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Research Article

Keywords: dental caries, 8-hydroxy-2'-deoxyguanosine, oxidative stress, saliva, children, permanent dentition, biomarker, Mongolia

Posted Date: September 22nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10718762/v1

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Additional Declarations: No competing interests reported.

Association between untreated permanent-tooth caries burden and salivary 8-hydroxy-2′-deoxyguanosine in twelve-year-old children: an exploratory cross-sectional biomarker study

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Abstract

Background: Dental caries may alter salivary oxidative-stress biomarkers, but evidence for salivary 8-hydroxy-2′-deoxyguanosine (8-OHdG) in children with permanent dentition is limited. We examined whether upper-tail untreated permanent-tooth caries burden was associated with salivary 8-OHdG in twelve-year-old children.

Methods: This exploratory cross-sectional study included an oral-health survey of 190 children from two schools in Ulaanbaatar, Mongolia, with a nested non-representative biomarker substudy of 45 children enriched for low and high untreated caries burden. Untreated decay was represented by the number of decayed permanent teeth (D); D ≥ 7 defined the parent cohort's upper tail. The primary HC3-robust log-linear model compared D ≥ 7 with D < 7, adjusting for school and salivary flow. Sensitivity models additionally adjusted for plaque, gingivitis and sex and restricted analyses by residential duration and observed caries phenotype.

Results: The biomarker sample comprised 27 children with D = 0, three with D = 1–2 and 15 with D ≥ 7; none had D = 3–6. D ≥ 7 was associated with 2.62-fold higher salivary 8-OHdG (geometric mean ratio [GMR] 2.619, 95% CI 2.213–3.099; $p < 0.001$). Estimates remained similar after plaque adjustment (GMR 2.564), adjustment for plaque, gingivitis and sex (GMR 2.465), restriction to residence ≥ 3 years (GMR 2.576), residence ≥ 5 years (GMR 2.539), and D = 0 or D ≥ 7 only (GMR 2.713); all $p < 0.001$. The school indicator was not independently associated with 8-OHdG. A complementary continuous model gave a GMR of 1.105 per additional decayed tooth, but crossed the unobserved D = 3–6 interval.

Conclusions: Upper-tail untreated permanent-tooth caries burden was strongly associated with salivary 8-OHdG in this criterion-enriched biomarker sample. The finding extends pediatric oral-redox evidence to twelve-year-old children with permanent dentition and supports careful measurement of active caries when interpreting salivary oxidative DNA-damage markers. The

cross-sectional, non-representative design does not establish causality, diagnostic utility or a population dose-response.

Keywords: dental caries; 8-hydroxy-2′-deoxyguanosine; oxidative stress; saliva; children; permanent dentition; biomarker; Mongolia

Background

Dental caries remains a major childhood oral-health problem, and national data indicate a substantial burden of untreated disease among Mongolian children [1]. Caries is not only a mineral-loss process but also a biofilm-mediated inflammatory disease occurring in a saliva-rich environment. Systematic reviews of pediatric studies suggest that caries is accompanied by altered salivary oxidative-stress profiles, although effect directions, assays and sampling methods vary substantially across studies [2,3]. Total antioxidant capacity (TAC) has been especially heterogeneous across the literature [4].

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is a widely used marker of oxidative DNA damage [5]. Recent pediatric studies have increased interest in its oral-health relevance. Yergin and colleagues reported higher salivary 8-OHdG in children with severe early-childhood caries [6], while Birant et al. demonstrated changes in salivary oxidative markers after treatment of severe early-childhood caries [7]. Other work has shown that antioxidant responses and oxidative damage may vary with carious-lesion severity [8]. Together, these findings support a relation between active caries and oral redox biology, but most available evidence concerns younger children and primary dentition.

Evidence in twelve-year-old children, when permanent dentition predominates and untreated decay can be quantified directly as decayed permanent teeth, is limited. This age is widely used for international oral-health surveillance and offers a clinically relevant setting for non-invasive salivary biomarker research. F_2-isoprostanes provide a complementary index of lipid peroxidation

[9], but coherent interpretation requires attention to analyte-specific behavior and to oral disease itself when salivary biomarkers are used for research purposes.

The present study reanalyzed a cross-sectional oral-health survey of twelve-year-old children from two Ulaanbaatar schools with a nested salivary biomarker substudy. The parent project had selected the schools in the context of a historical area-level $PM_{2.5}$ contrast, but the original station-level series and participant collection dates were not preserved. In this oral-health analysis, that historical environmental classification is therefore treated only as background to school selection; no participant-level pollution exposure or environmental effect is estimated.

Our primary objective was to quantify the association between upper-tail untreated permanent-tooth caries burden ($D \geq 7$) and salivary 8-OHdG. Secondary objectives were to assess the stability of this association after adjustment for oral-health covariates and in residence- and phenotype-restricted sensitivity analyses, to describe the complementary continuous-D association, and to explore TAC and F_2-isoprostane patterns. We hypothesized that untreated caries burden would be strongly associated with salivary 8-OHdG independent of school context and salivary flow.

Methods

Study design and setting

This exploratory cross-sectional study comprised a clinical oral-health survey and a nested salivary biomarker substudy. The analytic parent cohort included 190 twelve-year-old children: 107 attending School A in the Sharkhad area of Bayanzurkh District and 83 attending School B in the Khailaast area of Chingeltei District, Ulaanbaatar, Mongolia. For publication, the two schools are referred to as School A and School B throughout the manuscript and figures. Archived dissertation records document laboratory authorization on 4 June 2025 (No. 40/25),

permission from the Ulaanbaatar education authority for research in the two schools on 6 June 2025 (No. 01/843), and research-ethics approval on 12 June 2025 (No. 24-25/10-01), and state that the study was conducted under these permissions. Exact participant examination and saliva-collection dates were not retained in the analytic archive. The source project had selected the schools for a historical higher/lower $PM_{2.5}$-area contrast based on multi-year information from the National Agency for Meteorology and Environmental Monitoring (NAMEM). Surviving records do not preserve the exact monitoring-station identifiers, source concentration series, or a consistent four- versus five-year source period. Consequently, no quantitative $PM_{2.5}$ concentration was assigned to any participant in this reanalysis. School was treated as a contextual covariate; because school identity exactly maps to the historical binary area label, no model can distinguish a school effect from that historical contextual classification. Published and official sources were used only to describe the broader Ulaanbaatar setting [10-12].

