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A COMPARATIVE EVALUATION OF SALIVARY SUPEROXIDE DISMUTASE ENZYME LEVEL AMONG SMOKERS AND NON-SMOKERS IN CHRONIC PERIODONTITIS

Periodontology

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ABSTRACT

Background: Periodontal disease (PD) is a long-lasting inflammatory disorder resulting in the progressive worsening of the periodontal ligament, alveolar bone, and the tissues supporting the teeth. PD is initiated by microbial dysbiosis and sustained by an exaggerated host immune–inflammatory response. Identification of reliable biomarkers in gingival crevicular fluid (GCF) may enhance early diagnosis, assessment of disease severity, and monitoring of treatment outcomes. **Aim:** The present study aimed to evaluate selected inflammatory biomarkers in GCF and to correlate their levels with clinical periodontal parameters in healthy controls and patients with chronic periodontitis (CP).**Materials and methods:** The study included systemically healthy participants divided into healthy and periodontitis groups based on clinical periodontal examination. GCF samples were collected using standardized techniques and analyzed for inflammatory and oxidative stress–related biomarkers using appropriate biochemical and immunological assays. Statistical analysis was performed to compare biomarker levels between groups and to assess correlations with clinical parameters.**Results:** Periodontitis patients exhibited significantly elevated levels of inflammatory biomarkers in gingival crevicular fluid compared to healthy controls. A positive correlation was detected between biomarker expression and clinical periodontal parameters, indicating an association between increased inflammatory activity and disease severity. **Conclusion:** The study suggests that GCF inflammatory biomarkers play a significant part in the pathogenesis and progression of PD. Biomarker analysis, when used alongside conventional clinical assessment, may serve as a valuable adjunct for early detection, disease monitoring, and evaluation of periodontal therapy outcomes.

KEYWORDS

Periodontal Disease; Gingival Crevicular Fluid; Inflammatory Biomarkers; Host Immune Response; Periodontitis

INTRODUCTION

PD is a long-term, multifaceted inflammatory disorder that leads to the gradual breakdown of tooth auxiliary structures due to interactions between an imbalanced microbial biofilm and the immune inflammatory response of the host [1,2]. Smoking is one of the most persuasive environmental risk factor for PD, exerting through toxic effects and immunomodulatory mechanisms [3,4]. Oxidative stress, a disproportion between reactive oxygen species (ROS) production and the host antioxidant defense system, plays a chief role in arbitrating the infective impact of smoking and periodontitis (Chapple and Matthews 2007a; Wang et al. 2017).

Saliva and GCF have gained importance as non-invasive sources of biomarkers for periodontal disease detection and monitoring 7,8. Biomarkers including catalase (CAT), glutathione peroxidase (GPx), malondialdehyde (MDA), metallothionein (MT), total antioxidant capacity (TAOC), and inflammatory cytokines reflect oxidative damage and antioxidant status in periodontal tissues [9–11].

During periodontitis, activated neutrophils generate excessive ROS that damage lipids, proteins, and DNA, thereby aggravating inflammation and tissue destruction [12,13]. Elevated oxidative stress markers such as MDA and 8-OHdG have been reported in periodontal tissues and systemic circulation of periodontitis patients [14,15]. Although antioxidant enzymes and molecules normally protect tissues from oxidative injury, excessive ROS can overwhelm these defenses and accelerate periodontal breakdown [16,17].

Cigarette smoke contains harmful compounds including nicotine, carbon monoxide, heavy metals, and tar that enhance oxidative stress and impair periodontal healing (National Center for Chronic Disease Prevention and Health Promotion 2014). Smoking increases ROS production, reduces blood supply to periodontal tissues, alters cytokine responses, and impairs fibroblast function and neutrophil activity [18,20]. Consequently, smokers display deeper periodontal pockets, greater attachment loss, and poorer treatment outcomes compared to non-smokers 3.

