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The salivary oxidative stress profile shifts in pediatric dentofacial infection: a multivariate analysis of seven markers

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Title Page

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Abstract

Objectives: Acute dentofacial infection is a common reason for pediatric emergency presentation, yet decisions on admission, drainage, and antibiotics rest on clinical assessment alone. This study evaluated whether a multivariate salivary oxidative stress profile can distinguish acute dentofacial infection from uncomplicated dental caries in children.

Materials and Methods: Saliva was collected from 80 children: 48 with clinically diagnosed dentofacial infection and 32 with caries only. Seven markers along with two derived indices were evaluated. Group separation was tested by permutational multivariate analysis, adjusting for age, sex, and number of extracted teeth, followed by principal component analysis and univariate testing with multiple-testing correction.

Results: The overall marker profile differed significantly between groups ($F(1,60) = 3.96$, $R^2 = 0.062$, $p = 0.003$), and remained robust after covariate adjustment. Two components explained 60.3% of the variance: PC1 opposed antioxidant enzymes (catalase -0.88; glutathione reductase -0.82) to lipid peroxidation (MDA +0.72). PC2 captured oxidant load (TOS +0.85; SOD Mn -0.65). In univariate analysis, only SOD CuZn ($\delta = -0.48$, $p = 0.001$) and MDA ($\delta = +0.48$, $p = 0.001$) reached significance, both loading onto PC1. Global indices (TAC, TOS, OSI) showed no group differences.

Conclusions: Acute dentofacial infection shifts the pediatric salivary oxidative stress profile along a specific enzyme-to-damage axis rather than global summary metrics.

Clinical Relevance: Because the redox response may be at least two-dimensional, single-marker salivary studies may yield conflicting results. Profile-level analysis is required before salivary redox measurement can inform triage decisions.

Keywords: Saliva; Oxidative stress; Dentofacial infection; Dental caries; Malondialdehyde; Multivariate analysis

Introduction

Dental caries is one of the most prevalent chronic diseases in children [1]. Untreated lesions can lead to pulpal necrosis and periapical inflammation, from which infection may extend into the surrounding soft tissues and fascial spaces of the head and neck [2, 3]. The resulting acute dentofacial infections, such as periapical abscess, odontogenic fistula, or cellulitis, are a common reason for emergency admission in pediatric dentistry and maxillofacial surgery [4, 5]. In children, although serious complications are uncommon, airway compromise and orbital or intracranial extension remain recognised problems [3]. Despite this, decisions on admission, drainage, or antibiotic use rely on clinical assessment alone, without an objective laboratory measure of local inflammatory burden [6].

Such a measure may lie in the biology of the infection itself. Acute suppurative infection recruits neutrophils and macrophages, whose respiratory burst generates reactive oxygen species (ROS) as an antimicrobial mechanism [7]. ROS damage host lipids, proteins and nucleic acids, and are counteracted by enzymatic and non-enzymatic antioxidant defences. A sustained imbalance between the two is termed oxidative stress [8, 9]. Saliva is a suitable medium for assessing it locally: it contacts the affected tissues directly, contains locally produced and serum-derived components, and can be collected non-invasively and repeatedly [10]. The last point is decisive in pediatric practice, where venepuncture is poorly tolerated. Salivary oxidative stress has been studied extensively in dental caries and in periodontal disease [11–14], but dentofacial infections, the next stage of the same disease process, have received less attention [15, 16], largely for practical reasons. Patients are acutely unwell, treatment cannot be delayed to obtain a sample, and the pre-analytical requirements of these assays are demanding in an emergency setting.

The existing caries literature cannot simply be extrapolated to fill this gap. Caries is a chronic, biofilm-mediated process of localised demineralisation, whereas acute dentofacial infection is a suppurative process with regional and occasionally systemic inflammatory involvement [3]. Whether the two produce comparable redox changes is an open question. Adult data are equally difficult to transfer, as children differ from adults in salivary composition, antioxidant capacity, and dentition [17].

A second difficulty concerns measurement. Oxidative stress cannot be observed directly in vivo, and every parameter that can be assayed is a proxy with its own biological meaning and analytical limitations [18, 19]. Assay protocols, sample handling, and normalization differ substantially between laboratories, making absolute values difficult to compare across studies. Consequently, reviews have been unable to pool results quantitatively [10, 12] and reported single-marker associations frequently conflict [12, 20].

These are, however, the limitations of individual markers, not of the underlying biology, since the association between oxidative stress and inflammatory disease is itself well established [21, 22]. One alternative is to treat oxidative stress as a latent construct of which the measured parameters are correlated realisations. Correlation between markers then becomes informative rather than redundant, and combining them attenuates marker-specific error. Eldridge et al. [23] modeled oxidative stress in this way in colorectal adenoma and found a strong association at the construct level, while the same biomarkers modeled separately were weakly and mostly non-significantly associated. We therefore specified profile-level multivariate analysis as the primary approach, using permutation-based methods appropriate to the available sample size.

This study asked whether the salivary oxidative stress profile differs between children with acute dentofacial infection and children with uncomplicated dental caries. The primary question was multivariate: whether the joint distribution of the measured parameters separates the two groups. Secondary questions were which individual markers differ, whether previously reported markers replicate in an independent cohort, how many dimensions the panel resolves into, and whether any marker is associated with clinical or demographic characteristics.

