ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at:www.ijdsir.com

Volume – 9, Issue – 3, June – 2026, Page No. : 59 – 76

Evaluation of Salivary Levels of 8-Hydroxyguanosine in Non-Smokers, Smokers, and Smokeless Tobacco Consumers: A Clinico-Biochemical Study

[1]Ruchi Singh, Former Postgraduate, Department of Periodontology, AECS Maaruti College of Dental Sciences and Research Centre, Bangalore, Karnataka, India

[2]Rachana C, Professor and HOD, Department of Periodontology, Bangalore Institute of Dental Sciences, Bangalore, Karnataka, India

[3]Cristal R.S, Senior Lecturer, Department of Periodontology, Bangalore Institute of Dental Sciences, Bangalore, Karnataka, India

Corresponding Author: Cristal R.S, Senior Lecturer, Department of Periodontology, Bangalore Institute of Dental Sciences, Bangalore, Karnataka, India

Citation of this Article: Ruchi Singh, Rachana C, Cristal R.S, "Evaluation of Salivary Levels of 8-Hydroxyguanosine in Non-Smokers, Smokers, and Smokeless Tobacco Consumers: A Clinico-Biochemical Study", IJDSIR – June – 2026, Volume – 9, Issue – 3, P. No. 59 – 76.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Background: Tobacco smoking and smokeless tobacco consumption are major risk factors for periodontal disease and are known to enhance oxidative stress within periodontal tissues. 8-Hydroxy-2′-deoxyguanosine (8-OHdG), a stable biomarker of oxidative DNA damage generated by reactive oxygen species, has been associated with periodontal inflammation and tissue destruction. Salivary estimation of 8-OHdG may therefore provide a non-invasive approach for assessing oxidative stress and periodontal disease activity.

Aim: To evaluate and compare salivary 8-OHdG levels among smokers, smokeless tobacco consumers, and non-smokers with chronic periodontitis.

Materials and Methods: A total of 90 subjects were recruited and categorized into five groups (n = 18 each): Group I – periodontally healthy non-smokers; Group II – non-smokers with chronic periodontitis; Group III – smokers with chronic periodontitis; Group IV – smokeless tobacco consumers with chronic periodontitis; and Group V – tobacco consumers without periodontitis. Clinical periodontal parameters including plaque index (PI), gingival index (GI), probing depth (PD), and clinical attachment loss (CAL) were recorded. Unstimulated saliva samples were collected, and salivary 8-OHdG levels were quantified using enzyme-linked immunosorbent assay (ELISA). Statistical analysis was performed using Kruskal–Wallis test, one-way ANOVA,

post hoc tests, Spearman correlation, and multiple linear regression analysis.

Results: Salivary 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels differed significantly among the study groups ($p < 0.001$). The highest salivary 8-OHdG levels were observed in smokers with periodontitis (12.296 ± 2.137 pg/ml), followed by smokeless tobacco consumers with periodontitis (10.126 ± 2.265 pg/ml), non-smokers with periodontitis (3.479 ± 0.620 pg/ml), tobacco consumers without periodontitis (2.044 ± 0.694 pg/ml), and periodontally healthy controls (1.669 ± 0.392 pg/ml). Smokers and smokeless tobacco consumers with periodontitis demonstrated significantly elevated 8-OHdG levels compared with healthy controls and non-smokers with periodontitis ($p < 0.001$). Salivary 8-OHdG levels exhibited significant positive correlations with plaque index, gingival index, probing depth, and clinical attachment loss in periodontitis groups, with probing depth demonstrating the strongest association.

Conclusion: Salivary 8-OHdG levels were significantly elevated in smokers and smokeless tobacco consumers with periodontitis, suggesting increased oxidative DNA damage associated with tobacco exposure and periodontal inflammation.

Clinical Significance: Salivary 8-OHdG may serve as a simple, non-invasive biomarker for assessing oxidative stress and periodontal disease severity in tobacco users with periodontitis.

Keywords*:* Chronic periodontitis, Smoking, Smokeless tobacco, 8-OHdG

Introduction

Periodontitis is a chronic multifactorial inflammatory disease characterized by progressive destruction of tooth-supporting tissues, including the gingiva, periodontal ligament, cementum, and alveolar bone [1]. The disease is initiated by a dysbiotic subgingival biofilm; however, the host's immune-inflammatory response to this microbial challenge largely determines the extent and severity of periodontal destruction [1]. The persistent activation of inflammatory pathways within the periodontal microenvironment leads to the degradation of connective tissue, apical migration of the junctional epithelium, formation of periodontal pockets, and resorption of alveolar bone, ultimately culminating in tooth loss if left untreated. Periodontitis continues to pose a significant global public health burden due to its high prevalence and its negative impact on oral health and quality of life.