Key oxidative stress biomarkers in smoking-associated periodontitis:

1. Metallothionein (MT): MT is an antioxidant protein involved in metal detoxification and protection against reactive oxygen species (ROS). Smokers with chronic periodontitis show elevated MT levels in saliva, serum, and gingival crevicular fluid, which correlate with periodontal destruction. MT levels decrease following nonsurgical periodontal therapy (NSPT), indicating reduced oxidative stress 21,22.

2. Antioxidant Defense Enzymes (CAT, GPx, GR): Catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) are key antioxidant enzymes that protect periodontal tissues from ROS. Smokers with periodontitis exhibit reduced CAT and GPx levels, while GR activity improves after NSPT, suggesting partial restoration of antioxidant defense 23,24.

3. OSI, TAOC, and TOS: Oxidative Stress Index (OSI), Total Antioxidant Capacity (TAOC), and Total Oxidant Status (TOS) are important indicators of oxidative balance. Smokers with periodontitis demonstrate lower TAOC and higher TOS, MDA, and OSI levels, reflecting increased oxidative burden and impaired recovery after therapy 25,26.

4. Malondialdehyde (MDA): MDA is a marker of lipid peroxidation and oxidative tissue damage. Elevated MDA levels in smokers with CP indicate enhanced oxidative stress and periodontal destruction 27,28.

5. Inflammatory Cytokines and Immune Mediators: Smoking increases pro-inflammatory mediators such as IL-1β and CRP while reducing antioxidant enzymes like SOD, thereby intensifying inflammation and oxidative damage in periodontal tissues 29,30.

6. Trace Elements: Trace elements including zinc, copper, chromium, and strontium regulate antioxidant enzyme activity. Smokers with periodontitis exhibit altered trace element balance, particularly disturbed Cu/Zn ratios, which weaken antioxidant defenses and aggravate periodontal tissue damage 31.

Aim: To evaluate the effect of smoking on periodontal health by comparing salivary SOD levels and to investigate the correlation with clinical periodontal parameters in smokers and non-smokers.

MATERIAL AND METHODS

Study Design

This cross-sectional randomized controlled clinical study evaluated SOD1 levels and their association with periodontal parameters in smokers and non-smokers with CP.

Study Population
Ninety systemically healthy subjects (25–60 years) were recruited from the Department of Periodontics, Inderprastha Dental College and Hospital, Ghaziabad, following ethical approval and informed consent.

Subjects were divided into:
- **Group I:** 30 non-smokers with CP
- **Group II:** 30 smokers with CP (≥10 cigarettes/day)
- **Group III:** 30 periodontally healthy controls

CP subjects had ≥20 natural teeth with probing pocket depth (PPD) ≥5 mm and/or clinical attachment loss (CAL) ≥3 mm.

Inclusion and Exclusion Criteria
Systemically healthy individuals without recent periodontal therapy, antibiotic/anti-inflammatory/antioxidant use, pregnancy, tobacco forms other than cigarettes, alcohol abuse, or systemic diseases affecting periodontal status were included. Subjects unwilling to participate were excluded.

Clinical Examination
All periodontal recordings were performed by a single calibrated examiner using a UNC-15 periodontal probe under adequate illumination.

Saliva Collection
Unstimulated whole saliva was collected 48 h after examination during morning hours. Subjects avoided eating, smoking, drinking, and oral hygiene procedures for 8 h before collection. Samples were centrifuged at 3000 rpm for 10 min, and supernatants were stored at −20°C until analysis.

Estimation of Salivary SOD1
Salivary SOD1 levels were estimated using a commercially available ELISA kit (Elabscience® Human SOD1 ELISA Kit; Catalog No. E-EL-H1113) based on the competitive ELISA principle. Absorbance was measured at 450 ± 2 nm, and concentrations were calculated using a standard calibration curve.

Assay characteristics: Sensitivity: 37.5 pg/mL; Detection range: 62.5–4000 pg/mL; Precision: CV <10%.

