicate substantial imprecision. Given the limited sample size and number of events, this interaction analysis should be considered exploratory and interpreted cautiously. In a separate Firth-penalized logistic regression analysis, the conditional MDA OR among adolescents with T1DM was 14.2 (95% CI: 1.8-112.4) (Table *6*).

Predictor	Model 0: T1DM only, OR (95% CI)	p-value	Model 1: MDA only, OR (95% CI)	p-value	Model 2: + T1DM, OR (95% CI)	p-value	Model 3: + interaction, OR (95% CI)	p-value
T1DM (vs. control)	2.86 (0.88, 9.27)	0.08	—	—	0.98 (0.24, 4.02)	0.975	1.38 (0.26, 7.39)	0.706
MDA (per 1 nmol/mg, centered)	—	—	4.97 (1.81, 13.63)	0.002*	5.00 (1.68, 14.87)	0.004*	1.04 (0.21, 5.26)	0.962
MDA × T1DM	—	—	—	—	—	—	44.81 (1.50, 1337.40)	0.028*
Plaque Index	0.35 (0.05, 2.55)	0.298	0.17 (0.01, 2.24)	0.177	0.17 (0.01, 2.30)	0.182	0.04 (0.00, 1.05)	0.053
Age (years)	1.20 (0.84, 1.72)	0.323	1.37 (0.90, 2.09)	0.142	1.37 (0.89, 2.12)	0.15	1.58 (0.94, 2.64)	0.085
Sex (male)	1.16 (0.36, 3.74)	0.807	1.22 (0.31, 4.77)	0.779	1.22 (0.31, 4.77)	0.778	0.76 (0.15, 3.84)	0.736
Frequent sugar intake	1.31 (0.39, 4.35)	0.661	1.09 (0.28, 4.32)	0.902	1.09 (0.28, 4.32)	0.902	0.56 (0.10, 3.23)	0.512
Brushing ≥ twice daily	1.05 (0.33, 3.33)	0.929	1.27 (0.32, 5.07)	0.739	1.26 (0.32, 5.08)	0.741	2.15 (0.43, 10.80)	0.352

TABLE 6: Multivariable logistic regression analysis of factors associated with high caries experience.

High caries experience was defined as DMFT > 2, corresponding to 27 of 56 participants. ORs are presented with 95% CIs. Model 0 included T1DM status as the primary predictor; Model 1 included salivary MDA; Model 2 included MDA and T1DM status; and Model 3 additionally included the MDA × T1DM interaction term. All models were adjusted for plaque index, age, sex, and frequent sugar intake and brushing frequency (≥ twice daily). MDA was centered before interaction analysis. DMFT: decayed, missing, and filled teeth; MDA: malondialdehyde; T1DM: type 1 diabetes mellitus; and *p < 0.05 was considered statistically significant.

Discussion

The present study demonstrated higher salivary concentrations of MDA and 8-OHdG among adolescents with T1DM compared with systemically healthy adolescents. The persistence of the association between T1DM and both oxidative damage biomarkers after adjustment for relevant oral and demographic factors suggests an altered salivary oxidative profile in adolescents with diabetes. This observation is consistent with the established role of oxidative stress in diabetes-related metabolic disturbances. Chronic hyperglycemia has been proposed to promote reactive oxygen species generation through several pathways, including glucose auto-oxidation, mitochondrial dysfunction, and the formation of advanced glycation end products [5,17]. Previous studies in children and adolescents with T1DM have also reported increased oxidative stress markers, supporting the relevance of oxidative damage in this population [18,19]. However, the present study specifically measured MDA and 8-OHdG and did not directly assess reactive oxygen species generation, antioxidant enzyme activity, or total antioxidant capacity. Therefore, the underlying mechanisms responsible for the observed differences cannot be established from these findings.

The elevation of salivary MDA is particularly noteworthy because it is a product of lipid peroxidation and reflects oxidative damage to lipids [12]. Previous studies have reported increased salivary oxidative stress markers in individuals with diabetes, suggesting that saliva may reflect alterations associated with systemic metabolic conditions and the local oral environment [7,9]. Nevertheless, MDA concentrations may be influenced by several systemic and local factors, and the present study did not evaluate its diagnostic accuracy or prognostic performance. Accordingly, salivary MDA should be considered a potential research biomarker of oxidative damage rather than an established clinical marker for identifying adolescents at

increased risk of dental caries.

