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Altered Salivary Oxidative Stress-Related Enzymatic Activities in Victims of Interpersonal Physical Violence: A Preliminary Case–Control Study

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Abstract

Interpersonal physical violence may trigger complex physiological responses involving psychological stress, inflammation, and altered redox regulation. However, salivary enzymatic activities associated with recent violence exposure remain poorly characterized. This preliminary study aimed to evaluate salivary myeloperoxidase (MPO), catalase (CAT), and superoxide dismutase (SOD) activities in individuals exposed to interpersonal physical violence and to explore their discriminatory performance. This observational case–control study included 52 participants: 26 individuals recently exposed to interpersonal physical violence and 26 healthy controls. Salivary MPO, CAT, and SOD activities were compared between groups. General linear models were applied to adjust for age, gender, body mass index, and sample collection time. Exploratory analyses examined enzymatic activities according to violence characteristics and sampling intervals. Receiver operating characteristic (ROC) analysis was performed to assess apparent discriminatory performance. Compared with controls, violence-exposed individuals exhibited significantly higher MPO activity (258.14 ± 32.89 vs. 163.25 ± 14.15 U/L; $p < 0.001$) and CAT activity (154.70 ± 25.99 vs. 71.02 ± 15.73 U/mL; $p < 0.001$), corresponding to increases of 58.1% and 117.8%, respectively. Conversely, SOD activity was significantly lower (6.91 ± 1.60 vs. 12.20 ± 1.34 U/mL; $p < 0.001$), representing a 43.4% reduction. These associations remained significant after multivariable adjustment (all $p < 0.001$). All three enzymes demonstrated complete apparent separation between groups (AUC = 1.000 for each marker); however, the reproducibility of this finding requires independent validation. Recent interpersonal physical violence was associated with a distinct salivary enzymatic profile characterized by increased MPO and CAT activities and decreased SOD activity. These findings suggest alterations in redox-related enzymatic pathways, potentially reflecting responses to acute

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physical and psychological stress. Nevertheless, larger longitudinal studies incorporating direct oxidative damage biomarkers and independent validation are warranted.

Keywords: interpersonal violence; oxidative stress; saliva; myeloperoxidase; catalase; superoxide dismutase; redox homeostasis; salivary biomarkers

1. Introduction

Interpersonal physical violence represents a major public health concern with consequences extending beyond immediate physical injuries to include potentially persistent psychological, neuroendocrine, inflammatory, and metabolic disturbances [1–3]. The World Health Organization estimated that approximately 840 million women aged 15 years or older worldwide had experienced physical and/or sexual intimate partner violence, non-partner sexual violence, or both at least once in their lives. This estimate illustrates the public health burden of these forms of violence [4]. Exposure to violence constitutes a complex stressor involving the simultaneous activation of physiological responses to tissue injury and psychological distress. Although the health consequences of interpersonal violence have traditionally been investigated in relation to traumatic injuries and mental health outcomes, increasing attention has been directed toward identifying measurable biological alterations associated with violent experiences [2,3,5]. In this context, saliva has emerged as a promising biological matrix because of its non-invasive collection, accessibility, and suitability for repeated measurements [6,7].

Recent interpersonal physical violence can trigger overlapping responses to perceived threat, pain, and tissue injury. Perceived threat activates the sympathetic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis, leading to the release of catecholamines and glucocorticoids. Previous investigations of violence-related biological responses have predominantly focused on neuroendocrine markers, particularly cortisol. In our recent systematic review, we identified slightly lower morning cortisol concentrations and significantly elevated evening concentrations in saliva of violence-exposed individuals, suggesting potential disturbances in HPA axis regulation [3]. Furthermore, investigations of violence exposure in different populations have demonstrated associations with alterations in cortisol regulation and other physiological stress responses [5,8]. Together, these findings support the potential value of salivary biomarkers in violence-related research while emphasizing the limitations of relying exclusively on neuroendocrine indicators. Salivary measurements may reflect aspects of these responses, alongside contributions from the salivary glands and the local oral environment.

When tissue injury occurs, local damage signals can recruit and activate innate immune cells, which release inflammatory mediators and generate reactive oxygen species. Neuroendocrine signaling can in turn modify immune cell activity and cellular metabolism, linking the response to psychological threat with inflammatory and redox regulation. Beyond hormonal alterations, acute physical and psychological stress may influence inflammatory signaling and oxidative processes. Oxidative stress is currently understood as a disturbance in redox signaling and control that may result in molecular damage when oxidant generation exceeds the capacity of antioxidant defense systems [9,10]. Reactive oxygen species (ROS), including superoxide anions and hydrogen peroxide, participate in physiological signaling, immune defense, and cellular homeostasis. However, excessive or inadequately regulated ROS production may promote oxidative modifications of proteins, lipids, and nucleic acids. The biological response to stress involves interactions between the HPA axis, sympathetic nervous system, inflammatory mediators, and redox-sensitive signaling pathways [11,12]. These mechanisms may be particularly relevant

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following interpersonal physical violence, where psychological distress and physical injury may simultaneously contribute to inflammatory and oxidative responses.

Experimental studies have demonstrated that acute psychological stress can influence salivary oxidative stress-related parameters [13,14]. These findings indicate that individual salivary redox-related biomarkers may exhibit distinct responses to psychological stress and that changes in specific enzymatic activities may not necessarily be reflected by global antioxidant indices. Nevertheless, the biological response to interpersonal physical violence may differ from experimentally induced psychological stress because of the additional contribution of tissue injury, pain, and inflammatory activation.

Among the enzymatic components involved in redox regulation, myeloperoxidase (MPO), catalase (CAT), and superoxide dismutase (SOD) represent functionally distinct but interconnected pathways. MPO is a heme-containing enzyme predominantly associated with neutrophils and other myeloid cells. In the presence of hydrogen peroxide, MPO catalyzes oxidation reactions, leading to the generation of reactive oxidants, particularly hypochlorous acid. Although these reactions are essential for antimicrobial defense, excessive MPO-dependent oxidant production may contribute to oxidative modification of biological molecules and inflammatory tissue injury [15–17].

In contrast to MPO, CAT and SOD constitute major components of enzymatic antioxidant defense. SOD catalyzes the dismutation of superoxide anions into hydrogen peroxide and molecular oxygen, whereas CAT facilitates the decomposition of hydrogen peroxide into water and oxygen [18–20]. These reactions contribute to maintaining redox homeostasis and limiting excessive ROS accumulation. Importantly, antioxidant enzyme activities may respond differently to physiological stress depending on oxidant availability, enzyme regulation, oxidative inactivation, and the biological compartment investigated. Consequently, the simultaneous measurement of MPO, CAT, and SOD may provide a broader characterization of redox-related enzymatic alterations than the assessment of individual biomarkers alone.

Interpersonal physical violence can elicit an early physiological response involving psychological distress, pain, tissue injury, and inflammation. These components may overlap in individuals assessed during the first 48 h after the incident. Despite increasing interest in salivary biomarkers of psychological stress and violence exposure, to our knowledge, no previous study has jointly assessed salivary MPO, CAT, and SOD activities during the early period after interpersonal physical violence. Building upon our previous research on salivary hormonal markers of violence and MPO-related inflammatory processes, the present study extends this investigative framework toward the enzymatic components of redox regulation. Measuring MPO, CAT, and SOD activities together allows us to examine whether an oxidant-generating pathway and two complementary antioxidant pathways show different patterns of activity. If independently validated against clinically relevant comparison groups, such a profile could complement the description of physiological responses during medico-legal assessment.