Statistical Analysis
Data were analyzed using appropriate statistical software. Descriptive statistics, intergroup comparisons, and Pearson's/Spearman's correlation analyses were performed. A p-value <0.05 was considered statistically significant.

RESULTS
The present study evaluated salivary SOD1 levels in healthy controls, non-smokers with CP, and smokers with CP using competitive ELISA. A total of 90 participants were equally divided into three groups (n = 30 each):

- **Group I:** Healthy controls
- **Group II:** Non-smokers with CP
- **Group III:** Smokers with CP (≥10 cigarettes/day)

Salivary SOD1 concentrations were estimated using a competitive ELISA assay with a standard calibration curve ranging from 0–4000 pg/mL (Table 1). The assay demonstrated an inverse relationship between optical density (OD) and SOD1 concentration, confirming proper assay performance.

Table 1: Standard curve values for salivary SOD1 estimation

Concentration pg/ml	OD
4000	0.248
2000	0.378
1000	0.614
500	0.964
250	1.361
125	1.705
62.5	1.947
0	2.364

(4000 pg/mL → lowest OD; 0 pg/mL → highest OD)

Salivary SOD1 Levels
Healthy controls showed the lowest salivary SOD1 levels, with OD values ranging from 1.30–1.39, corresponding to approximately 230–270 ng/mL.

Non-smokers with CP demonstrated moderately elevated SOD1 levels, with OD values of 0.90–0.96 and concentrations ranging from 490–560 ng/mL, indicating increased antioxidant activity associated with periodontal inflammation.

Smokers with CP exhibited the highest salivary SOD1 levels, with lower OD values (0.60–0.69) corresponding to concentrations of approximately 860–1060 ng/mL, suggesting enhanced oxidative stress associated with smoking.

Overall, salivary SOD1 levels increased progressively in the order: **Healthy controls < Non-smokers with CP < Smokers with CP**, indicating that periodontal inflammation and smoking both contribute to increased oxidative stress and antioxidant enzyme activity (Table 2, Figure1).

Table 2: Assessment of salivary SOD1 levels among healthy controls, non-smokers, and smokers with CP

Healthy OD	Healthy Concentration ng/ml	Nonsmokers OD	Nonsmokers Concentration ng/ml	Smokers OD	Smokers concentration ng/ml
1.351	247.37719	0.954	507.6850034	0.604	1059.393458
1.341	251.9119536	0.944	517.326649	0.614	1033.868092
1.331	256.5201441	0.934	527.1923902	0.624	1009.251959
1.321	261.2035125	0.924	537.2900211	0.634	985.4977389
1.311	265.963866	0.914	547.6277005	0.644	962.5613298
1.301	270.8030701	0.904	558.2139731	0.654	940.4015791
1.365	241.1486575	0.965	497.3291423	0.664	918.9800422
1.364	241.5889867	0.963	499.1929601	0.674	898.260763
1.363	242.0300118	0.962	500.1280185	0.684	878.2100767
1.362	242.4717346	0.961	501.0651858	0.694	858.7964299
1.360	243.3572793	0.960	502.0044691	0.604	1059.393458
1.355	245.5834622	0.955	506.7328744	0.610	1043.966366
1.344	250.5438929	0.954	507.6850034	0.611	1041.42799
1.333	255.5925486	0.944	517.326649	0.612	1038.898852
1.322	260.7317416	0.933	528.1915866	0.613	1036.378903
1.311	265.963866	0.922	539.3380854	0.614	1033.868092
1.390	230.36202	0.911	550.7770807	0.615	1031.366371
1.350	247.8274109	0.909	552.8892129	0.616	1028.87369
1.346	249.6355007	0.938	523.2187114	0.617	1026.390001
1.309	266.925338	0.946	515.3806355	0.618	1023.915256
1.311	265.963866	0.914	547.6277005	0.644	962.5613298
1.301	270.8030701	0.904	558.2139731	0.654	940.4015791
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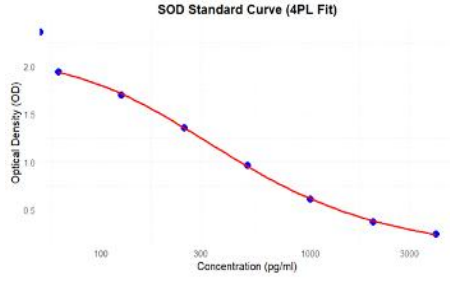