The current understanding of periodontal pathogenesis underscores the crucial role of host-mediated inflammatory tissue injury in disease progression [2]. Activated neutrophils, macrophages, and other immune cells release a wide spectrum of inflammatory mediators, proteolytic enzymes, and reactive molecular species during periodontal inflammation, which directly contribute to the breakdown of periodontal tissue [2]. Among these mechanisms, oxidative stress has emerged as a critical biological pathway implicated in the initiation and progression of periodontal destruction. Oxidative stress refers to a state of imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense capacity of the host [3]. Although reactive oxygen species (ROS) are essential for normal cellular signaling and antimicrobial defense, excessive and uncontrolled production may induce oxidative injury to surrounding host tissues [3].

Polymorphonuclear neutrophils serve as the primary cellular defense against periodontal pathogens and represent a major source of ROS generation within inflamed periodontal tissues. During the phagocytosis of periodontal microorganisms, activated neutrophils undergo respiratory burst activity, producing superoxide

radicals, hydrogen peroxide, hydroxyl radicals, and other reactive intermediates in substantial quantities[3]. Although these reactive species play a pivotal role in microbial elimination, their excessive release may simultaneously damage adjacent periodontal tissues.

ROS-mediated injury has been associated with the degradation of the extracellular matrix, lipid peroxidation, activation of matrix metalloproteinases, oxidation of cellular proteins, stimulation of pro-inflammatory cytokines, and oxidative modification of nucleic acids [3]. Consequently, oxidative stress is now recognized as a significant contributor to connective tissue destruction and alveolar bone loss in chronic periodontitis [3].

Among the biomarkers investigated for assessment of oxidative stress, 8-hydroxy-2'-deoxyguanosine (8-OHdG) has gained considerable importance as a sensitive and stable indicator of oxidative DNA damage [4]. 8-OHdG is formed following oxidative modification of guanine bases within mitochondrial and nuclear DNA and is subsequently released during DNA repair processes. Elevated levels of 8-OHdG have been reported in various chronic inflammatory, autoimmune, degenerative, and malignant conditions, indicating an increased oxidative burden at the cellular level [4]. In periodontal research, saliva has emerged as a promising diagnostic medium due to its non-invasive collection method and its capacity to reflect biochemical alterations occurring within the periodontal environment. Previous investigations have demonstrated significantly elevated salivary 8-OHdG levels in individuals with chronic periodontitis, suggesting a strong correlation between oxidative DNA damage and periodontal inflammatory activity [4].

Among the various environmental and behavioral risk factors associated with periodontal disease progression, tobacco consumption remains the most significant modifiable determinant. Both smoking and smokeless tobacco use have consistently been associated with increased prevalence, severity, and progression of periodontitis [5]. Tobacco exposure adversely affects periodontal tissues through multiple biologic mechanisms, including impairment of neutrophil function, suppression of fibroblast activity, altered vascular response, disruption of host immune regulation, and delayed wound healing [5]. Additionally, tobacco products contain abundant oxidants and free radicals capable of amplifying oxidative stress within periodontal tissues [6]. Cigarette smoke itself serves as a major exogenous source of reactive oxygen and nitrogen species, while smokeless tobacco products such as gutka, paan, and betel quid possess considerable pro-oxidant potential. These alterations may intensify oxidative tissue injury and accelerate periodontal destruction [7].

Although previous studies have independently demonstrated the association of chronic periodontitis with oxidative stress and tobacco exposure, comparative evidence evaluating oxidative DNA damage among smokers, smokeless tobacco users, and non-tobacco users with chronic periodontitis remains limited. Furthermore, the influence of different forms of tobacco consumption on salivary 8-hydroxy-2’-deoxyguanosine (8-OHdG) levels and their relationship with clinical periodontal destruction has not been comprehensively elucidated. Therefore, the present cross-sectional study was undertaken to evaluate and compare salivary 8-OHdG levels among smokers, smokeless tobacco users, and non-tobacco users diagnosed with chronic periodontitis.