Therefore, the aim of this preliminary observational study was to compare salivary MPO, CAT, and SOD activities between individuals recently exposed to interpersonal physical violence and healthy controls. Additionally, we evaluated whether the observed associations persisted after adjustment for age, gender, BMI, and saliva collection time; explored differences according to violence characteristics and the elapsed time between the violent incident and sample collection; and assessed the apparent exploratory discriminatory performance of the investigated enzymes. We hypothesized that recent interpersonal physical violence would be associated with a distinct pattern of salivary oxidative stress-related enzymatic activities, potentially reflecting alterations in oxidant-generating and antioxidant defense pathways.

2. Materials and Methods

The present study is a continuation of the investigations performed as part of the SALIVA (Saliva Analysis in Interpersonal Violence Assessment) project. The following section outlines the fundamental methodological aspects required for a comprehensive evaluation of the study. A detailed account of the experimental design and procedures is provided in the opening paper that launches the project series [21]. The present study included the same participant cohort, with identical characteristics and sample size, but extended the analysis to additional markers, specifically enzymes involved in salivary oxidative and antioxidant status. These measurements therefore extend the investigation beyond the endocrine stress response, providing complementary information on the biological changes associated with recent exposure to physical violence. The contribution of the present study lies in the assessment of a different biological domain within an already characterized cohort.

2.1. Participants

Participants in the study group were recruited from individuals referred to the Department of Forensic Medicine, Poznan University of Medical Sciences, for medico-legal examination following interpersonal physical violence. Patient inclusion and exclusion criteria are presented in Table 1. Eligibility was limited to individuals assessed within 48 h of the incident and included those assessed during either the first or the second 24-h period. The control group was recruited concurrently from healthy volunteers, age-, gender- and BMI-frequency-matched. The study was conducted between March 2026 and June 2026, and was approved by the institutional Bioethics Committee (approval no. 311/25; 8 May 2025).

Table 1. Participants' inclusion and exclusion criteria.

Aspect	Inclusion	Exclusion
Consent	written informed consent	lack of consent
Purpose of examination	patient referred by police after physical violence	other medico-legal examinations
Age	18 and over	less than 18
Gender	male, female	gender not disclosed
Chronic occupational stress	no	yes (e.g., soldiers, police officers, members of other uniformed services, prosecutors)
Interval of violent incident	48 h or less	more than 48 h
Time of sampling	between 8:30 and 10:30 a.m.	except from 8:30 and 10:30 a.m.
Corticosteroid use	no	yes
Endocrine disease	no	yes
Autoimmune disorders	no	yes
Oral hygiene	proper oral hygiene	unsatisfactory oral hygiene or damage to the mucosa of oral cavity

Given the exploratory nature of the SALIVA project and the specific recruitment setting involving individuals undergoing forensic examination shortly after violent incidents, no formal a priori sample-size calculation was performed. The sample size was determined by the number of eligible participants recruited during the predefined study period. The present analysis included the same 26 cases and 26 controls as the previous SALIVA study.

2.2. Interview and Examination

Following the qualification process, eligible participants underwent a thorough clinical evaluation, which included a physical examination and a review of medical history. Data concerning the context of the violent event were systematically documented using a standardized questionnaire. The medical assessment captured details on pre-existing comorbidities, current pharmacotherapy, smoking habits, and last oral intake interval. The clinical examination encompassed basic measurements including height and weight, basic hemodynamic parameters including blood pressure and heart rate, and an inspection of the oral cavity and its mucosa. Furthermore, incident-specific parameters were recorded, detailing the date, location, and interpersonal relationship between the victim and the perpetrator. Incidents were grouped as domestic violence (DV), intimate partner violence (IPV), urban violence (UV)/assault, or other. Subsequent medico-legal examinations were conducted to document external injuries alongside a comprehensive review of all incident-related medical documentation. Cases involving oral mucosal damage or dental trauma that risked blood contamination of the collected specimens were excluded. The interval between the incident and saliva collection was used in the exploratory timing analysis.

Severity of the injuries was classified as minor, moderate, or severe in accordance with Articles 156 and 157 of the Polish Criminal Code and established forensic medical practice in Poland. When no external injuries were found during examination, despite a confirmed incident of physical violence, they were defined as a violation of physical integrity in accordance with Article 217 of the Polish Criminal Code. The injuries and circumstances of the incident were also evaluated to determine whether they had posed an immediate risk of death or grievous bodily harm, as defined in Article 160 of the Polish Criminal. Participants in the control group underwent the same medical history assessment and physical examination protocol.

2.3. Sample Collection and Preparation

Saliva samples were obtained using the passive drool technique. A physician explained the collection procedure to each participant beforehand to ensure appropriate sampling. Participants were asked to lean forward and allow saliva to drain into sterile, graduated 50 mL Falcon tubes for 10 min. Collection took place at least 1 h after the last oral intake to limit food-related interference. Sampling began no sooner than 15 min after the forensic medical examination had commenced, providing time for participants to become accustomed to the setting and reducing the possible effects of examination-related psychological stress. Before sampling, participants rinsed their mouths freely with a moderate amount of water to reduce the risk of contamination. Samples were excluded if their volume was below 0.5 mL or if blood and/or food contamination was present.

After collection, samples were kept temporarily at 4 °C in a dedicated refrigerator before being transported to the laboratory of the Department of Forensic Medicine at Poznan University of Medical Sciences for processing. Saliva was centrifuged at 3000× *g* for 20 min at 4 °C, and the supernatant was retained for analysis. Aliquots were stored at −70 °C until antioxidant enzyme activities were measured. All measurements were performed in duplicate. Colorimetric assays and ELISA tests were conducted using an AMR-100T microplate reader (Hangzhou Allsheng Instruments Co., Hangzhou, China).

Saliva samples were pseudonymized and labelled with unique study codes. Assay results were linked to the corresponding participant records using these codes. Laboratory personnel were blinded to group allocation.

2.4. Biochemical Analysis of Enzyme Activity

Salivary myeloperoxidase activity was determined using a commercially available Myeloperoxidase Activity Assay Kit (Elabscience, Wuhan, China, SKU No. E-BC-K074-M), in accordance with the manufacturer's instructions. MPO assay was based on the enzymatic oxidation–reduction (redox) reaction principle. Myeloperoxidase reduced hydrogen peroxide to a complex. The complex reacts with o-dianisidine (as hydrogen donor) to produce a yellow product which had a maximum absorption peak at 460 nm. The activity of MPO was calculated indirectly by measuring the OD value at 460 nm. The saliva sample volume used for analysis was 2 μL, diluted with 50 μL of PBS buffer. The amount of MPO in 1 L of sample that catalyzes decomposition of 1 μmol H_2O_2 at 37 °C for 30 min was defined as 1 unit. Detection range was from 19.42–893.31 U/L. Intra-assay was 5.4%. Inter-assay was 7.3%.