Methods

Study population and sample collection

The study was conducted at the Children's Maxillofacial Surgery Clinic, Chair of Pediatric Surgery, Medical University of Silesia, Katowice, Poland. Children presenting with a clinically diagnosed acute dentofacial infection were eligible for the disease group. Those with uncomplicated dental caries and no history of dentofacial infection served as controls. No participant had received pharmacological treatment, antibiotics or anti-inflammatory drugs in particular, in the month before admission. Unstimulated whole saliva was collected fasting between 8:00 and 11:00 a.m., after rinsing the mouth with water and a 10-minute wait. Samples were centrifuged at 3,000 rpm for 10 min at 4 °C in a Centurion centrifuge (Centurion Scientific Ltd., Chichester, UK) and stored at -80 °C. The cohort comprised 80 children (disease n = 48, controls n = 32; Table 1 and Online Resource 1, Table S1).

Analysis of the oxidant-antioxidant balance

Superoxide dismutase (SOD) isoenzymes (SOD Mn, SOD CuZn) were determined according to Oyanagui [24], with absorbance read at 550 nm and potassium cyanide used to inhibit SOD CuZn. One nitrite unit (NU) was the amount of enzyme producing 50% inhibition of nitrite ion formation. Activities are expressed as NU/mg protein. Catalase was measured after Johansson and Borg [25], quantifying the formaldehyde generated spectrophotometrically at 550 nm after derivatization with Purpald® (Avantor Performance Materials Poland, Gliwice, Poland) and expressed as IU/g protein. Glutathione reductase (GR) was assayed after Richterich and Colombo [26] and expressed as IU/g protein. Total antioxidant capacity (TAC) and total oxidant status (TOS) were determined by Erel's methods [27] on an automated analyser (JANUS™; PerkinElmer Inc., Waltham, USA): TAC from the absorbance change at 660 nm against Trolox® (Sigma-Aldrich Chemie, Steinheim, Germany) (mmol/g), TOS from ferric ions quantified with xylenol orange at 560 nm against H_2O_2 (µmol/g). Malondialdehyde (MDA) was quantified fluorometrically as thiobarbituric acid-reactive substances (TBARS) after Ohkawa et al. [28] (excitation 515 nm, emission 552 nm; tetraethoxypropane standard), and is reported as MDA equivalents in µmol/g.

Statistical analysis

We measured seven independent oxidative stress parameters (catalase, glutathione reductase, TAC, TOS, SOD Mn, SOD CuZn, MDA) and computed two indices (total SOD = SOD Mn + SOD CuZn; oxidative stress index OSI = TOS / TAC / 10). Two subjects returned a negative TAC reading (below the assay floor). TAC and OSI were therefore recoded as missing for them in all inferential analyses. Univariate analyses used pairwise-complete cases. Multivariate analyses require a full distance matrix and are listwise-complete unless specified otherwise. OSI and total SOD were used in the univariate tests only.

Distributions were characterised by group and tested for normality (Online Resource 1, Table S1). Twelve of the 18 cells rejected normality and most markers were substantially right-skewed, so all inference is rank- and permutation-based. Continuous variables were summarised as median (IQR) and compared with a rank-based two-sample independence test with a Monte Carlo null (B = 25 000, two-sided) and Cliff's δ as the effect size. Categorical variables were summarised as n (% of column total) and compared with Fisher's exact test on the full levels × groups table, with Cramér's V.

The primary multivariate test was PERMANOVA (*adonis2* from *vegan*, 24 999 permutations) on Euclidean distances between subjects computed from the seven independent markers standardised to Z-scores. The primary model specified before analysis consisted only of the grouping variable. Several covariates were also tested in secondary models. PERMANOVA is sensitive to differences in multivariate dispersion as well as in location [29], so the homogeneity of dispersion was tested explicitly with betadisper and a permutation test of the distances to the group centroid (24 999 permutations).

Principal component analysis described the latent structure shared by the markers, using the same seven markers but all 80 subjects. Each was first Yeo-Johnson transformed, with a single maximum-likelihood λ per marker (Online Resource 1, Fig. S1). Missing values were filled by regularised iterative PCA on the standardised matrix, the number of components in the reconstruction chosen

by cross-validation: 5% of observed cells were blanked at random and imputed at each candidate number over 100 replications, and the number minimising prediction error (four) was retained. Because imputed cells are shrunk towards the fitted structure and lie closer to the components than real measurements, the decomposition was repeated with NIPALS fitted directly to the incomplete matrix; the two solutions agreed on every component (loading congruence ≥ 0.991, absolute score correlation ≥ 0.976).

The PCA used the correlation matrix, so that components are not driven by the markers with the largest units, and the seven eigenvalues sum to seven. Factorability was assessed before extraction with Bartlett's test of sphericity and the Kaiser-Meyer-Olkin measure, overall and per marker (Online Resource 1, Table S2). Component retention was specified as Horn's parallel analysis against a permutation null (25 000 permutations of each marker's own values), retaining components whose eigenvalue exceeded the 95th percentile of the corresponding null eigenvalue. Loadings are the eigenvector scaled by the square root of its eigenvalue, i.e. the marker-component correlation on [-1, 1]. Communalities, squared cosines and percentage contributions on the retained plane are given in (Online Resource 1, Table S2).

Each of the nine markers was related to eight items of subject metadata, pooled across groups and pairwise-complete; oral anomaly was excluded because it is strongly confounded with group (Table 1). Effect sizes were Spearman's ρ for continuous metadata, with p obtained by permuting the pairing (B = 25 000), and Cliff's δ for categorical metadata, using the same permutation test as above. Both are rank-based and bounded on [-1, 1], and are therefore plotted on a common axis. Precision was assessed with 95% bootstrap confidence intervals (B = 25 000) and multiplicity controlled by Benjamini-Hochberg within each metadata column.