Duration of residence at the current address was recorded in completed years and was used in sensitivity analyses to test whether the principal biomarker association depended on very short residence at the current location.

Participants and biomarker-substudy selection

The parent analytic cohort consisted of all 190 analyzable twelve-year-old records retained after data cleaning. Parent or legal-guardian permission and child assent were required. No separate prospective power calculation for the biomarker substudy was retained in the archived documentation; its size was fixed by the 45 participants with recoverable salivary measurements. Archived protocol and dissertation materials describe an intended phenotype-based biomarker strategy contrasting clinically healthy/caries-free children with children having high untreated decay within each school; later records specify $D \geq 7$ and residence for at least three years among intended criteria. Recorded exclusions included dental anomalies or retained deciduous teeth,

regular medication or underlying disease, antibiotic use or respiratory illness within the preceding two weeks, and oral mucosal lesions.

The final analysis archive contains biomarker measurements for 45 children but does not preserve the exact final selection sequence, and some participants do not satisfy every criterion appearing in the evolving study documents. We therefore describe the biomarker substudy as criterion-enriched and non-representative rather than random or population-representative. It comprised School A/D < 7 (n = 15), School A/D ≥ 7 (n = 7), School B/D < 7 (n = 15), and School B/D ≥ 7 (n = 8). Twenty-seven participants had D = 0, three had D = 1–2, fifteen had D ≥ 7, and none had D = 3–6. Phenotype-enriched sampling can increase the apparent contrast between groups, so population-prevalence, diagnostic-accuracy and conventional dose-response claims were not made [13,14].

Oral-health assessment

Oral examinations followed the World Health Organization basic oral-health survey framework [15]. The study protocol specified that one dentist perform examinations in the school physician's room under natural light using single-use examination instruments. Caries experience was recorded as decayed (D), missing (M), and filled (F) permanent teeth, and DMFT was calculated as D + M + F. Oral hygiene was evaluated using the Simplified Oral Hygiene Index [16]. Gingival status followed the Löe–Silness approach [17]; the dissertation record specifies assessment of teeth 16, 11, 26, 31, 36 and 46 on four surfaces, including bleeding observed 30 seconds after probing. The protocol specified periodontal pocket-depth (PPD) measurement with a periodontal probe at six sites per tooth. The final analysis workbook retained six PPD measurements per child, from which participant-level mean and maximum PPD were derived; the archive does not permit reconstruction of every tooth/site-level measurement. Pediatric periodontal findings were interpreted in the context of current guidance [18].

Active caries was defined as D > 0. Upper-tail untreated caries burden was defined analytically as D ≥ 7, corresponding to 46/190 children (24.2%) in the parent cohort and aligning with the high-decay phenotype in later study records. Poor/very poor plaque was defined as plaque index ≥ 2. Because one examiner was specified, inter-examiner agreement was not applicable; no intra-examiner repeatability statistic was retained in the final archive.

Saliva collection, processing and biomarker assays

Resting unstimulated whole saliva was collected under standardized two-hour pre-collection restrictions that prohibited eating or drinking, toothbrushing, mouth rinsing, and dental treatment. Children were seated upright with feet supported, the head slightly inclined, and relaxed facial muscles. Saliva was allowed to drain into a sterile graduated container for five minutes; salivary flow (mL/min) was calculated as collected volume divided by five. Samples were transported in a dedicated cold container. The laboratory standard operating procedure specified centrifugation at 2000–3000 rpm for 20 minutes, repeat centrifugation if precipitate remained, and storage at −70 °C. The approved protocol stated delivery to the laboratory within 10 minutes, whereas a later dissertation narrative stated within one hour; participant-level transport timestamps were not retained, so the exact interval cannot be verified.

Salivary 8-OHdG and F_2-isoprostane were assayed using Sunlong Biotech Co., Ltd. (Hangzhou, China) products SL2044Hu and SL3468Hu, respectively [19,20]. TAC was measured with the Sunlong AK0455-100T-96S total antioxidant capacity assay, which uses a ferric-reducing antioxidant power (FRAP) colorimetric principle [21]. Current manufacturer information is cited for product traceability only and is not treated as validation of the unavailable study lot. The archived ELISA standard operating procedure specified 1:5 sample dilution, 30-minute incubation at 37 °C, five wash cycles, HRP-conjugate/chromogen development, and absorbance reading at 450 nm. Surviving analytic and laboratory records do not establish whether the

participant-level 8-OHdG values stored in the analysis file were back-calculated for the 1:5 dilution. Accordingly, 8-OHdG values are reported exactly as recorded in the analytic dataset and should not be interpreted as verified dilution-corrected absolute salivary concentrations. A uniform multiplicative dilution correction applied equally to all samples would add a constant to log-transformed concentrations and therefore would not change the relative GMR estimates, but it would change absolute concentrations used for cross-study comparison.