Salivary total superoxide dismutase activity was determined using a commercially available Superoxide Dismutase Activity Assay Kit (Elabscience, Wuhan, China, SKU No. E-BC-K020-M, WST-1 method), in accordance with the manufacturer's instructions. This assay was based on the inhibition of water-soluble tetrazolium salt-1 (WST-1) reduction by superoxide dismutase. Superoxide anions generated in the reaction system reduced WST-1 to a water-soluble formazan dye. SOD activity negatively correlated with the formazan dye formation, which was quantified colorimetrically at 450 nm. The saliva volume used for analysis was 20 μL. When the SOD inhibition ratio in this reaction system reached 50%, the corresponding enzyme level was 1 SOD activity unit (U). Analytical range was from 0.2–14.4 U/mL. The analytical sensitivity of the assay was 0.2 U/mL. SOD intra-assay was 2.9%. Inter-assay was 3.7%.

Salivary catalase activity was determined using a commercially available Catalase Activity Assay Kit (Elabscience, Wuhan, China, SKU No. E-BC-K031-M), in accordance with the manufacturer's instructions. CAT catalyzed the decomposition of H_2O_2 into oxygen and water. After a defined reaction period, the reaction was stopped by addition of the chromogenic reagent system. Residual H_2O_2 reacted with ammonium molybdate to form a yellowish complex. The absorbance of this complex was measured at 405 nm. Catalase activity was determined from the difference between the control and sample absorbance values ($\Delta A = OD_{control} - OD_{sample}$) using the slope of an H_2O_2 standard curve (1 mmol/mL H_2O_2 standard solution with double-distilled water diluent to a serial concentration). A larger decrease in residual H_2O_2 corresponded to greater catalase activity. The sample volume used for analysis was 20 μL. Sample concentrations were calculated using a standard calibration curve (0, 10, 20, 30, 40, 50, 60, 100 μmol/mL). The amount of CAT in 1 mL of sample that decomposed 1 μmol H_2O_2 per minute at 37 °C was defined as 1 unit. Analytical range was up to 150 U/mL with a quantification limit of 1.12 U/mL. CAT concentrations exceeding the stated analytical range were calculated by extrapolating the calibration curve. Intra-assay was 3.9% and inter-assay was 7.7%.

2.5. Diagnostic Test Framework

Classification of exposure status served as the reference standard and was determined independently of the biomarker measurements. The violence-exposed cohort comprised individuals referred by law enforcement for medico-legal examination subsequent to reported interpersonal physical violence; these participants underwent a standardized evaluation encompassing medical history, physical examinations, injury documentation, and review of relevant incident records. Control subjects reported no recent exposure to interpersonal physical violence. Results were not used to establish exposure status in terms of legal procedural activities. The reporting of this preliminary diagnostic accuracy study was guided, where applicable, by the Standards for Reporting Diagnostic Accuracy Studies (STARD) recommendations.

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2.6. Statistical Analysis

Continuous variables are presented as means with standard deviations (SDs). Distributional normality was evaluated using the Shapiro–Wilk test. Because the analyzed variables were consistent with a normal distribution, differences between the study and control groups were assessed using the independent-samples Student's *t*-test. Enzyme activities across violence categories were compared using the Kruskal–Wallis test because normality was not supported within the small subgroups.

General linear models (GLMs) were fitted to determine whether associations between violence exposure and salivary enzyme activities persisted after adjustment for potential confounders. Each enzyme activity was treated as the dependent variable, with group (study vs. control), age, gender, body mass index (BMI), and sample collection time included as predictors. Group and gender were specified as categorical factors, while age, BMI, and collection time were entered as continuous covariates. Regression coefficients (β) were reported alongside their standard errors and 95% confidence intervals (95% CIs).

Distributions of enzyme activities were illustrated using violin plots overlaid with box plots and individual data points, both for the study and control groups and across violence categories: assault, domestic violence (DV), intimate partner violence (IPV), and other.

Associations between enzyme activities and the interval since the violent incident were examined using Spearman's rank correlation coefficient. Within the study group, these relationships were also displayed in scatter plots with locally estimated scatterplot smoothing (LOESS) curves to explore possible nonlinear patterns.

Receiver operating characteristic (ROC) analysis was used to assess the ability of salivary enzyme activities to distinguish individuals exposed to violence from matched controls. Discrimination was quantified using the area under the ROC curve (AUC) and its 95% CI. Optimal cut-off values were selected by maximizing the Youden index, and the associated sensitivity and specificity were calculated. Apparent sensitivity and specificity at the selected cut-offs were reported with two-sided exact Clopper–Pearson 95% CIs, treating these thresholds as fixed. Bootstrap resampling with 1000 replicates was applied to quantify uncertainty in ROC-derived estimates and obtain 95% CIs for the optimal cut-offs. For markers showing complete separation in the observed data, bootstrap resampling retained this separation, resulting in degenerate AUC confidence intervals.

Statistical significance was set at alfa = 0.05. Data were analyzed using Statistica 13.3 software (StatSoft, Cracow, Poland) and MedCalc 23.4.0 software (MedCalc Software Ltd., Ostend, Belgium).

No artificial intelligence tools were used for literature screening, study selection, data extraction, scientific content generation, evidence synthesis, interpretation, or any methodological or scientific decision-making. Artificial intelligence tools were used exclusively for post-writing linguistic refinement (grammar and stylistic editing), as disclosed in the Acknowledgements [22].

3. Results

3.1. Characteristics of Study Population

The study included 52 participants, comprising 26 individuals exposed to interpersonal physical violence and 26 healthy controls. The groups were comparable in terms of age (38.46 ± 14.83 vs. 37.54 ± 14.53 years, respectively; $p = 0.822$) and body mass index (BMI; 23.34 ± 5.07 vs. 24.62 ± 4.49 kg/m^2, respectively; $p = 0.338$).

Significant differences were observed in cardiovascular parameters. Individuals exposed to physical violence exhibited higher systolic blood pressure (139 ± 14 vs. 122 ± 10 mmHg; $p < 0.001$), diastolic blood pressure (86 ± 11 vs. 77 ± 7 mmHg; $p = 0.002$), and pulse

rate (85 ± 11 vs. 76 ± 10 beats/min; $p = 0.005$) compared with controls (Table 2). The mean time from violent incident to saliva collection was 21.8 h (range: 8.5–43.5).

Table 2. Demographic, clinical and biochemical characteristics in individuals exposed to interpersonal physical violence and healthy controls.

	Study Group $n = 26$		Control Group $n = 26$		p-Value
	Mean	SD	Mean	SD	
age	38.46	14.83	37.54	14.53	0.822
BMI	23.34	5.07	24.62	4.49	0.338
systolic pressure	139	14	122	10	<0.001
diastolic pressure	86	11	77	7	0.002
pulse	85	11	76	10	0.005
MPO, U/L	258.14	32.89	163.25	14.15	<0.001
CAT, U/mL	154.70	25.99	71.02	15.73	<0.001
SOD, U/mL	6.91	1.60	12.20	1.34	<0.001

3.2. Differences in Salivary Enzymatic Activities Between Groups

Significant differences in the salivary activities of all three investigated enzymes were observed between individuals exposed to interpersonal physical violence and healthy controls (Table 2, Figure 1).