All analyses were performed using R 4.5.2. The full analysis code is available at https://github.com/Polymerase3/oxidation-stress.

Results

Multivariate oxidative stress profile

The oxidative stress profile taken as a whole separated the two groups (Fig. 1a). Among the 62 subjects with a complete set of the seven independent markers (21 control, 41 disease), PERMANOVA on the standardised Euclidean distance gave $F(1, 60) = 3.96$, $R^2 = 0.062$, $p = 0.003$ (B = 24 999). The Mahalanobis sensitivity analysis, which accounts for the correlation among markers, agreed ($F = 3.53$, $p < 0.001$).

The accompanying test of multivariate dispersion was not significant (Fig. 1b): mean distance to the group centroid was 2.16 in controls and 2.49 in the disease group, and the difference was not statistically significant ($F = 1.77$, $p = 0.185$; Mahalanobis $F = 0.25$, $p = 0.614$). No evidence of unequal dispersion was found, so the significant PERMANOVA is most consistent with a genuine shift in the center of the oxidative stress profile: subjects in the disease group have systematically different marker values.

The separation is not attributable to the measured covariates. Age, sex, number of teeth extracted, and number of teeth with caries were each not significant when fitted alone ($p = 0.968$, 0.424, 0.497, and 0.989), and the group term retained its magnitude and significance under every adjustment (marginal tests: $p = 0.002$ adjusted for age, $p = 0.003$ for sex, $p = 0.001$ for teeth extracted, $p = 0.002$ in the full group + age + sex + teeth extracted model). $R^2 = 0.062$ indicates that the group difference is robust to adjustment, but moderate in magnitude.

Individual markers

Two of the nine markers reached significance across the marker panel (Table 1). SOD CuZn was lower in the disease group (Fig. 1c), whereas MDA was higher in the disease group (Fig. 1d). For SOD CuZn, $\delta = -0.483$ means that if one draws one disease and one control subject at random, the disease subject has the lower SOD CuZn activity in about 74% of such pairs and the higher value in about 26%. For MDA the picture is the mirror

image. Both values sit just above 0.474, the conventional cutoff for a large effect, so these are substantial separations by rank-based standards.

Structure of the marker panel

The seven independent markers were intercorrelated enough to support a component solution: Bartlett's test rejected sphericity ($\chi^2(21) = 195.1$, $p < 0.001$), while the Kaiser-Meyer-Olkin measure was 0.604 overall, with per-marker values from 0.702 down to 0.444 (Online Resource 1, Table S2). Horn's parallel analysis retained two components, carrying 36.6% and 23.7% of the total variance (60.3% cumulatively; Online Resource 1, Fig. S2).

The correlation matrix (Fig. 2a) is dominated by one strong pair: catalase and glutathione reductase, $\rho = 0.85$, both of which correlate negatively with MDA (-0.47 and -0.34). TOS runs negatively against all three SOD measures (-0.35 to -0.45) and TAC correlates positively with MDA ($\rho = 0.42$). The two components divide the panel almost cleanly (Fig. 2b-d). PC1 is defined by catalase (-0.88) and glutathione reductase (-0.82) opposed to MDA (+0.72), which together contribute 76.9% of it (Fig. 2d). In the correlation circle these three lie along the x axis, with catalase and GR nearly superimposed and MDA pointing opposite (Fig. 2b). PC1 therefore contrasts antioxidant enzyme activity with lipid peroxidation. PC2 is built predominantly by TOS (+0.85, 43.9%) and SOD Mn (-0.65, 25.4%), which contribute essentially nothing to PC1 (0.25% and 0.05%) and run near-vertically in the circle - it represents oxidant load. SOD CuZn alone loads appreciably on both (-0.60 and -0.49). Communalities ranged from 0.823 for catalase to 0.394 for TAC, so the retained plane describes catalase and GR well and TAC poorly (Online Resource 1, Table S2). The solution was stable: NIPALS fitted directly to the incomplete matrix reproduced it (loading congruence ≥ 0.991), no arch artefact was present (PC2 on PC1 + PC1², $R^2 = 0.004$, $p = 0.564$).

Exploratory associations with clinical metadata

Seventy-two marker × metadata associations were examined (9 markers × 8 metadata variables), pooled across groups. Two reached nominal significance at $p < 0.05$, and neither survived multiplicity correction. No cell in the entire matrix reached BH $q < 0.10$ (Fig. 3a).

Glutathione reductase and atopic comorbidity (n = 80; 75 without, 5 with): $\delta = -0.60$ means that in about 80% of (non-atopic, atopic) subject pairs the atopic child has lower glutathione reductase activity, compared with 20% in which they have higher (Fig. 3b). Nominally, it is a large effect, and the direction is consistent (the point estimate and the whole confidence interval lie below zero). Catalase and the number of teeth extracted (n = 80): Spearman's $\rho = 0.24$, 95% CI [0.03, 0.43]. This is a weak positive monotone association (Fig. 3c), meaning higher catalase activity in children with more extracted teeth, with roughly 6% of the rank variance shared.