Materials and Methodology

Study design and study population:

This cross-sectional observational study was conducted in the Department of Periodontology at A.E.C.S. Maaruti College of Dental Sciences and Research Centre. The

study protocol was approved by the Institutional Ethics Committee of the institution, and all procedures were performed in accordance with the Declaration of Helsinki (1975, revised 2013). Written informed consent was obtained from all participants prior to enrolment. A total of 90 systemically healthy subjects aged 30–75 years were recruited from patients attending the outpatient Department of Periodontology. Detailed medical and dental histories were recorded using a structured questionnaire that included demographic characteristics, oral hygiene practices, tobacco usage, medication history, and family history. Participants were categorized into five groups, comprising 18 subjects each: Group I, periodontally healthy non-smokers; Group II, non-smokers with chronic periodontitis; Group III, smokers with chronic periodontitis; Group IV, smokeless tobacco users with chronic periodontitis; and Group V, tobacco users without chronic periodontitis.

Periodontal diagnosis and eligibility criteria

The diagnosis of chronic periodontitis was established according to the 1999 International Classification of Periodontal Diseases and Conditions of the American Academy of Periodontology. Subjects with generalized chronic periodontitis exhibiting clinical attachment loss (CAL) of ≥5 mm affecting more than 30% of sites, along with radiographic evidence of alveolar bone loss, periodontally healthy subjects demonstrating probing depth (PD) of ≤3 mm, absence of clinical attachment loss, absence of bleeding on probing, and no radiographic evidence of alveolar bone loss and subjects with ≥20 functional teeth were included.

Subjects were excluded if they had active infections other than periodontitis, systemic diseases known to influence periodontal status, immunological disorders, alcohol dependence, or were pregnant or lactating women; or had been individuals receiving oral contraceptives, antibiotics, corticosteroids, or non-steroidal anti-inflammatory drugs within the previous 6 months, as well as those who had undergone periodontal therapy within 6 months.

Smoking status was assessed according to the criteria of the Centers for Disease Control and Prevention. Current smokers were defined as individuals who had smoked ≥100 cigarettes during their lifetime and continued smoking at the time of examination. Former smokers had smoked ≥100 cigarettes during their lifetime but had ceased smoking, whereas never smokers had not smoked 100 cigarettes during their lifetime. Smokeless tobacco users included subjects habitually consuming paan with tobacco, khaini, mawa, gutkha, or related smokeless tobacco products for a minimum duration of 12 months.

Clinical periodontal examination

A single calibrated examiner conducted all clinical examinations using a Williams graduated periodontal probe (Hu-Friedy, Chicago, IL, USA), with third molars excluded from the assessment. The clinical periodontal parameters recorded included Gingival Index (GI) (Löe & Silness, 1963), Plaque Index (PI) (Silness & Löe, 1964), probing depth (PD), and clinical attachment level (CAL). GI and PI were assessed at four sites per tooth, whereas PD and CAL were measured at six sites per tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual/palatal, midlingual/palatal, and distolingual/palatal). Probing depth was measured from the gingival margin to the base of the periodontal pocket, whereas CAL was measured from the cementoenamel junction to the base of the periodontal pocket. To minimize variability in measurements, all clinical recordings were obtained under standardized conditions by the same examiner throughout the study period.

Saliva sample collection

Unstimulated whole saliva samples were obtained between 09:00 and 11:00 AM to minimize variations associated with circadian rhythm. The participants were advised to avoid eating, drinking, smoking, and performing oral hygiene procedures for a minimum of 2 hours prior to sample collection. Saliva samples were collected using the passive drooling technique. Participants were seated in an upright position and instructed to allow saliva to accumulate in the floor of the mouth before expectorating into sterile polypropylene tubes over a 10-minute collection period. Immediately following collection, the samples were stored at −80°C until further analysis. Prior to biochemical evaluation, the samples were thawed and centrifuged at 14,000 × g for 5 minutes for separation of cellular debris. The clear supernatant obtained was subsequently used for biochemical estimation.

Estimation of salivary 8-hydroxy-2′-deoxyguanosine levels

The levels of salivary 8-hydroxy-2′-deoxyguanosine (8-OHdG) were determined using a commercially available enzyme-linked immunosorbent assay kit (ELabscience, USA) in accordance with the manufacturer's recommendation. The assay was based on sandwich ELISA technology employing microplates pre-coated with monoclonal antibodies specific for 8-hydroxy-2′-deoxyguanosine. All standards and samples were analyzed in duplicate to ensure reproducibility. Briefly, diluted standards and saliva samples were dispensed into the appropriate wells and incubated at 37°C. After washing, horseradish peroxidase-conjugated detection antibodies were added, followed by tetramethylbenzidine substrate solution. The reaction was terminated using the stop solution, and the optical density was measured at 450 nm using a microplate reader. Salivary 8-OHdG concentrations were determined from the standard curve generated using known concentrations of the analyte.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS version 11.5; SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation (SD), whereas categorical variables were presented as frequencies and percentages. The distribution of data was assessed for normality prior to inferential statistical analysis.