Salivary myeloperoxidase (MPO) activity was approximately 1.58-fold higher in the violence-exposed group than in controls (258.14 ± 32.89 vs. 163.25 ± 14.15 U/L; $p < 0.001$), corresponding to a relative increase of approximately 58.1%.

An even greater difference was observed for catalase (CAT) activity, which was approximately 2.18-fold higher among violence-exposed individuals compared with controls (154.70 ± 25.99 vs. 71.02 ± 15.73 U/mL; $p < 0.001$), representing an increase of approximately 117.8%.

In contrast, superoxide dismutase (SOD) activity was significantly lower in the violence-exposed group than in controls (6.91 ± 1.60 vs. 12.20 ± 1.34 U/mL; $p < 0.001$), corresponding to a reduction of approximately 43.4%.

The violin plots demonstrated marked separation between the study and control groups for all three enzymes, with higher MPO and CAT activities and lower SOD activity consistently observed among violence-exposed individuals (Figure 1). These findings indicate a distinct pattern of salivary oxidative stress-related enzymatic activities associated with recent exposure to interpersonal physical violence.

3.3. General Linear Model Analysis

General linear models (GLMs) were applied to assess whether the observed differences in salivary MPO, CAT, and SOD activities remained significant after adjustment for age, BMI, sample collection time, and gender (Table 3).

For MPO, exposure to physical violence remained strongly and positively associated with enzymatic activity after adjustment for the included covariates ($\beta = 0.853$; 95% CI: 0.721–0.984; $p < 0.001$). The model demonstrated substantial explanatory power, with an adjusted R^2 of 0.796. Sample collection time was also positively associated with MPO activity ($\beta = 0.146$; 95% CI: 0.010–0.283; $p = 0.037$), whereas age, BMI, and gender were not statistically significant predictors (all $p > 0.05$).

Similarly, exposure to physical violence was significantly associated with higher CAT activity after adjustment for potential confounders ($\beta = 0.886$; 95% CI: 0.756–1.016; $p < 0.001$). The corresponding model yielded an adjusted R^2 of 0.801. No statistically

significant associations were observed for age, BMI, sample collection time, or gender (all $p > 0.05$).

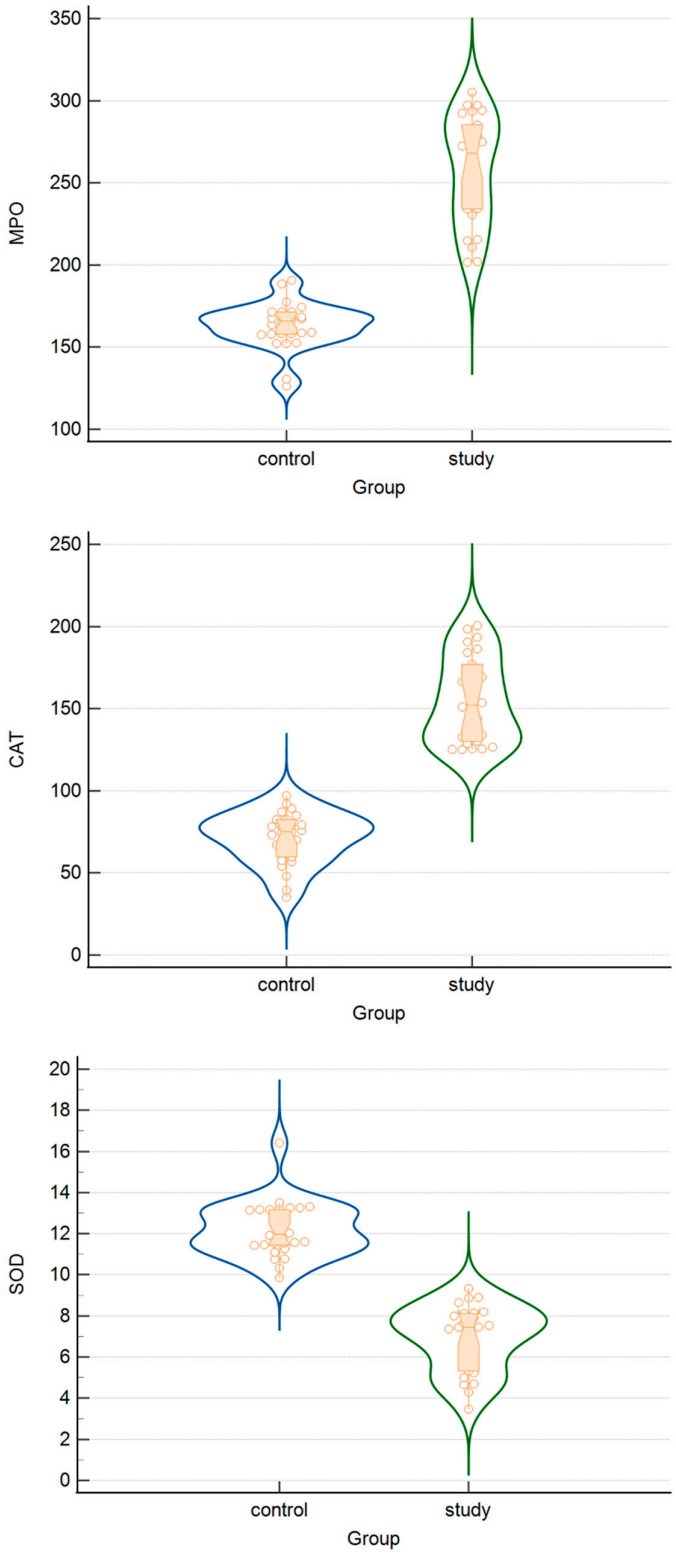


Figure 1. Violin plots illustrating the distributions of salivary MPO [U/L], CAT [U/mL], and SOD [U/mL] activities in individuals exposed to interpersonal physical violence and healthy controls.

For SOD, exposure to physical violence remained strongly associated with lower enzymatic activity (β = −0.869; 95% CI: −1.011 to −0.726; $p < 0.001$). The model demonstrated an adjusted R^2 of 0.762. None of the remaining covariates showed statistically significant associations with SOD activity (all $p > 0.05$).

Overall, exposure to interpersonal physical violence was consistently associated with altered salivary enzymatic activities across all three GLMs. These associations remained statistically significant after adjustment for demographic characteristics, BMI, and sample collection time, indicating that the observed between-group differences were not explained by the included covariates.

Table 3. General linear models (GLMs) with standardized regression coefficients (β) evaluating the associations between exposure to interpersonal physical violence and salivary MPO, CAT, and SOD activities after adjustment for age, body mass index, sample collection time, and gender.