Discussion

The present study demonstrates that salivary oxidative stress profiles in children with acute dentofacial infections differ from those in children with uncomplicated dental caries, and this profile-level separation is driven by a shift in multivariate location ($F = 3.96$, $p = 0.003$; betadisper $p = 0.185$; Fig. 1). While the proportion of explained variance (6.2%) represents a moderate effect size, this is consistent with modeling a complex, latent biological construct using downstream proxy markers. It also aligns with previous literature. Tóthová et al. [30] reported that the carious index (CIdx) accounted for 8.6% of the variance in salivary advanced oxidation protein products (AOPPs) among pediatric participants. Similarly, studies in adult non-smokers demonstrated modest relationships, with the CIdx explaining 3.2% of the variance in AOPPs and 2.5% variance in thiobarbituric acid reactive substances (TBARS) [31]. A study of 46 children conducted by Poimenidou et al. [32] using 8-isoprostane reported an effect size of $r = 0.163$ for the caries-active vs caries-free comparison (≈2.7% of variance) and a correlation with caries experience of $r = -0.204$ (≈4%), neither significant [32]. A larger pediatric case-control study (n = 163) [33] found no significant differences in AOPP, TBARS, or TAC between caries-free and caries-affected children aged 3-18. Only in the adolescent subgroup did TAC differ significantly. That subgroup difference corresponded to roughly 15-16% of variance [33].

Several biological and methodological factors explain why individual salivary oxidative stress markers consistently yield low R^2 values in observational regression models. First, salivary redox parameters suffer from extreme baseline variability. In healthy individuals sampled longitudinally, inter-individual variation reaches approximately 60% for markers such as AOPPs and advanced glycation end-products, and 30-40% for TAC [34]. When the between-subject coefficient of variation ranges from 30% to over 85% [35], and single measurements cannot reliably capture unstable, short-lived markers like MDA [35], baseline physiological scatter leaves little remaining variance for a disease to explain. Second, the very large effect sizes reported in some meta-analytic reviews - such as a standardised mean difference of 2.66 for salivary TAC in pediatric caries [11], which would theoretically imply roughly 64% of variance explained - appear inflated and can reflect small-study effects and extreme methodological heterogeneity ($I^2 > 99\%$) [36]. Indeed, primary clinical studies frequently report conflicting directions for TAC and fail to replicate single-marker differences across independent cohorts [11, 32, 33, 36].

In univariate analyses, two of the nine markers survived correction, and they moved in opposite directions: SOD CuZn was lower in children with acute infection ($\delta = -0.48$, $p = 0.001$) and MDA was higher ($\delta = +0.48$, $p = 0.001$). Both reach the conventional boundary for a large rank separation, and both carry their largest loading on PC1 (-0.60 and +0.72), the axis opposing antioxidant enzyme activity to lipid peroxidation. SOD CuZn regulates the superoxide/hydrogen peroxide balance in neutrophils during the NADPH oxidase-driven respiratory burst, and loss of its activity increases lipid peroxidation [7, 19]. As a capacity measure, its level falls through consumption, reduced replacement, and oxidative inactivation [19, 37]. Because this oxidative modification persists until the enzyme is degraded, whereas radical-derived species clear within minutes, SOD CuZn reflects oxidant exposure accumulated over a longer period. MDA, conversely, is a stable end product of polyunsaturated fatty acid peroxidation and a widely used index of membrane damage, but its rapid formation and clearance integrate a much shorter timeframe [9, 19]. The two are not successive steps in one causal chain, since neutrophil-initiated peroxidation proceeds largely through myeloperoxidase-derived oxidants rather than superoxide directly [7, 37] - they are better read as parallel indices. Reduced antioxidant capacity alongside active damage is what would be expected where a chronically stressed compartment meets an acute suppurative event, i.e., a child with dentofacial infection on a background of long-standing caries. The PCA supports this reading as a coherence check: the same two markers sit at opposite ends of PC1, a component extracted without reference to group membership and from a differently constituted sample (all 80 subjects, transformed and partly imputed). This is not a second independent test, but it indicates that the contrast is a structural property of how these markers covary across children generally, which matters in a literature where single-marker findings reverse between cohorts [11, 12, 14, 36].

Parallel analysis retained two components carrying 60.3% of the variance, and the markers divided between them almost without overlap (Fig. 2). PC1 was defined by catalase (-0.88) and glutathione reductase (-0.82) opposed to MDA (+0.72), which together contribute 76.9% to this principal component: an antioxidant-enzyme versus lipid-peroxidation axis, on which a child high in peroxidation product is low in enzymatic capacity. It is also consistent with the results presented in the univariate analysis, where the two markers that differed between groups, CuZn-SOD and MDA, load on PC1 with opposite sign. PC2 was built by TOS (+0.85) and SOD Mn (-0.65), both essentially absent from PC1 (0.25% and 0.05%), and reads as an oxidant-load axis. SOD CuZn alone loaded on both (-0.60 and -0.49), sitting between them rather than on either - consistent with an enzyme that both consumes superoxide and is degraded by sustained exposure to it. That a second dimension survived the permutation null at all is a notable point: a panel measuring one underlying quantity with different instruments would have collapsed onto a single component. This is the empirical counterpart to the argument set out in the Introduction. If the markers indexed a single latent state, as for example in the model assumed by Eldridge et al. [23] they would be interchangeable, and disagreement between studies would reflect only measurement error. A two-dimensional structure implies instead that a study sampling one axis can legitimately fail to replicate a study sampling the other. It also explains the flat global indices, since TAC and TOS sit on opposite sides of the retained plane and OSI is their ratio.