Participant-level means from two or three technical measurements were analyzed: 30 participants had three measurements and 15 had two. Median technical coefficients of variation (CVs) were 22.2% for 8-OHdG, 23.4% for F_2-isoprostane, and 10.2% for TAC. Lot-specific inserts, analyte-specific lot numbers, plate identifiers, participant-level freeze–thaw histories and complete lot-specific calibration records were not retained. Raw participant-level repeat values were also unavailable in the final reanalysis workbook, preventing a reproducible high-CV-exclusion analysis. Because plate and run assignment cannot be reconstructed, it is not possible to show that low- and high-caries participants were balanced across assay runs; a systematic run effect aligned with caries phenotype could therefore bias the observed GMR in either direction. The magnitude of that potential bias cannot be bounded from intra-assay CVs alone. Surviving records also use inconsistent physical units for TAC; TAC is therefore reported as assay units. Absolute biomarker concentrations and all secondary-biomarker findings were interpreted cautiously.

Data quality and statistical analysis

The participant-level workbook was independently audited before analysis. One source DMFT entry did not equal D + M + F; DMFT was therefore recalculated from its components for every participant. Continuous clinical variables are presented as mean ± standard deviation and were compared between schools with Mann–Whitney U tests. Binary outcomes were compared using

two-sided Fisher exact tests with exact conditional 95% confidence intervals. Available-case denominators were used for variables with missing data.

Biomarker distributions were right-skewed and natural-log transformed. The primary model was $\ln(8\text{-OHdG}_i) = \beta_0 + \beta_1 I(D_i \geq 7) + \beta_2 \text{SchoolB}_i + \beta_3 \text{Flow}_i + \varepsilon_i$. Ordinary least-squares models used HC3 heteroscedasticity-robust standard errors, and exponentiated coefficients are reported as geometric mean ratios (GMRs). Salivary flow was included as a physiological concentration-related covariate to account for variation in measured analyte concentration associated with saliva output; it was not interpreted as a mediator of the caries association. The school indicator is a contextual adjustment variable and is not interpreted as a causal school or $PM_{2.5}$ effect. Sensitivity models additionally adjusted for plaque index and then for plaque, gingivitis and sex. A complementary model substituted continuous D for $I(D \geq 7)$; because no biomarker participant had D = 3–6, its per-tooth coefficient was interpreted as a model-based summary across a discontinuous, phenotype-enriched distribution rather than a densely observed linear dose-response.

Robustness analyses refit the primary categorical model among participants resident at the current address for at least three years (n = 40), at least five years (n = 33), and after excluding the three participants with D = 1–2 so that only the observed D = 0 and D ≥ 7 phenotypes remained (n = 42). These were post hoc sensitivity analyses and did not redefine the primary estimand. Leave-one-out analyses assessed influence of individual observations. Exploratory analyses evaluated TAC and F_2-isoprostane and used Spearman correlations for unadjusted monotonic associations. Because secondary biomarkers and multiple sensitivity analyses were exploratory, they were interpreted with attention to multiplicity rather than as independent confirmatory hypotheses. Two-sided $p < 0.05$ was used for the primary analysis. No a priori power calculation was available for the biomarker substudy; its size was fixed by the archived

45-person biomarker sample, so sensitivity-subset estimates are interpreted for robustness rather than independent statistical power. Analyses were performed in Python 3.13.5 using NumPy 2.3.5, SciPy 1.17.0 and statsmodels 0.14.6. Reporting followed STROBE [22] (see Additional file 1).

Use of generative artificial intelligence

OpenAI ChatGPT (OpenAI; accessed July–August 2026) was used for English-language editing, improvement of readability, and assistance with reproducible statistical code. No generative-AI system was used as a source of study data or listed as an author. All numerical outputs, source data, references, analyses and interpretations included in the manuscript were reviewed and verified by the authors, who take full responsibility for the work.

Results

Clinical cohort

The analytic cohort comprised 190 children. Sex was recorded for 189, of whom 102 (54.0%) were female. Mean recalculated DMFT was 5.29 ± 3.49, with untreated decayed teeth contributing most of the index. Mean D and D ≥ 7 did not differ significantly between schools. Caries experience (DMFT > 0) was more frequent in School B, although this comparison was based on sparse caries-free counts and was not accompanied by a difference in mean D. The largest between-school imbalance was plaque: poor/very poor plaque affected 33.7% of School B versus 72.9% of School A. These school comparisons are descriptive and are not interpreted as environmental effects (Table 1).

Residential-duration data were available for 179/190 children. Mean residence duration was 8.54 ± 3.58 years at School B and 7.13 ± 3.94 years at School A (Welch $p = 0.014$). A total of 134 children (59 at School B and 75 at School A) had lived at their current address for at least five

years. In this subgroup, D, DMFT, mean PPD, active caries, D ≥ 7 and gingivitis still did not differ significantly by school, while plaque remained lower at School B (1.27 ± 0.64 versus 1.79 ± 0.76; $p < 0.001$; Additional file 2).

Table 1 Clinical characteristics and oral-health outcomes by school

Variable	Overall	School A	School B	p value
Participants, n	190	107	83	—
Female sex	102/189 (54.0%)	56/106 (52.8%)	46/83 (55.4%)	0.769
Decayed teeth (D)	4.46 ± 3.51	4.70 ± 3.78	4.16 ± 3.12	0.454
Missing teeth (M)	0.06 ± 0.28	0.06 ± 0.27	0.06 ± 0.29	0.963
Filled teeth (F)	0.78 ± 1.42	0.60 ± 1.34	1.01 ± 1.49	0.021
DMFT	5.29 ± 3.49	5.34 ± 3.98	5.23 ± 2.76	0.913
Caries experience (DMFT > 0)	173/190 (91.1%)	93/107 (86.9%)	80/83 (96.4%)	0.038
Upper-tail caries burden (D ≥ 7)	46/190 (24.2%)	30/107 (28.0%)	16/83 (19.3%)	0.176
Plaque index	1.57 ± 0.81	1.84 ± 0.79	1.22 ± 0.68	<0.001
Poor/very poor plaque	106/190 (55.8%)	78/107 (72.9%)	28/83 (33.7%)	<0.001
Gingivitis	44/190 (23.2%)	27/107 (25.2%)	17/83 (20.5%)	0.491
PPD mean, mm	1.92 ± 0.82	1.94 ± 0.77	1.90 ± 0.88	0.893
PPD max > 3 mm	9/190 (4.7%)	9/107 (8.4%)	0/83 (0.0%)	0.005