#	Beta	SE	−95% CI	+95% CI	*p*-Value
MPO—adjusted R^2 0.796					
intercept					<0.001
age	−0.110	0.070	−0.251	0.030	0.121
BMI	−0.076	0.068	−0.214	0.062	0.272
collection time	0.146	0.068	0.010	0.283	0.037
group—study	0.853	0.065	0.721	0.984	<0.001
gender—female	−0.013	0.065	−0.143	0.118	0.848
CAT—adjusted R^2 0.801					
intercept					<0.001
age	−0.067	0.069	−0.206	0.072	0.335
BMI	−0.094	0.068	−0.231	0.042	0.169
collection time	−0.022	0.067	−0.157	0.114	0.751
group—study	0.886	0.065	0.756	1.016	<0.001
gender—female	−0.053	0.064	−0.183	0.076	0.410
SOD—adjusted R^2 0.762					
intercept					<0.001
age	−0.092	0.075	−0.244	0.060	0.230
BMI	−0.023	0.074	−0.172	0.126	0.758
collection time	−0.045	0.074	−0.193	0.103	0.545
group—study	−0.869	0.071	−1.011	−0.726	<0.001
gender—female	0.051	0.070	−0.090	0.193	0.469

3.4. Exploratory Analysis of Enzymatic Activities According to Violence Characteristics and Sampling Interval

The distributions of salivary MPO, CAT, and SOD activities according to the recorded categories of interpersonal violence are illustrated in Figure 2. Visual inspection revealed variability in enzymatic activities across the categories, although no statistical differences between these subgroups were found.

The relationships between the time interval from the violent incident to saliva collection and salivary enzymatic activities were additionally explored using scatterplots with locally estimated scatterplot smoothing (LOESS) curves (Figure 3). No significant monotonic correlations were observed.

The fitted curves suggested modest non-linear variations in MPO and CAT activities, with decreases at intermediate sampling intervals followed by increases at longer intervals. SOD activity appeared relatively stable across much of the observed interval, with a slight upward trend toward longer intervals.

These observations were descriptive and were not supported by formal statistical tests of temporal associations. Therefore, no definitive conclusions regarding time-dependent changes in salivary enzymatic activities can be drawn from these exploratory analyses.

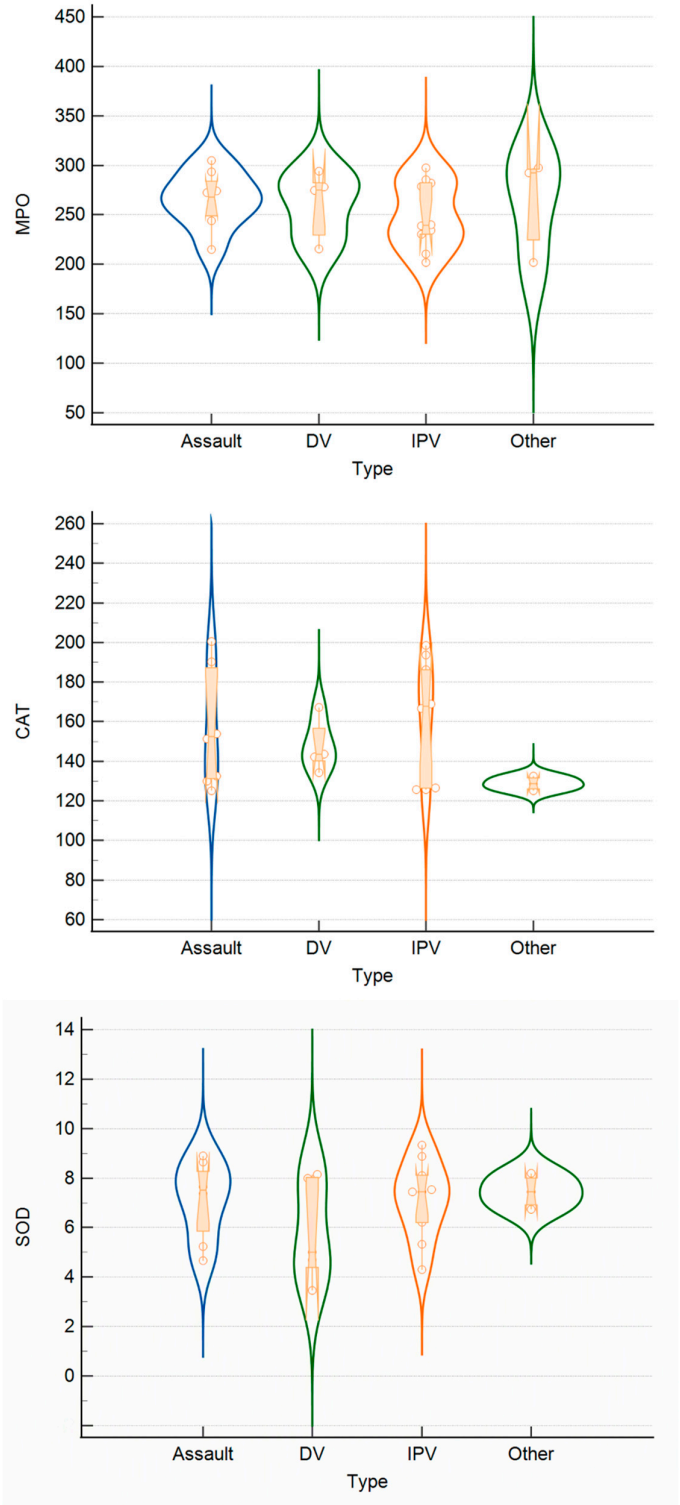


Figure 2. Violin plots illustrating the distributions of salivary MPO [U/L], CAT [U/mL], and SOD [U/mL] activities according to the recorded categories of interpersonal violence.