Fig. 1: Mean salivary SOD1 concentration across study groups

Table 3: Pairwise intergroup comparison of SOD

Comparison	Mean difference	p-value
Healthy vs Nonsmokers	↑	<0.001*
Healthy vs Smokers	↑↑	<0.001*
Nonsmokers vs Smokers	↑	<0.001*

Table 4: Percentage increase in SOD levels

Comparison	% Change
Nonsmokers vs Healthy	~100%
Smokers vs Healthy	~300%
Smokers vs Nonsmokers	~85–90%

From the above results, it is showed that salivary SOD1 concentration was lowest in healthy subjects, representing normal redox homeostasis (Table 3). Non-smokers with CP displayed higher SOD1 concentration, exhibiting an inflammatory oxidative response. Smokers with CP represented the maximum SOD1 levels, emphasizing the synergistic effect of smoking and periodontal inflammation on oxidative stress (Table 4). These outcomes indicates that smoking significantly modifies salivary antioxidant profiles, possibly inducing periodontal disease severity and progression.

DISCUSSION

The present study demonstrated a progressive increase in salivary SOD1 levels from healthy controls to non-smokers with CP, with the highest levels observed in smokers with CP. These findings suggest that oxidative stress increases with periodontal inflammation and is further amplified by cigarette smoking 5,32. Healthy individuals exhibited the lowest salivary SOD1 levels, indicating physiological oxidative balance, whereas non-smokers with CP showed elevated SOD1 levels, reflecting a compensatory antioxidant response to inflammation-induced ROS generation[33,34].

Smokers with CP demonstrated significantly higher salivary SOD1 concentrations than non-smokers with CP, indicating an enhanced oxidative burden associated with tobacco exposure. Cigarette smoke contains free radicals, nicotine, heavy metals, and reactive aldehydes that increase ROS production and impair antioxidant defense systems 35,36. Elevated SOD1 levels in smokers may therefore represent a compensatory response to persistent oxidative stress rather than effective antioxidant protection[37,38].

The present findings are consistent with previous reports showing increased oxidative stress markers such as MDA, TOS, OSI, and metallothionein, along with reduced antioxidant capacity in smokers with periodontitis 24,26,39. Furthermore, worsening clinical periodontal parameters, including probing pocket depth and clinical attachment loss, were associated with elevated salivary SOD1 levels, supporting the role of oxidative stress in periodontal disease progression[40,41].

Saliva serves as a reliable and non-invasive diagnostic medium for evaluating oxidative stress biomarkers in periodontal disease 42,43. The clear differentiation of salivary SOD1 levels among healthy controls, non-smokers with CP, and smokers with CP highlights its potential as an adjunctive biomarker for periodontal disease assessment.

The strengths of this study include standardized clinical examination, strict inclusion criteria, and sensitive ELISA-based analysis. However, the cross-sectional design and evaluation of a single biomarker limit causal interpretation. Future longitudinal studies incorporating multiple oxidative stress biomarkers and smoking cessation outcomes are warranted.

CONCLUSION

Salivary SOD1 levels increased progressively from healthy controls to non-smokers with CP and smokers with CP, indicating increasing oxidative stress burden with periodontal inflammation and smoking. Cigarette smoking significantly alters antioxidant responses and contributes to enhanced periodontal tissue destruction. Salivary SOD1 may therefore serve as a promising non-invasive biomarker for oxidative stress assessment and periodontal disease severity, particularly in smokers.

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