Continuous-variable p values are from Mann–Whitney U tests; categorical p values are from two-sided Fisher exact tests. DMFT was recalculated as D + M + F. School comparisons are descriptive. The schools had historical $PM_{2.5}$-area labels in the source study, but school and historical area label are inseparable and no causal pollution interpretation is made.

Biomarker substudy

The biomarker substudy included 45 children, 23 from School B and 22 from School A. Figure 1 shows study flow and the concentration of the biomarker sample at the lower and upper tails of D.

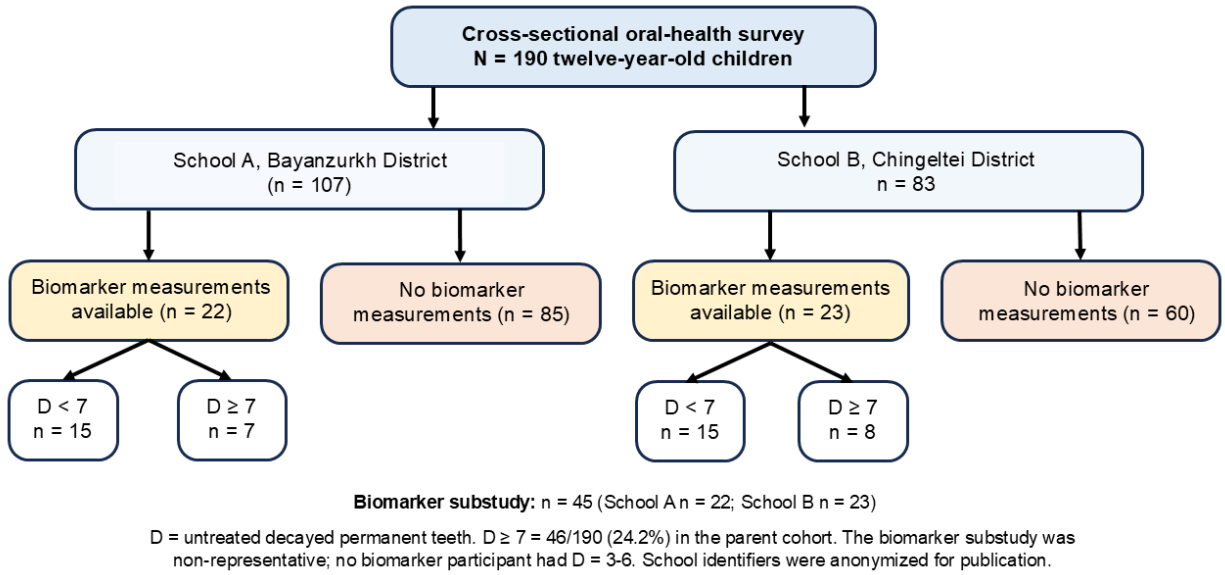


Fig.1 Study flow and biomarker-substudy composition. The clinical survey included 190 twelve-year-old children from School A (n = 107) and School B (n = 83). Biomarker measurements were available for 45 children and unavailable for 145. D denotes untreated decayed permanent teeth. D ≥ 7 represents the parent cohort's upper tail (46/190, 24.2%). The biomarker substudy was non-representative and concentrated at D = 0 and D ≥ 7. School identifiers were anonymized for publication.

Biomarker participants differed from nonparticipants in caries burden, confirming that the substudy was non-representative (Additional file 2). Mean 8-OHdG was substantially higher in both D ≥ 7 strata than in their corresponding D < 7 strata (Table 2 and Figure 2).

Table 2 Biomarker characteristics by school and untreated caries burden

Stratum	n	D	Recorded 8-OHdG assay concentration, pg/mL	F_2-isoprostane, pg/mL	TAC, assay units
School A, D < 7	15	0.20 ± 0.56	168.67 ± 67.88	30.39 ± 7.17	0.724 ± 0.388
School A, D ≥ 7	7	8.57 ± 1.51	458.71 ± 60.00	24.07 ± 5.02	0.550 ± 0.144
School B, D < 7	15	0.07 ± 0.26	203.41 ± 66.00	38.28 ± 7.53	0.833 ± 0.417
School B, D ≥ 7	8	10.00 ± 1.60	430.41 ± 29.00	21.19 ± 6.09	0.438 ± 0.133

Values are mean ± standard deviation. D denotes untreated decayed permanent teeth. The strata describe the observed criterion-enriched biomarker sample and are not population-representative groups.