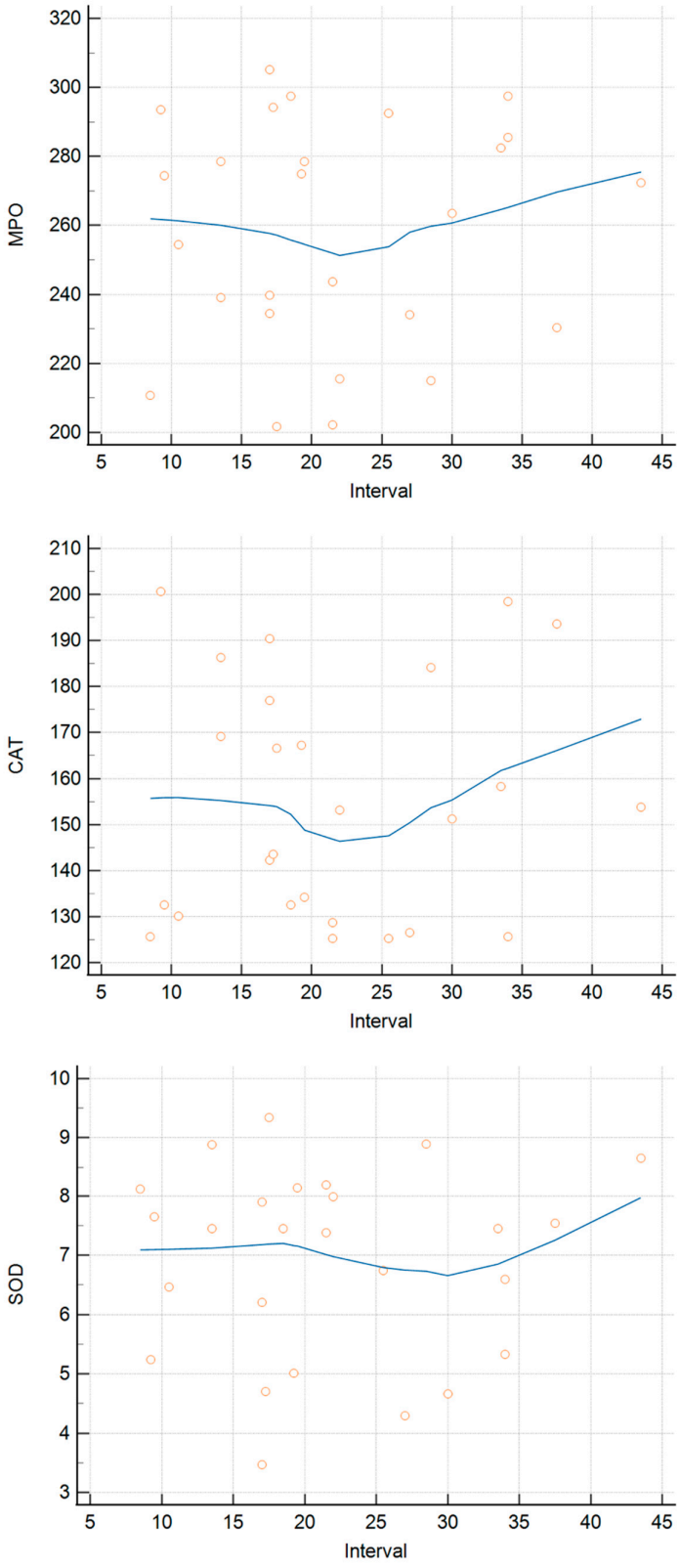


Figure 3. Scatterplots with locally estimated scatterplot smoothing (LOESS) curves illustrating the relationships between the interval from the violent incident to saliva collection and salivary MPO [U/L], CAT [U/mL], and SOD [U/mL] activities among violence-exposed individuals.

3.5. Exploratory Diagnostic Performance of Salivary Enzymatic Activities

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the apparent discriminatory performance of salivary MPO, CAT, and SOD activities in distinguishing individuals recently exposed to interpersonal physical violence from healthy controls. Each enzyme showed complete separation between the 26 violence-exposed participants and the 26 healthy controls in this sample (empirical AUC = 1.000 for each marker). Bootstrap resampling preserved the observed separation and yielded degenerate AUC intervals. At the sample-derived Youden thresholds, apparent sensitivity and specificity were each 100.0% (exact binomial 95% CI: 86.8–100.0% for each)—Table 4.

Thus, the activities of all three enzymes completely separated violence-exposed individuals from healthy controls within the analyzed dataset, with increased MPO and CAT activities and decreased SOD activity characterizing the violence-exposed group.

Nevertheless, despite the bootstrap-based estimation of confidence intervals, these findings represent apparent in-sample discrimination rather than internally validated diagnostic performance. Given the relatively small sample size and the absence of independent validation, the observed perfect classification should be interpreted cautiously.

Table 4. Exploratory receiver operating characteristic (ROC) analysis of salivary MPO, CAT, and SOD activities for distinguishing individuals exposed to interpersonal physical violence from healthy controls.

	MPO		CAT		SOD	
AUC (95%CI) [a]	1.000	1.000–1.000	1.000	1.000–1.000	1.000	1.000–1.000
p-value	<0.0001		<0.0001		<0.0001	
cut-off (95%CI) [b]	>190.59	188.64–190.59	>97.12	92.12–97.12	≤9.34	8.89–9.34
sensitivity (95%CI) [c]	100.0	86.8–100.0	100.0	86.8–100.0	100.0	86.8–100.0
specificity (95%CI) [c]	100.0	86.8–100.0	100.0	86.8–100.0	100.0	86.8–100.0

[a] complete empirical separation was preserved in every bootstrap replicate, yielding a degenerate AUC interval of 1.000–1.000; [b] confidence intervals were estimated using bootstrapping; [c] two-sided exact Clopper–Pearson interval based on 26/26 correctly classified participants, calculated at the selected threshold.

4. Discussion

4.1. Principal Findings and Their Relevance to Redox Biology

The present preliminary study identified pronounced alterations in salivary oxidative stress-related enzymatic activities among individuals recently exposed to interpersonal physical violence. Compared with healthy controls, violence-exposed individuals exhibited significantly higher MPO and CAT activities and lower SOD activity. Specifically, MPO activity was approximately 58.1% higher, CAT activity was 117.8% higher, and SOD activity was 43.4% lower in the violence-exposed group. These associations remained statistically significant after adjustment for age, gender, BMI, and saliva collection time, suggesting that the observed enzymatic differences were not explained by the included covariates.

The principal finding is the identification of a directionally heterogeneous enzymatic profile characterized by increased activity of an oxidant-generating enzyme, increased activity of a hydrogen peroxide-degrading antioxidant enzyme, and decreased activity of an enzyme responsible for superoxide dismutation. Rather than indicating a uniform increase or decrease in antioxidant defense, this pattern suggests differential alterations in salivary redox-related enzymatic pathways.

The biological interpretation of these findings should be considered within the broader framework of stress-induced redox regulation. Oxidative stress is not simply

characterized by increased concentrations of reactive species but involves complex interactions between oxidant generation, antioxidant responses, redox signaling, and oxidative molecular damage [9–12]. As emphasized by Ghezzi et al. [11], biomarkers reflecting biological responses to stress should be distinguished from direct indicators of oxidative damage. This distinction is particularly important in the present study because MPO, CAT, and SOD activities represent functional enzymatic measurements rather than direct evidence of lipid, protein, or DNA oxidation.

Our findings are broadly consistent with the concept that acute stressors may influence salivary redox-related processes, although the available literature demonstrates substantial heterogeneity. Stojanović et al. [13] observed increased salivary nitrite/nitrate levels following experimentally induced psychological stress without significant changes in total antioxidant capacity. Similarly, another investigation demonstrated increased salivary CAT activity accompanied by reduced protein oxidation following acute psychosocial stress [14]. These observations support the importance of assessing specific enzymatic pathways rather than interpreting oxidative stress solely through composite antioxidant indices.

The present study extends our previous research on salivary biomarkers (e.g., cortisol) associated with interpersonal violence [21] by shifting the focus from neuroendocrine indicators toward enzymatic pathways involved in oxidant metabolism and antioxidant defense. These findings complement this earlier work by demonstrating that recent violence exposure is associated not only with potentially relevant neuroendocrine processes but also with measurable differences in salivary oxidative stress-related enzymatic activities. However, these results should not be interpreted as evidence that hormonal and enzymatic alterations occur through a single common mechanism. Indeed, the relationships between HPA-axis activation, inflammatory signaling, and redox regulation are complex and potentially bidirectional [11,12]. Cortisol may modulate inflammatory responses, while inflammatory mediators and oxidative processes may influence neuroendocrine regulation.

4.2. Increased Salivary MPO Activity: Inflammatory Activation and Oxidant Generation

One of the most prominent findings was the significantly higher salivary MPO activity in violence-exposed individuals. MPO participates in host defense by catalyzing the formation of reactive oxidants, particularly hypochlorous acid, from hydrogen peroxide and chloride ions. Although these reactions contribute to microbial killing, MPO-derived oxidants can also promote oxidative modification of proteins, lipids, and other biological structures [15–17].