The two available direct comparisons are with our own earlier cohort [15] and with Zarban et al. [16]. The former sampled the same clinical groups at the same clinic using the same assays, and reported catalase and glutathione

reductase as the discriminating markers, both substantially higher in infection, with MDA, TOS, TAC and all SOD measures flat. The present cohort reverses this: catalase and glutathione reductase did not differ ($p = 0.334$ and 0.208), while SOD CuZn and MDA, previously null, now survive correction, though in both cases the earlier point estimates already pointed the same way (SOD CuZn 1.68 vs 2.53 NU/mg; MDA 0.26 vs 0.23 μmol/g), consistent with a real but modest difference that a smaller sample could not detect. Notably, all four markers sit on PC1, with catalase, GR and MDA anchoring its opposite poles (-0.88, -0.82, +0.72), so the two studies are not reporting four independent disagreements but one contrast read with the disease group displaced towards a different end of it. Zarban et al. [16] supplies the one point of agreement: in 20 children with primary tooth abscess compared with 21 caries-free controls, salivary total antioxidant capacity did not differ before or after extraction ($p = 0.81$ and 0.75), matching our flat TAC ($\delta = 0.00$) and the flat TOS and OSI that follow from it. Global capacity assays appear insensitive to acute dentofacial infection across methods and cohorts. What replicates, then, is that the salivary redox state is altered and that summary indices fail to capture it - not which individual enzyme moves.

These findings should be considered in light of several limitations. The design is cross-sectional and single-center, with saliva sampled once at presentation, so the temporal ordering implied above cannot be established. The sample is modest (80 children, 62 with a complete marker set for the multivariate test), limiting power for covariate-adjusted models. Group allocation rested on clinical diagnosis without microbiological confirmation, and controls had caries rather than being caries-free, so the contrast isolates the acute suppurative component rather than caries itself. Oral anomaly was unevenly distributed between groups and excluded from the metadata set. Three markers (TOS, TAC and SOD Mn) returned values at the assay floor, including two negative TAC readings, and PC2 is built largely from two of them, so it may partly reflect a shared detection limit rather than shared biology.

Conclusions

Acute dentofacial infection is associated with a shift in the salivary oxidative stress profile of children. It is carried by a specific enzyme-to-damage contrast, lower SOD CuZn with higher MDA, while the global indices TAC, TOS and OSI remain flat. The panel resolves into two independent dimensions, which may explain the inconsistency of single-marker studies: two cohorts sampling different axes may disagree without either being wrong. Salivary redox measurement cannot yet inform decisions on admission or drainage, but it should be pursued at profile level rather than marker by marker, and with repeated sampling across treatment.

Human Ethics and Consent to Participate

This study was performed in line with the principles of the Declaration of Helsinki (1964) and its later amendments. Approval was granted by the Bioethical Committee of the Medical University of Silesia in Katowice, Poland (reference number PCN/0022/KB1/1/20). All participant recruitment, clinical procedures, sample collection and laboratory analyses were carried out in Poland. Written informed consent was obtained from the parents or legal guardians of all individual participants included in the study. Assent was obtained from participating children where age-appropriate.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Availability of data and materials

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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CRediT author contribution statements

- Conceptualization: BEOW, RD, JZF, NSI
- Data curation: BEOW, MFK
- Formal analysis: MFK
- Investigation: BEOW, RD, JZF, NSI
- Software: MFK
- Supervision: BEOW
- Visualization: MFK
- Writing - original draft: MFK, JZF, BEOW
- Writing - review & editing: BEOW, RD, JZF, NSI

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487

488 **Table 1. Subject characteristics and salivary oxidative-stress markers by group.** Continuous variables were
489 tested with a two-sided Wilcoxon-Mann-Whitney permutation test (B = 25 000 Monte Carlo resamples) and
490 summarised by Cliff's δ, oriented disease vs control, so a positive δ means the value runs higher in the disease
491 group. Categorical variables were tested with Fisher's exact test on the full levels × groups table and summarised
492 by Cramér's V. The p-values shown are unadjusted. Column headers give each group's maximum n (32 control,
493 48 disease). Rows are pairwise-complete.

Variable		Control (*n* = 32)	Disease (*n* = 48)	*p*	Effect size
Age (years)		8.21 (6.19)	7.17 (5.29)	0.366	-0.12
Sex					
	F	11 (34.38%)	23 (47.92%)	0.257	0.13
	M	21 (65.62%)	25 (52.08%)		

Teeth extracted (n)	2.00 (3.25)	4.00 (5.00)	0.053	0.25
Teeth with dental caries (n)	1.00 (2.25)	2.00 (3.00)	0.422	0.10
Neurodevelopmental comorbidity				
No	26 (81.25%)	46 (95.83%)	0.054	0.24
Yes	6 (18.75%)	2 (4.17%)		
Atopic comorbidity				
No	30 (93.75%)	45 (93.75%)	>0.999	0.00
Yes	2 (6.25%)	3 (6.25%)		
Gastrointestinal comorbidity				
No	30 (93.75%)	47 (97.92%)	0.561	0.11
Yes	2 (6.25%)	1 (2.08%)		
Other comorbidity				
No	28 (87.50%)	42 (87.50%)	>0.999	0.00
Yes	4 (12.50%)	6 (12.50%)		
Oral anomaly				
No	24 (75.00%)	47 (97.92%)	0.002	0.36
Yes	8 (25.00%)	1 (2.08%)		
Catalase (IU/g protein)	66.30 (51.24)	60.21 (64.72)	0.334	-0.13
Glutathione reductase (IU/g protein)	7.03 (6.76)	5.20 (6.87)	0.208	-0.17
TAC (mmol/g protein)	0.21 (0.55)	0.28 (0.44)	0.980	0.00
TOS (µmol/g protein)	0.00 (0.76)	0.00 (1.00)	0.871	0.02
SOD total (NU/mg protein)	10.14 (4.06)	7.88 (4.51)	0.054	-0.28
SOD Mn (NU/mg protein)	2.14 (3.48)	3.62 (4.49)	0.430	0.12
SOD CuZn (NU/mg protein)	6.38 (3.63)	4.89 (2.35)	0.001	-0.48
MDA (µmol/g protein)	0.32 (0.25)	1.23 (1.87)	0.001	0.48

OSI (AU)	0.00 (0.67)	0.00 (0.45)	0.812	-0.03

Note: data are presented as median (*IQR)* for continuous variables and *n* (% of column) for categorical variables. Cliff's delta was used as the effect size coefficient for continuous variables, and Cramér's *V* for categorical variables.