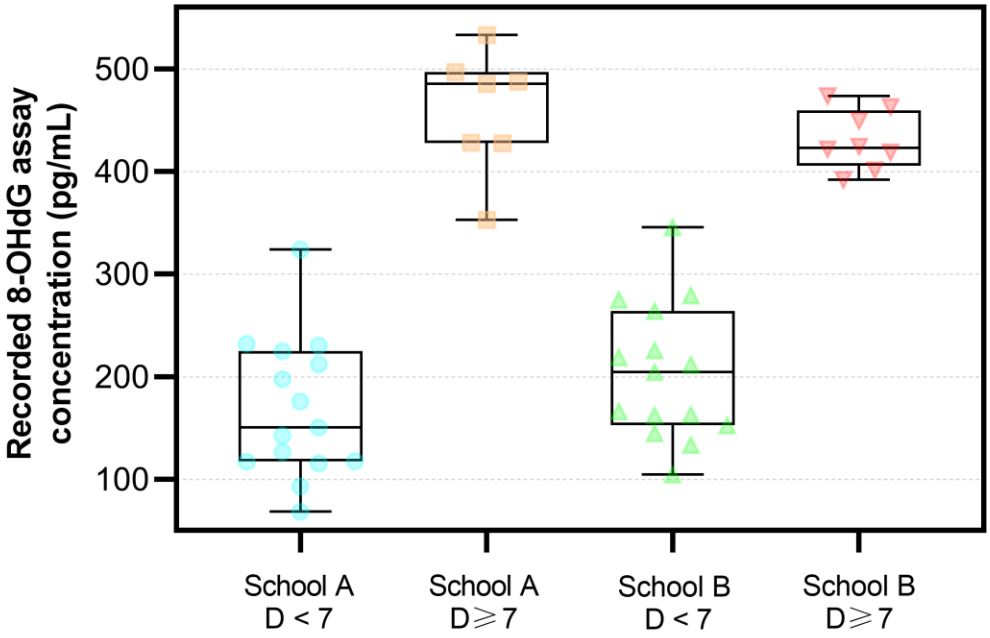


Fig.2 Recorded 8-OHdG by school and untreated caries burden. Boxes show the interquartile range, center lines show medians, whiskers extend from minimum to maximum, and symbols show individual participants. D denotes untreated decayed permanent teeth. 8-OHdG values are reported as recorded in the analytic dataset because dilution back-calculation could not be verified.

Primary 8-OHdG association and sensitivity analyses

In the primary HC3-robust log-linear model, D ≥ 7 was associated with 2.62-fold higher salivary 8-OHdG (GMR 2.619, 95% CI 2.213–3.099; $p < 0.001$) after adjustment for school and salivary flow. The school indicator was not independently associated with 8-OHdG (School B versus School A: GMR 1.167, 95% CI 0.960–1.418; $p = 0.121$). The D ≥ 7 estimate remained similar after plaque adjustment (GMR 2.564, 95% CI 2.109–3.118) and fuller adjustment for plaque,

gingivitis and sex (GMR 2.465, 95% CI 2.034–2.987), both $p < 0.001$. Leave-one-out estimates ranged from 2.535 to 2.683, indicating that no single participant determined the association.

In the complementary continuous model, each additional decayed tooth was associated with 10.5% higher recorded 8-OHdG concentration (GMR 1.105, 95% CI 1.082–1.128; $p < 0.001$). Figure 3 shows the absence of biomarker observations at D = 3–6; accordingly, the per-tooth coefficient summarizes a model across a discontinuous distribution and should not be interpreted as evidence for a densely observed linear dose-response.

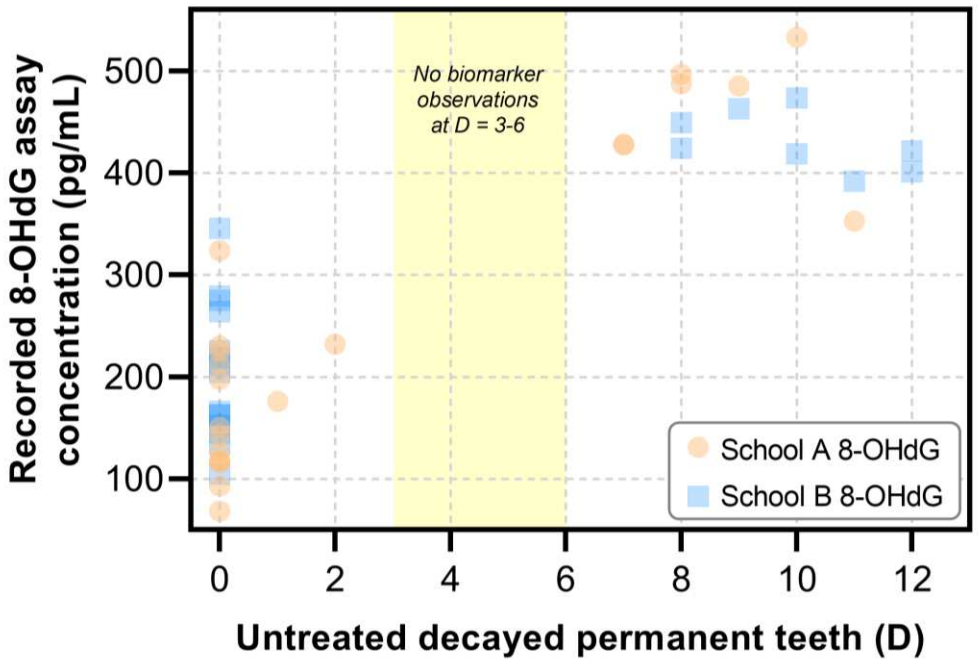


Fig.3 Observed untreated caries burden and recorded 8-OHdG. Symbols show individual biomarker-substudy participants by school. D denotes untreated decayed permanent teeth. No biomarker participant had D = 3–6, although this range was present in the parent cohort. No fitted curve is displayed.

The association remained stable in protocol-informed post hoc sensitivity analyses: residence ≥ 3 years (n = 40; GMR 2.576, 95% CI 2.151–3.086), residence ≥ 5 years (n = 33; GMR 2.539, 95%

CI 2.078–3.101), and the observed D = 0 or D ≥ 7 phenotypes only (n = 42; GMR 2.713, 95% CI 2.264–3.250); all p < 0.001. Figure 4 summarizes the primary, adjusted and restriction-based estimates, and complete coefficients are provided in Additional file 2. The primary and complementary log-linear model estimates are summarized in Table 3.