The approximately 1.58-fold increase in MPO activity observed in the present study may therefore be compatible with enhanced oxidant-generating enzymatic activity associated with acute inflammatory responses. Physical injuries may trigger the recruitment and activation of inflammatory cells, whereas psychological stress may influence immune regulation through neuroendocrine and autonomic mechanisms [11,12]. Both processes provide plausible explanations for the association between recent violence exposure and increased salivary MPO activity.

However, MPO activity in saliva should not be interpreted as a direct measure of systemic neutrophil activation. Salivary MPO originates substantially from oral neutrophils, and its activity may be influenced by gingival inflammation, periodontal status, and the composition of oral fluids. In our previous investigation involving apparently healthy adults, salivary MPO concentrations were associated with BMI, inflammatory cytokines, oral hygiene, gingival status, and salivary flow [23]. Importantly, BMI and stimulated salivary flow emerged as major predictors in multivariable analysis. These findings

demonstrate that salivary MPO may be influenced by subtle inflammatory and physiological factors even in individuals without clinically apparent oral inflammatory lesions.

The present findings should also be interpreted in light of our systematic review of salivary MPO in systemic diseases [24]. That review identified altered salivary MPO levels in several systemic conditions, particularly cardiovascular and gastrointestinal diseases. The direction of these alterations was not uniform, highlighting the context-dependent nature of MPO responses. Furthermore, in our subsequent study involving patients with inflammatory bowel diseases, salivary MPO concentrations varied according to disease activity and treatment-related factors [25]. These findings reinforce the interpretation that MPO is a biologically responsive but nonspecific marker whose salivary measurements may reflect interactions between local inflammation, systemic disease, and physiological regulation.

Another relevant consideration is the temporal variability of salivary MPO. In our previous investigation involving repeated saliva collection over 36 h, we observed evidence suggesting ultradian variation in salivary MPO concentrations, with a possible approximately 12-h rhythmic component [26]. These findings underline the importance of controlling or accounting for sample collection time when investigating salivary MPO in relation to acute physiological stressors. Nevertheless, the distinction between MPO concentration and catalytic activity should be emphasized, as changes in enzyme abundance do not necessarily correspond directly to changes in enzymatic function.

In the present study, collection time was positively associated with MPO activity in the adjusted model ($\beta = 0.146$; $p = 0.037$), whereas no statistically significant associations were observed for CAT or SOD. This finding may indicate sampling-related variability in MPO activity, although the relatively small sample size and exploratory nature of the analysis preclude definitive conclusions. The interval between the violent incident and saliva collection was additionally explored using LOESS curves. The resulting plots suggested modest nonlinear variations in MPO and CAT activities, whereas SOD activity appeared comparatively stable across much of the observed interval. However, these patterns were descriptive and were not supported by formal statistical testing.

A methodological distinction is essential when comparing the present findings with our earlier work. Previous investigations primarily measured salivary MPO concentration, whereas the current study assessed enzymatic activity. MPO concentration reflects the abundance of the enzyme, while activity depends additionally on its catalytic state, substrate availability, potential inhibitors, and assay conditions. Consequently, the present increase in MPO activity cannot be directly equated with the concentration changes reported previously.

4.3. Increased Catalase Activity and Potential Compensatory Antioxidant Responses

CAT activity demonstrated the largest relative difference between groups, being approximately 2.18-fold higher among individuals exposed to interpersonal physical violence. CAT is an important antioxidant enzyme involved in the decomposition of hydrogen peroxide into water and molecular oxygen, thereby limiting excessive accumulation of this reactive intermediate [18–20].

The marked elevation in CAT activity may represent a compensatory or adaptive response to altered hydrogen peroxide metabolism following acute physical and psychological stress. Increased oxidant generation may stimulate antioxidant defense mechanisms, although the direction and magnitude of enzymatic responses depend on several factors, including the intensity and duration of the oxidative challenge, enzyme regulation, and the biological compartment investigated.

Interestingly, previous experimental research has demonstrated increased salivary CAT activity following acute psychosocial stress, accompanied by reduced protein

oxidation [14]. This observation provides a particularly relevant comparison with the present findings and supports the biological plausibility of enhanced CAT activity during acute stress responses. However, the stress paradigm, study population, and biological context differed substantially from those of the present investigation, preventing direct conclusions regarding shared mechanisms.

The simultaneous elevation of MPO and CAT is particularly noteworthy because both enzymes interact with hydrogen peroxide through distinct catalytic mechanisms. MPO utilizes hydrogen peroxide to generate highly reactive oxidants, whereas CAT facilitates its decomposition. Therefore, increased activities of both enzymes may indicate altered regulation of hydrogen peroxide-associated pathways rather than a straightforward predominance of either oxidative or antioxidant processes. This interpretation is consistent with the broader understanding of redox regulation as a dynamic system in which oxidant generation and antioxidant defenses are functionally interconnected [9,18,19]. However, it should not be assumed that increased CAT activity directly compensates for increased MPO activity. Such a relationship would require evidence regarding hydrogen peroxide availability, enzyme localization, and the kinetics of competing reactions, none of which were assessed in the present investigation.

Our previous study involving patients with inflammatory bowel diseases also incorporated salivary CAT measurements alongside MPO and total antioxidant status [25], providing a relevant methodological and biological context for investigating multiple redox-related markers in saliva. Nevertheless, differences in study populations, clinical conditions, and analytical endpoints limit direct comparisons between these investigations.

4.4. Reduced Superoxide Dismutase Activity and Differential Antioxidant Regulation

In contrast to MPO and CAT, salivary SOD activity was substantially reduced among violence-exposed individuals. SOD represents a major enzymatic defense against superoxide radicals by catalyzing their dismutation into hydrogen peroxide and molecular oxygen. Through this reaction, SOD contributes to the regulation of superoxide availability and the maintenance of redox homeostasis [18–20].

The approximately 43.4% reduction in SOD activity may indicate altered regulation of superoxide-related antioxidant defenses. Several mechanisms could potentially contribute to reduced enzyme activity, including oxidative inactivation, changes in enzyme expression, altered extracellular release, or differences in the cellular composition of saliva. However, these mechanisms remain speculative because the present study did not include measurements of SOD expression, isoenzyme distribution, or direct indicators of oxidative enzyme modification.

The opposing directions of CAT and SOD activities deserve particular attention. Although these enzymes participate in interconnected antioxidant pathways, their regulation is not necessarily synchronized. Reduced SOD activity accompanied by increased CAT activity may reflect differential responses of enzymatic systems involved in superoxide and hydrogen peroxide metabolism. Nevertheless, the observed pattern cannot establish the direction of ROS flux or demonstrate a specific biochemical compensation between these enzymes.

Previous research has demonstrated that salivary antioxidant parameters may differ according to the nature and chronicity of physiological or psychological stress. For example, an investigation involving patients with myogenous temporomandibular disorders reported higher salivary total antioxidant capacity and lower total oxidant status than in controls, despite no significant differences in SOD activity [27]. Similarly, studies involving recurrent aphthous stomatitis have demonstrated alterations in salivary antioxidant enzyme activities, although the direction of SOD changes has varied between populations [28,29]. These findings illustrate the context-dependent nature of salivary antioxidant

responses and suggest that reduced SOD activity should not be interpreted as a universal marker of oxidative stress.