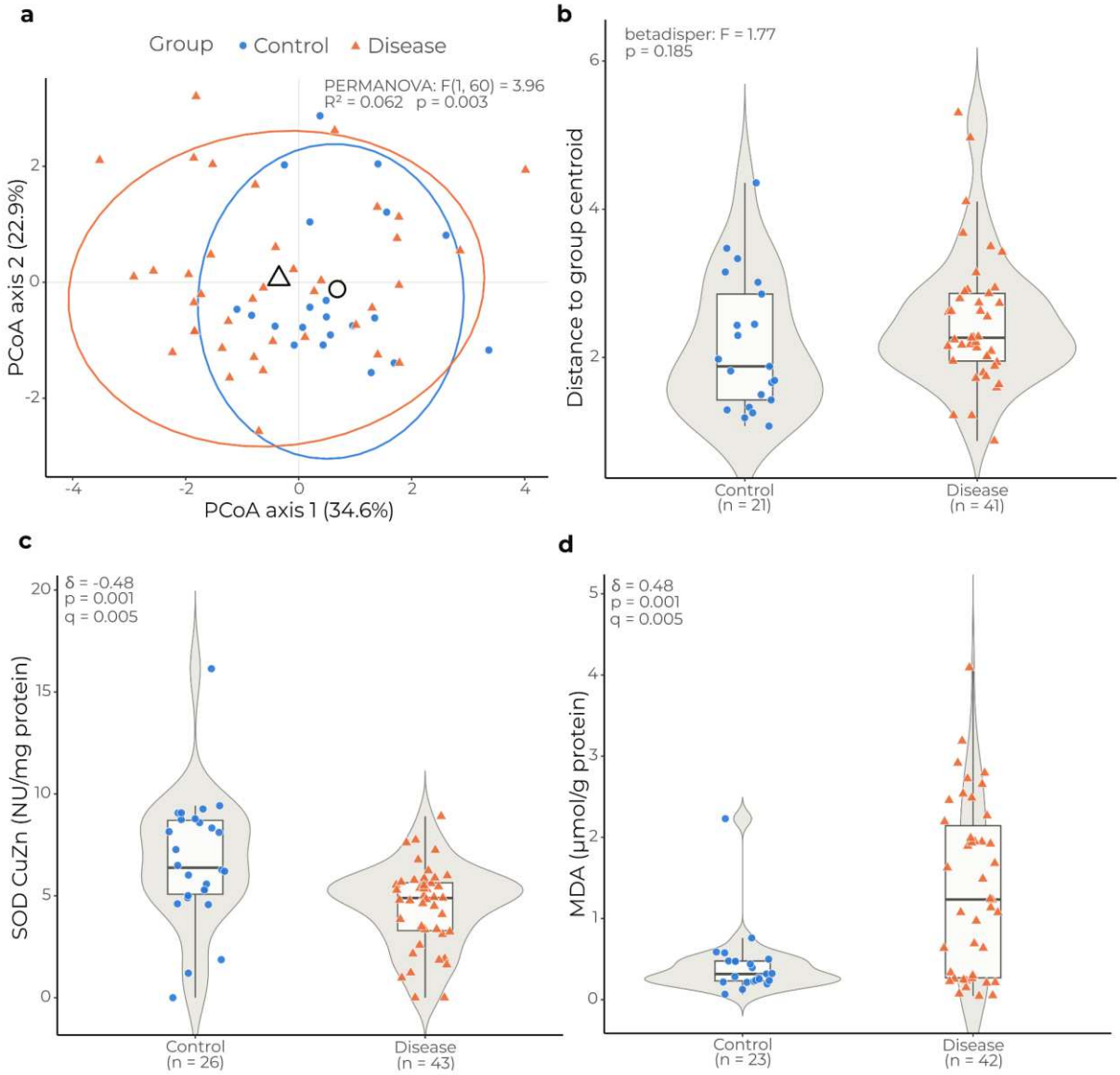


Fig. 1 Salivary oxidative stress profiles differ between groups, and the difference is a shift in location carried by SOD CuZn and MDA (a) Principal coordinates analysis of the seven independent markers (Euclidean distance on standardised values; OSI and total SOD excluded as linear combinations of the other markers, n = 62). Points are individual subjects, ellipses are 95 % multivariate-*t* regions, and the large outlined markers are group centroids. Axis labels give the percentage of total variance each coordinate carries. The panel presents all listwise-complete observations. (b) Distance from each subject to its own group centroid. PERMANOVA responds to differences in dispersion as well as in location, so the non-significant test here is what licenses reading panel A as a shift in center. Violin = kernel density, box = median and IQR, points = individual subjects. (c, d) The two markers of the nine that show statistical significance: SOD CuZn, lower in the disease group, and MDA, higher. Both panels are pairwise-complete.