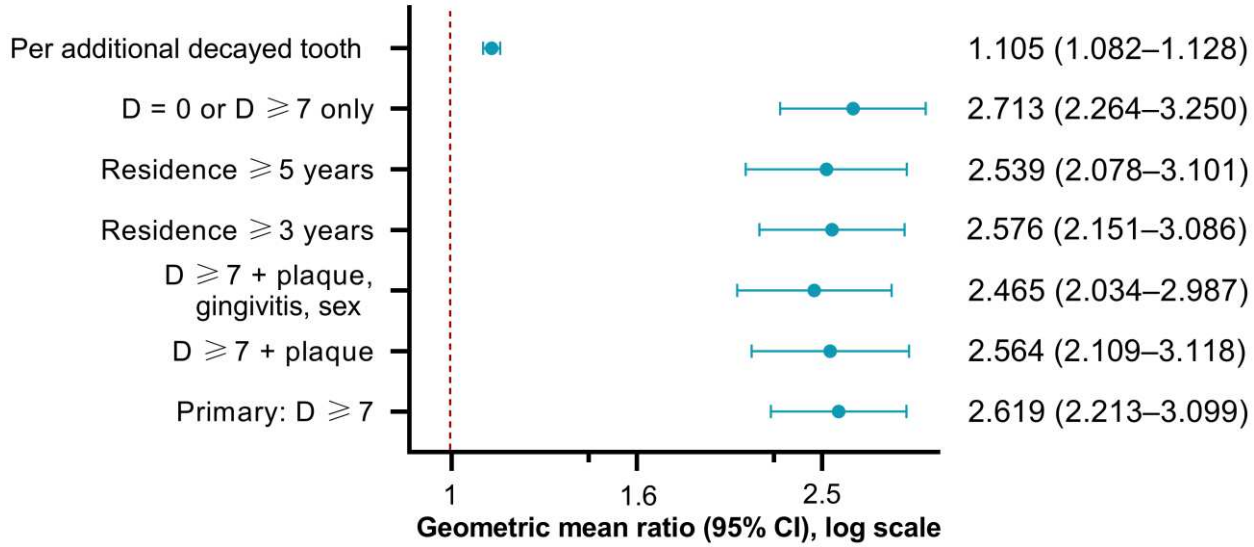


Fig.4 Primary association and robustness analyses for salivary 8-OHdG. Points represent geometric mean ratios (GMRs) and horizontal lines represent HC3-robust 95% confidence intervals. The primary model compared D ≥ 7 with D < 7 and adjusted for school and salivary flow. Sensitivity models additionally adjusted for plaque, gingivitis and sex or restricted the analysis by residential duration and observed caries phenotype. The final row shows the complementary continuous-D model per additional untreated decayed permanent tooth. The vertical dashed line indicates the null value (GMR = 1).

Table 3 Robust log-linear models for salivary 8-OHdG

Model	Predictor	GMR (95% CI)	p value	n; R^2
Primary categorical	D ≥ 7	2.619 (2.213–3.099)	<0.001	45; 0.684
Primary categorical	School B vs A	1.167 (0.960–1.418)	0.121	45; 0.684
Plaque-adjusted	D ≥ 7	2.564 (2.109–3.118)	<0.001	45; 0.687

Fully adjusted	D ≥ 7	2.465 (2.034–2.987)	<0.001	45; 0.724
Complementary continuous	D, per tooth	1.105 (1.082–1.128)	<0.001	45; 0.664

All models included school and salivary flow. The plaque-adjusted model additionally included plaque index; the fully adjusted model included plaque, gingivitis and sex. HC3 heteroscedasticity-robust standard errors were used. The school coefficient is a contextual adjustment term and cannot distinguish school from the historical area classification.

Exploratory secondary biomarkers and laboratory quality control

In exploratory continuous models, TAC decreased with increasing D (GMR per tooth 0.951, 95% CI 0.924–0.979; p = 0.001). F_2-isoprostane also decreased (GMR 0.949, 95% CI 0.925–0.974; p < 0.001), a direction that did not corroborate the higher 8-OHdG/lower TAC pattern. Potential explanations include analyte-specific saliva-matrix effects, assay cross-reactivity, pre-analytical or batch variation, and selection-induced correlations in the criterion-enriched sample. Because the archived data cannot distinguish among these possibilities, the inverse F_2-isoprostane association is considered hypothesis-generating rather than evidence of reduced lipid peroxidation in children with high caries burden. Spearman correlations and full secondary results are provided in Additional file 2.

Median technical CVs were 22.2% for 8-OHdG, 23.4% for F_2-isoprostane and 10.2% for TAC. The source data dictionary preserves the original technical-repeat variable structure, but raw participant-level repeat values and plate/run identifiers were not present in the final reanalysis workbook. A reproducible high-CV-exclusion or inter-plate sensitivity analysis therefore could not be performed. The direction of any resulting bias is not assumed: random outcome imprecision would mainly increase uncertainty, whereas systematic run effects aligned with phenotype could bias the GMR in either direction. The primary estimate is consequently robust to the observed covariates and restriction analyses, but not analytically validated against unrecorded batch effects.

Discussion

The principal finding was a large association between upper-tail untreated permanent-tooth caries burden and salivary 8-OHdG in twelve-year-old children. D ≥ 7 was associated with approximately 2.6-fold higher 8-OHdG after adjustment for school context and salivary flow. The estimate remained similar after adjustment for plaque, gingivitis and sex, after restricting analyses by residential duration, and after limiting the sample to the observed D = 0 and D ≥ 7 phenotypes. These convergent analyses support the stability of the association within this selected biomarker sample, while not converting that sample into a population-representative cohort.