4.5. Integrated Interpretation of the MPO–CAT–SOD Profile

The simultaneous assessment of MPO, CAT, and SOD represents an important aspect of the present investigation. These enzymes participate in interconnected but functionally distinct components of redox regulation, allowing a broader characterization of salivary enzymatic alterations than would be possible through the measurement of a single biomarker. The observed profile of increased MPO, increased CAT, and decreased SOD may be compatible with altered regulation of oxidant-generating and antioxidant pathways following recent interpersonal physical violence. Increased MPO activity suggests greater capacity for MPO-dependent oxidant formation under appropriate substrate conditions, while elevated CAT activity indicates increased measured hydrogen peroxide-degrading activity. Reduced SOD activity, in turn, suggests lower measured superoxide-dismutating capacity.

However, the simultaneous occurrence of these alterations does not establish their causal or functional interdependence. In particular, the relative activities of MPO, CAT, and SOD cannot be used to infer intracellular ROS concentrations or the magnitude of oxidative damage without complementary biochemical measurements. This distinction is consistent with methodological recommendations emphasizing that oxidative stress should be characterized using appropriately selected biomarkers reflecting different biological processes [7,9,11]. Direct markers of oxidative molecular damage, such as lipid peroxidation products, protein carbonyls, or oxidized nucleic acid derivatives, would provide complementary information that was not available in the present study.

An additional challenge arises from the complex biological composition of whole saliva. The measured activities of MPO, CAT, and SOD may originate from different cellular and extracellular sources, including salivary glands, oral epithelial cells, leukocytes, and microorganisms. Consequently, the observed enzymatic profile may reflect combined local and systemic influences rather than a single coordinated biological response. The combination of higher MPO and CAT activities and lower SOD activity may reflect changes in distinct redox pathways. Because we measured enzyme activities at a single time point and did not assess direct markers of oxidative damage, the data cannot establish whether these changes reflect an adaptive response, oxidative injury, or a combination of processes. Comparisons with individuals assessed after accidental injury or acute psychological distress, together with serial saliva sampling, would help distinguish these possibilities.

Salivary oxidative stress-related biomarkers may be influenced by periodontal inflammation, salivary flow, oral hygiene, systemic conditions, and sampling procedures [7,23,30,31]. Studies investigating oral inflammatory diseases have demonstrated alterations in salivary oxidative and antioxidant parameters, although the direction and magnitude of these changes vary across biomarkers and populations [28–31]. Therefore, the identification of exposure-associated enzymatic patterns requires careful consideration of both local and systemic determinants.

4.6. Exploratory Discriminatory Performance and Potential Biomarker Applications

The exploratory ROC analyses demonstrated complete apparent separation between violence-exposed individuals and healthy controls for all three investigated enzymes. MPO, CAT, and SOD each achieved an AUC of 1.000, with apparent sensitivity and specificity of 100% at the identified sample-derived thresholds.

Although these results indicate pronounced differences between the investigated groups, they should be interpreted cautiously. The relatively small sample size and the

absence of independent validation raise concerns regarding potential overestimation of discriminatory performance. Previous methodological research has demonstrated that diagnostic and prediction models may exhibit optimistic performance when evaluated in the same data used for their development, emphasizing the importance of internal and external validation [32,33].

Importantly, bootstrap confidence intervals calculated for apparent ROC estimates do not necessarily correct for optimism arising from sample-derived threshold selection. Accordingly, the narrow AUC confidence intervals observed in the present study should not be interpreted as evidence that perfect discrimination would be reproduced in independent populations. Complete separation across all three markers in a small sample warrants caution. Recruitment of medico-legal cases and healthy volunteers may have produced a stronger contrast than would be observed in a forensic referral population, where acute distress, accidental injury, and inflammation may also influence salivary enzyme activities.

4.7. Strengths, Limitations and Future Directions

The principal strength of the present study is the simultaneous investigation of three functionally distinct enzymes involved in redox regulation in individuals examined following recent interpersonal physical violence. This approach enabled the identification of a characteristic enzymatic pattern involving increased MPO and CAT activities and decreased SOD activity. Another strength is the application of multivariable models accounting for selected demographic and sampling-related factors. The persistence of the observed associations after adjustment for age, gender, BMI, and saliva collection time supports the consistency of the group differences within the investigated population.

Nevertheless, several limitations should be acknowledged. First, the sample size was relatively small, comprising 26 violence-exposed individuals and 26 controls. This limits the precision of effect estimates, the assessment of subgroup differences, and the generalizability of the findings. The observational cross-sectional design prevents causal inference. Because pre-exposure enzymatic activities were unavailable, it cannot be established whether the observed differences developed as a consequence of the violent incident. Also, the absence of comparison groups exposed to non-violent physical trauma or acute psychological stress limits conclusions regarding the specificity of the observed enzymatic profile for interpersonal violence.

Moreover, the study did not include direct markers of oxidative molecular damage or measurements of ROS concentrations. Therefore, the observed enzymatic differences cannot establish the presence or magnitude of oxidative damage. Finally, the ROC analyses were performed in the same dataset used to identify the optimal thresholds, without independent validation. Accordingly, the apparent perfect classification should not be interpreted as evidence of clinical diagnostic performance. Despite these limitations, the present findings provide preliminary evidence supporting further investigation of salivary MPO, CAT, and SOD activities as components of the biological response associated with recent interpersonal physical violence.

Future research should prioritize larger prospective studies with longitudinal saliva collection at standardized intervals following acute violence exposure. Such investigations would help determine whether the observed enzymatic alterations are transient, persistent, or influenced by the timing of sample collection. Also, the inclusion of individuals experiencing non-violent physical injuries and acute psychological stress without physical trauma would be particularly valuable for evaluating the specificity of the observed enzymatic profile.

Furthermore, combining MPO, CAT, and SOD activity measurements with salivary cortisol, alpha-amylase, inflammatory mediators, and direct oxidative damage

biomarkers could provide a more comprehensive understanding of the relationships between neuroendocrine activation, inflammation, and redox regulation. Finally, the reproducibility and discriminatory performance of the investigated enzymes should be evaluated using independent populations and appropriately validated statistical approaches before any potential clinical applications are considered.

5. Conclusions

Recent exposure to interpersonal physical violence was associated with significantly increased salivary MPO and CAT activities and decreased SOD activity compared with healthy controls. These differences remained statistically significant after adjustment for age, gender, BMI, and saliva collection time, suggesting a distinct salivary enzymatic profile involving oxidant-generating and antioxidant defense pathways.

The observed pattern may reflect altered redox-related biological responses accompanying acute physical and psychological stress. However, the cross-sectional design, absence of pre-exposure measurements, and lack of direct oxidative damage biomarkers preclude causal or mechanistic conclusions. Although all three enzymes demonstrated complete apparent separation within the investigated dataset, these findings require independent validation and should not be interpreted as evidence of established diagnostic utility. Larger longitudinal studies incorporating comprehensive redox, inflammatory, neuroendocrine, and oral health assessments are warranted.

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