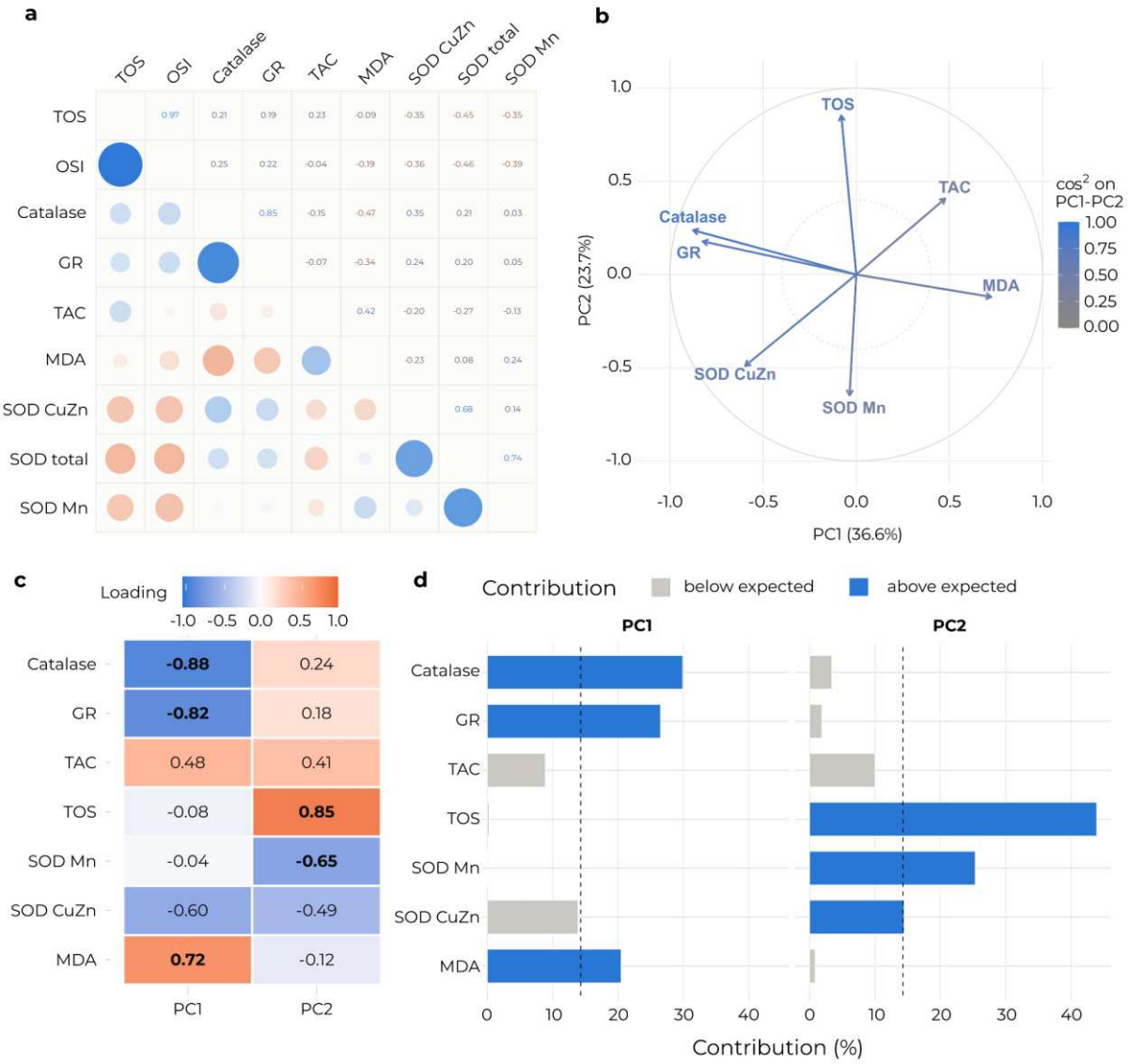


Fig. 2 The seven-marker panel reduces to two components carrying 60.3 % of total variance, and the markers divide sharply between them: an antioxidant-enzyme axis and an oxidant-load axis (a) Spearman correlation matrix of all nine markers in the whole sample. Below the diagonal, circle area and colour give the strength and direction of each association. Above the diagonal correlation coefficients are presented as numbers. Variables are ordered by hierarchical clustering of the matrix (Ward D2). (b) Correlation circle: each marker's loadings on the retained PCA plane, drawn as arrows from the origin. The angle between two arrows approximates the correlation between the markers (near-parallel = strongly positive, opposed = strongly negative, perpendicular = uncorrelated). Distance from the origin is the arrow's square root of the communality – the proportion of a marker's variance retained by the two components solution. The outer circle is defined by a radius |loading| = 1, whereas the inner dashed circle marks the |0.40| threshold. Colour of the arrow gives each marker's $\cos^2$ (its quality of representation on the retained plane). Catalase and GR lie almost parallel to each other along the negative PC1 axis, consistent with their ρ = 0.85 in panel a. MDA points in the opposite direction along the same axis: PC1 separates the antioxidant enzymes from lipid peroxidation. TOS and SOD Mn run almost parallel to the ordinate in opposite directions and are nearly absent from PC1, so PC2 is essentially the axis these two define. (c) Loadings as a heat map. A loading is the correlation between a marker and a component. Values with |loading| ≥ 0.60 are in bold. (d) Each marker's percentage contribution to PC1 and PC2. The dashed line marks 14.3 %, the contribution expected if every