The finding is consistent with a growing pediatric literature linking caries severity with altered salivary redox biology. Yergin et al. observed higher salivary 8-OHdG in severe early-childhood caries [6], and Birant et al. reported changes in salivary oxidative-stress markers after treatment of severe early-childhood caries [7]. Araujo and colleagues also demonstrated severity-related changes in salivary antioxidant activity and oxidative damage [8]. Our data extend this question to twelve-year-old children with permanent dentition, in whom untreated decay can be quantified directly through the D component of DMFT. Adult periodontal literature independently supports salivary 8-OHdG as responsive to oral inflammatory disease, but a meta-analysis found very high between-study heterogeneity [23]; direct numerical benchmarking of the present extreme-enriched pediatric GMR against those studies would therefore be inappropriate. Kurgan et al. showed that salivary 8-OHdG tracked periodontal status using both ELISA and LC–MS/MS, underscoring the value of orthogonal assay validation [24]. The cross-sectional design cannot establish whether elevated 8-OHdG is a cause, consequence or correlate of caries. Plaque-related host inflammation, bacterial metabolic activity, dietary exposures, altered salivary physiology and tissue oxidative responses could all contribute.

352 The magnitude of the categorical association is striking, but the sampling structure is essential to
353 interpretation. The biomarker sample was enriched for caries extremes: 27 children had D = 0,
354 only three had D = 1–2, and 15 had D ≥ 7, with no observations from D = 3 to 6. Importantly, the
355 parent cohort did contain substantial mid-range disease—79/190 children (41.6%) had D = 3–6
356 (Additional file 2)—showing that the gap arose from biomarker-substudy selection rather than
357 from the underlying clinical population. The primary D ≥ 7 comparison is therefore more
358 defensible than treating the continuous coefficient as a conventional dose-response. The nearly
359 unchanged estimate after restricting to D = 0 or D ≥ 7 supports internal robustness within the
360 selected phenotypes, but the very limited overlap between strata also makes unrecorded batch/run
361 alignment particularly important to exclude in future studies. The point estimate should not be
362 interpreted as a population effect size. Future studies should sample the full caries distribution
363 consecutively or randomly and prespecify the functional form of D.
364 The secondary redox markers were not fully concordant. Lower TAC with increasing D could be
365 consistent with diminished antioxidant capacity, but the literature is heterogeneous: Ahmadi-
366 Motamayel et al. reported higher salivary TAC in caries-active adolescents than in caries-free
367 controls [25]. The inverse F_2-isoprostane association in our data is likewise inconsistent with a
368 simple unified oxidative-stress mechanism. Given the relatively high technical CV, unrecorded
369 plate/run information and criterion-enriched sampling, analyte-specific matrix, assay, pre-
370 analytical, batch and selection effects remain plausible. Current manufacturer information
371 identifies SL2044Hu as an ELISA product, but current catalog specifications cannot validate the
372 unavailable lot used in this study; absolute recorded concentrations therefore should not be
373 compared directly across lots or analytical platforms. Prior work also demonstrates appreciable
374 within-subject variability in salivary oxidative-stress markers, including 8-OHdG, supporting
375 repeated standardized sampling in future studies [26]. Replication with retained plate-level data,

prespecified quality thresholds and an orthogonal method such as LC–MS/MS would materially strengthen analytical validity.

The two-school context should not be interpreted as an environmental exposure analysis. The source project originally selected one school in each historical $PM_{2.5}$ category, but the original monitoring stations, quantitative source series and participant collection dates are unavailable, and school identity is inseparable from the historical category. School was therefore used only as a contextual covariate. The marked plaque difference between schools demonstrates that school populations differed in oral-health determinants, further precluding any pollution-effect inference. Published and official sources document spatial and seasonal particulate variability in Ulaanbaatar [10-12], but those external data were not assigned to participants. The practical implication for oral-health biomarker research is that local disease status and contextual factors should be characterized before salivary redox variation is attributed to other exposures.

Strengths of the study include participant-level data auditing, display of individual biomarker observations, HC3-robust inference, adjustment for key available oral-health covariates, residence- and phenotype-restricted sensitivity analyses, recovery of detailed clinical and saliva-handling documentation, and explicit separation of the primary categorical estimand from the sparse continuous-D model. The study also contributes pediatric oral-redox data from Mongolia, where analogous 8-OHdG data in twelve-year-old children with permanent dentition are scarce.

Several limitations remain. First, the biomarker substudy was small, criterion-enriched and non-representative, and its final selection sequence cannot be fully reconstructed; effect magnitudes may therefore be amplified by phenotype selection and are not generalizable as population estimates. Second, the cross-sectional design precludes temporal or causal inference. Third, socioeconomic status, parental education, dietary sugar, fluoride exposure, household smoking, heating fuel and other potential confounders were not available. Fourth, no intra-examiner

repeatability statistic was retained. Fifth, technical CVs for 8-OHdG and F_2-isoprostane were relatively high, and lot-specific inserts, plate/run identifiers, freeze–thaw histories and participant-level transport timestamps were unavailable; systematic batch differences aligned with caries phenotype cannot be excluded or quantitatively bounded. Sixth, the archived SOP documents 1:5 dilution but the final records do not establish whether stored 8-OHdG values were back-calculated, limiting interpretation of absolute concentrations; a uniform scale factor would not alter the log-linear GMR. Seventh, the available immunoassay result has not been replicated with an orthogonal analytical method. Eighth, residence duration was missing for 11 children, although the primary association remained strong in ≥3-year and ≥5-year residence subsets. Finally, the two-school design prevents separation of school context from the historical environmental area label.