The reduction in salivary flow rate and pH observed in adolescents with T1DM represents another important finding. Saliva contributes to oral protection through lubrication, buffering, antimicrobial activity, clearance of fermentable carbohydrates, and remineralization of early enamel lesions. Alterations in salivary secretion and composition may reduce these protective functions [20]. A previous meta-analysis reported impaired salivary parameters in individuals with T1DM, supporting the present observations [21]. Although the precise mechanisms underlying the reduced salivary flow and pH were not investigated, these findings highlight the importance of evaluating salivary function alongside conventional clinical oral health parameters in adolescents with diabetes.

The pattern of caries findings also warrants consideration. Although active caries was significantly more prevalent among adolescents with T1DM, the difference in overall DMFT scores between the groups did not reach statistical significance. This apparent discrepancy may reflect the different aspects of caries experience captured by these measures. Active caries reflects clinically detectable disease at the time of examination, whereas DMFT represents cumulative disease experience, incorporating previous decay, tooth loss attributed to caries, and restorative treatment. Consequently, differences in previous dental treatment and healthcare utilization may influence cumulative DMFT independently of current caries activity. In the present study, dental visits during the preceding 12 months were reported by 71.4% of adolescents with T1DM compared with 46.4% of healthy controls, although this difference did not reach statistical significance.

More frequent dental attendance among adolescents with T1DM may have increased opportunities for restorative treatment and potentially influenced the filled component of DMFT. However, the individual decayed, missing, and filled components were not recorded separately; therefore, the contribution of dental attendance and previous restorative treatment to the observed DMFT findings could not be directly evaluated. Previous systematic reviews have reported variable associations between T1DM and dental caries, with differences potentially influenced by age, glycemic control, oral hygiene, dietary habits, fluoride exposure, and access to dental care [22,23]. These findings emphasize the importance of distinguishing current caries activity from cumulative caries experience when interpreting oral health outcomes in adolescents with T1DM.

An important finding was the positive association between salivary MDA and caries experience among adolescents with T1DM. The correlation was stronger in the T1DM group than in healthy controls, and the between-group comparison suggested a difference in the strength of this association. The exploratory interaction analysis also indicated a possible variation in the relationship between MDA and high caries experience according to diabetes status. Previous research has reported relationships between salivary oxidative stress markers and dental caries in children and adolescents, although the direction and biological basis of these associations remain uncertain [24]. Oxidative processes have been proposed to influence salivary components, epithelial integrity, host defense mechanisms, and microbial homeostasis, while active carious lesions and associated local inflammatory processes may themselves contribute to oxidative damage [6]. However, these mechanisms were not directly evaluated in the present study. Importantly, the cross-sectional design does not establish the temporal direction of the observed association or determine whether increased lipid peroxidation contributes to caries development or whether established carious lesions contribute to increased MDA concentrations. Furthermore, the relatively small sample and wide confidence intervals surrounding the interaction estimate indicate substantial statistical uncertainty. Therefore, the observed association should be interpreted as exploratory and hypothesis-generating rather than evidence of a diabetes-specific causal mechanism.

In contrast, 8-OHdG did not demonstrate a significant association with caries experience in the present study. MDA and 8-OHdG represent different forms of oxidative damage, with MDA reflecting lipid peroxidation and 8-OHdG serving as a marker of oxidative DNA damage [19,25]. Their differing associations with caries may reflect differences in the biological processes represented by these biomarkers and their relationships with systemic and local oral conditions. Previous studies have also emphasized that salivary oxidative biomarkers do not necessarily behave uniformly across different diseases and clinical outcomes [26]. Nevertheless, the absence of an association between 8-OHdG and caries should not be interpreted as evidence that oxidative nucleic acid damage is biologically unimportant, particularly because the underlying oxidative pathways were not directly investigated.

The lack of significant associations between oxidative biomarkers and HbA1c or diabetes duration is also noteworthy. HbA1c represents average glycemic exposure over approximately the preceding 2-3 months and may not fully capture short-term glucose fluctuations or other metabolic factors that influence oxidative damage [27]. Although glycemic variability, nutritional status, inflammatory activity, and individual antioxidant capacity have been proposed as potential contributors to oxidative imbalance in individuals with T1DM [5], these factors were not directly assessed in the present study. Consequently, their possible contribution to the observed salivary biomarker differences remains speculative. Future investigations incorporating repeated glycemic measurements, inflammatory mediators, antioxidant enzyme activity, and total antioxidant capacity may provide a more comprehensive understanding of the relationship between metabolic control and salivary oxidative damage.