525 marker weighted the component equally. Catalase (29.9 %), GR (26.5 %) and MDA (20.5 %) build PC1,
526 together accounting for 76.9 % of it. The direction reverses on PC2, where TOS (43.9 %) and SOD Mn
527 (25.4 %) dominate. SOD CuZn is the only marker contributing near-equally to both (13.9 % and 14.5 %).
528

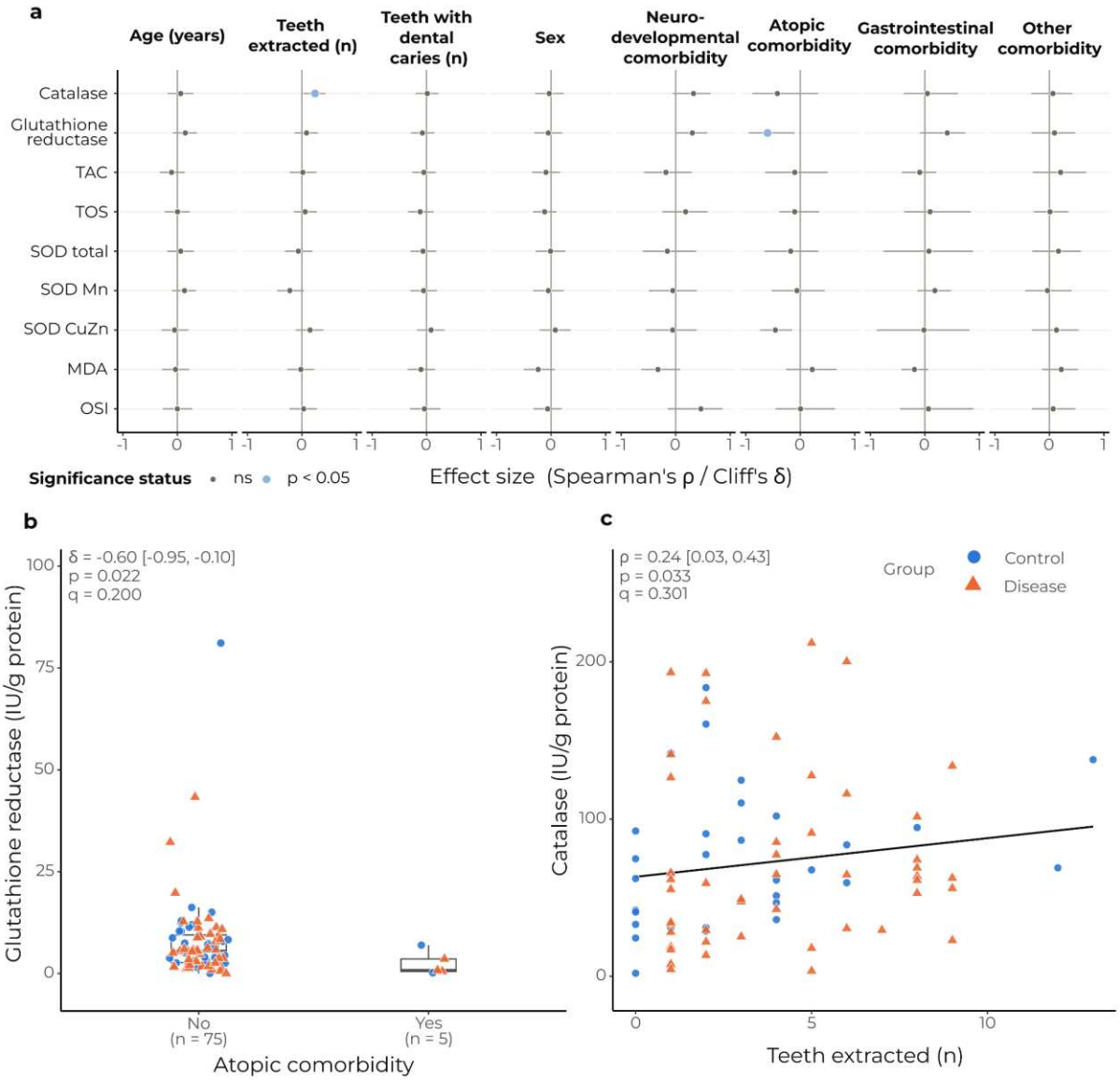


Fig. 3 No oxidative-stress marker is associated with subject metadata once multiplicity within each metadata variable is accounted for (a) All 72 marker × metadata associations as one dot-and-whisker grid: nine markers (rows) against eight metadata variables (columns), pooled across groups and pairwise-complete (n = 57-80). Point = effect size, whisker = 95 % bootstrap interval (B = 25 000). Spearman's ρ is used for continuous metadata and Cliff's δ for categorical; the two share an axis because both are rank-based and bounded on [-1, 1]. Fill marks the evidence tier from the two-sided permutation p-value (*B* = 25 000; grey - ns; blue - $p < 0.05$). Two cells reach $p < 0.05$ uncorrected, and neither survives correction: no cell in the matrix reached $q < 0.10$ (smallest $q = 0.200$). Oral anomaly is excluded from the metadata set because it is confounded with group (25 % of controls vs 2 % of cases, Fisher $p = 0.002$), so a pooled association with it would largely re-express the group difference. (b, c) The two cells with the smallest uncorrected p, shown as raw data. (b) Glutathione reductase by atopic comorbidity. δ is oriented as Yes vs No, so the negative value means lower activity in children with an atopic comorbidity. (C) Catalase by number of teeth extracted: line = ordinary least-squares fit, no confidence band, line is descriptive only.

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