Prospective work should enroll representative samples across the full caries distribution, retain detailed pre-analytical and plate-level quality-control data, collect dietary, fluoride and socioeconomic covariates, and repeat salivary 8-OHdG after caries treatment. Longitudinal within-child measurements would be particularly valuable for determining whether successful treatment is followed by a reduction in oxidative DNA-damage markers.

Conclusions

In this exploratory, criterion-enriched biomarker substudy of twelve-year-old children, upper-tail untreated permanent-tooth caries burden ($D \geq 7$) was strongly associated with higher salivary 8-OHdG, and the association remained similar across covariate-adjusted, residence-restricted and phenotype-restricted sensitivity analyses. A complementary continuous-D model showed the same direction but crossed an unobserved mid-range of caries burden and should not be interpreted as a conventional population dose-response. These findings extend pediatric oral-redox evidence to permanent dentition and support careful assessment of active caries when

salivary oxidative DNA-damage markers are interpreted. Representative longitudinal studies are required before causal, diagnostic or population-level conclusions can be drawn.

List of abbreviations

8-OHdG: 8-hydroxy-2′-deoxyguanosine; CI: confidence interval; CV: coefficient of variation; D: decayed permanent teeth; DMFT: decayed, missing and filled permanent teeth; F: filled permanent teeth; FRAP: ferric-reducing antioxidant power; GMR: geometric mean ratio; HC3: heteroscedasticity-consistent covariance estimator type 3; M: missing permanent teeth; NAMEM: National Agency for Meteorology and Environmental Monitoring; $PM_{2.5}$: particulate matter with aerodynamic diameter ≤2.5 μm; PPD: periodontal pocket depth; TAC: total antioxidant capacity; STROBE: Strengthening the Reporting of Observational Studies in Epidemiology.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Review Committee of the Mongolian National University of Medical Sciences (Approval No. 24-25/10-01; 12 June 2025). The dissertation archive also records laboratory authorization (No. 40/25; 4 June 2025) and permission from the Ulaanbaatar education authority for research in the two schools (No. 01/843; 6 June 2025). Written informed consent for participation was obtained from parents or legal guardians, and assent was obtained from participating children.

Consent for publication

Not applicable. No identifiable individual participant information is reported.

Availability of data and materials

The datasets used and/or analysed during the current study are not publicly available because this small, school-based pediatric dataset carries a potential risk of participant re-identification and is subject to institutional and ethics-related data-sharing restrictions. De-identified data necessary to verify the reported analyses are available from the corresponding authors on reasonable request and subject to approval by the responsible institution and ethics committee.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was funded by the Science and Technology Support Fund of the Mongolian National University of Medical Sciences, grant number 25-07. The funder had no role in study design; data collection, analysis or interpretation; preparation of the manuscript; or the decision to submit the work for publication.

Authors' contributions

S.B. and D.G. conceptualized the study, formal analysis, and drafted the manuscript. N.-E.M., T.S., M.-E.U.-O., E.L., B.B. and M.J. contributed to methodology. S.B., I.B., N.B.-O., B.G.-O. and E.-O.B. contributed to investigation. S.B., D.G., N.-E.M., T.S., M.-E.U.-O., I.B., N.B.-O., B.G.-O. and E.-O.B. contributed to data curation. D.G., I.B., N.B.-O., B.G.-O. and E.-O.B. contributed to formal analysis. D.G. and D.J. critically reviewed and edited the manuscript. E.L., M.J. and D.J. supervised the work. All authors read and approved the final manuscript.

Acknowledgements

The authors thank the participating children and families, the two participating schools, and the clinical and laboratory personnel who supported data collection and sample processing. The authors also thank colleagues who assisted with archival source verification.

Additional files

Additional file 1 (.docx). STROBE checklist for cross-sectional studies. Completed reporting checklist for the present manuscript.

Additional file 2 (.docx). Supplementary statistical results and methodological provenance. Expanded parent-cohort comparisons and D distribution, biomarker distributions, complete primary and sensitivity model coefficients, correlations, laboratory quality-control summaries, residential-duration analyses, and a methodological-provenance audit.

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546 **Figure legends**

547 **Fig.1** Study flow and biomarker-substudy composition. The clinical survey included 190 twelve-
548 year-old children from School A (n = 107) and School B (n = 83). Biomarker measurements were
549 available for 45 children and unavailable for 145. D denotes untreated decayed permanent teeth.
550 D ≥ 7 represents the parent cohort's upper tail (46/190, 24.2%). The biomarker substudy was
551 non-representative and concentrated at D = 0 and D ≥ 7. School identifiers were anonymized for
552 publication.

553 **Fig.2** Recorded 8-OHdG by school and untreated caries burden. Boxes show the interquartile
554 range, center lines show medians, whiskers extend from minimum to maximum, and symbols
555 show individual participants. D denotes untreated decayed permanent teeth. 8-OHdG values are

reported as recorded in the analytic dataset because dilution back-calculation could not be verified.

Fig.3 Observed untreated caries burden and recorded 8-OHdG. Symbols show individual biomarker-substudy participants by school. D denotes untreated decayed permanent teeth. No biomarker participant had D = 3–6, although this range was present in the parent cohort. No fitted curve is displayed.

Fig.4 Primary association and robustness analyses for salivary 8-OHdG. Points represent geometric mean ratios (GMRs) and horizontal lines represent HC3-robust 95% confidence intervals. The primary model compared $D \geq 7$ with $D < 7$ and adjusted for school and salivary flow. Sensitivity models additionally adjusted for plaque, gingivitis and sex or restricted the analysis by residential duration and observed caries phenotype. The final row shows the complementary continuous-D model per additional untreated decayed permanent tooth. The vertical dashed line indicates the null value (GMR = 1).

Supplementary Files

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- 03AdditionalFile1STROBEChecklist0816.docx
- 04AdditionalFile2SupplementaryResults0816.docx