From a clinical perspective, the findings emphasize the importance of preventive oral healthcare in adolescents with T1DM. Dental assessment should include evaluation of active caries, salivary function, oral hygiene, dietary practices, and gingival health rather than focusing exclusively on cumulative caries experience. Patients with reduced salivary protection or increased caries activity may benefit from individualized preventive strategies, including reinforcement of fluoride use, dietary counselling, plaque control, and appropriate dental recall intervals. Although salivary MDA demonstrated an association with caries experience in this study, its diagnostic accuracy and ability to predict future caries development remain unknown. Sensitivity, specificity, receiver operating characteristic curve analysis, area under the curve, predictive values, and external validation were not evaluated. Therefore, MDA should currently be regarded as a potential research biomarker rather than an established clinical marker for caries-risk assessment. Prospective studies with appropriate diagnostic and prognostic validation are required before any adjunctive clinical role can be considered.

Several limitations should be considered. The cross-sectional design prevents determination of temporal or causal relationships between diabetes, oxidative damage, and dental caries. In particular, reverse causation cannot be excluded because established carious lesions and associated local inflammatory processes may contribute to increased oxidative damage, rather than oxidative damage necessarily preceding caries development. The relatively small sample and single-center, hospital-based recruitment may limit generalizability and introduce selection bias, as hospital-attending adolescents may not represent the wider adolescent population. Furthermore, the sample-size calculation was primarily designed to detect between-group differences in salivary MDA and was not powered to detect interaction effects or develop multivariable caries prediction models. Therefore, the interaction analysis should be considered exploratory, particularly given the wide confidence intervals and substantial imprecision of the estimates.

Potential confounders, including socioeconomic status, parental education, and access to dental care, were not comprehensively assessed. Although dental visits during the preceding 12 months were recorded, differences in healthcare-seeking behaviour and previous restorative treatment may have influenced the observed caries outcomes. The individual decayed, missing, and filled components of DMFT were not recorded separately, limiting further evaluation of these differences. Dietary sugar exposure was assessed using reported frequency rather than a detailed dietary assessment, limiting evaluation of the quantity, type, and timing of sugar consumption. Additionally, the clinical examiner was aware of participants' diabetes status, which may have introduced observer bias despite examiner calibration and standardized diagnostic criteria.

Although participants were individually matched by age and sex during recruitment, the matched-pair structure was not incorporated into the statistical analyses, which treated the groups as independent. This may have affected the precision of the estimates. The DMFT >2 cutoff was also exploratory rather than based on an established clinical threshold, and dichotomization resulted in some loss of information; therefore, continuous DMFT findings should receive greater emphasis. A single HbA1c measurement may not adequately reflect long-term glycemic variability. Furthermore, reactive oxygen species, antioxidant enzyme activity, total antioxidant capacity, inflammatory mediators, salivary glucose, and cariogenic microbiota were not evaluated. Consequently, the study could not characterize the overall balance between oxidant production and antioxidant defense or establish the specific biological pathways underlying the observed biomarker differences.

Although pre-collection conditions were standardized across groups, continuous glucose monitoring data and formal measures of hydration status were not obtained on the day of saliva collection. Consequently, short-term glycemic excursions or differences in hydration cannot be completely excluded as potential sources of variability in salivary flow rate or biomarker concentrations. A further methodological limitation is that the TBARS assay used for MDA quantification is not completely specific, as thiobarbituric acid can react with other compounds; the reported values therefore represent MDA equivalents rather than pure MDA. Finally, the diagnostic and prognostic performance of MDA was not assessed, and no external validation was performed. Larger, multicenter prospective studies incorporating comprehensive metabolic, dietary, inflammatory, antioxidant, and microbiological assessments, together with diagnostic and predictive validation, are warranted to clarify these relationships and determine whether salivary oxidative biomarkers have any potential clinical relevance in caries-risk assessment among adolescents with T1DM.

Conclusions

Adolescents with T1DM demonstrated higher salivary oxidative stress, reduced salivary flow rate and pH, and a higher prevalence of active caries compared with healthy controls. Salivary MDA was positively associated with caries experience in adolescents with T1DM; however, this association should be interpreted cautiously given the cross-sectional design. These findings do not establish the clinical utility of salivary oxidative stress assessment or support its routine use in oral health monitoring. Larger, longitudinal studies are needed to clarify the potential adjunctive role of salivary oxidative stress biomarkers in caries risk assessment among adolescents with T1DM.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Disclosures

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