Intergroup comparisons for normally distributed variables were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Non-normally distributed variables were analyzed using the Kruskal–Wallis test followed by Dunn's post hoc analysis. Associations between categorical variables were evaluated using the chi-square test.

Correlations between clinical periodontal parameters and salivary 8-OHdG levels were assessed using Spearman's rank correlation coefficient. Multiple linear regression analysis was performed to evaluate the influence of independent variables on salivary 8-OHdG concentrations while controlling for potential confounding factors. A p-value <0.05 was considered statistically significant throughout the analysis.

Results

The present study was conducted in the Department of Periodontology at A.E.C.S. Maaruti College of Dental Sciences and Research Centre. A total of 90 participants reporting to the outpatient department of Periodontology were categorized into five groups: healthy controls (Group I), chronic periodontitis patients without tobacco exposure (Group II), chronic periodontitis patients who smoked tobacco (Group III), chronic periodontitis patients using smokeless tobacco (Group IV), and

tobacco users without chronic periodontitis (Group V). The mean age of participants was 52.44 ± 11.99 years in Group I, 58.39 ± 5.44 years in Group II, 57.67 ± 8.38 years in Group III, 59.23 ± 9.02 years in Group IV, and 55.56 ± 6.12 years in Group V.

Intergroup comparison using the Kruskal–Wallis test demonstrated no statistically significant difference in age distribution among the five study groups ($p = 0.26$) (Table 1) (Graph no. 1). Gender distribution was comparable across all study groups, with males comprising the majority of participants in each group. The proportion of male participants ranged from 66.7% to 88.9%, while females accounted for 11.1%–33.3% of the study population. No statistically significant intergroup difference in sex distribution was observed following Chi-square analysis ($p = 0.61$) (Table 2) (Graph 2).

Comparison of mean Plaque index scores and comparison of mean difference in Plaque index scores between the groups

The plaque index (PI) scores differed significantly among the groups ($p < 0.001$). The highest PI scores were observed in Group IV (2.360 ± 0.214), followed by Group II (1.620 ± 0.214), Group III (1.345 ± 0.053), Group I (0.647 ± 0.066), and Group V (0.580 ± 0.158) (Table 3). Post hoc analysis demonstrated significantly higher PI scores in Group IV compared with all other groups ($p < 0.001$). No statistically significant difference was identified between Group I and Group V ($p = 0.70$) (Table 4) (Graph 3).

Comparison of mean Gingival index scores and comparison of mean difference in Gingival index scores between group

The mean gingival index (GI) scores demonstrated statistically significant differences among the study groups ($p < 0.001$). Group IV exhibited the highest mean GI score (2.268 ± 0.183), followed by Group III (1.649 ± 0.156), Group V (0.924 ± 0.099), Group II (0.735 ± 0.220), and Group I (0.403 ± 0.081) (Table 5). Post hoc analysis using Tukey's test revealed that Group IV demonstrated significantly higher GI scores compared with all other groups ($p < 0.001$). Group III also showed significantly elevated GI scores in comparison with Groups I, II, and V ($p < 0.001$). Furthermore, Group V exhibited significantly higher GI scores than Groups I and II ($p < 0.001$ and $p = 0.004$, respectively). In addition, Group II demonstrated significantly greater GI scores compared with Group I ($p < 0.001$). Overall, GI scores were highest in Group IV, followed sequentially by Groups III, V, II, and I (Table 6) (Graph 4).

Comparison of mean probing depth and comparison of mean difference in probing depth between group

The mean probing depth (PD) values demonstrated statistically significant differences among the study groups ($p < 0.001$). Group IV exhibited the highest mean PD value (7.270 ± 0.360 mm), followed by Group III (4.205 ± 0.053 mm) and Group II (4.065 ± 0.053 mm), whereas substantially lower PD values were observed in Group I (1.545 ± 0.053 mm) and Group V (1.511 ± 0.127 mm) (Table 7). Multiple intergroup comparisons using Tukey's post hoc analysis revealed that Group IV demonstrated significantly greater PD values compared with all other groups ($p < 0.001$). Group III exhibited significantly higher PD values than Groups I and V ($p < 0.001$), while Group II also demonstrated significantly elevated PD values compared with Groups I and V ($p < 0.001$). However, no statistically significant differences were observed between Groups II and III ($p = 0.13$) or between Groups I and V ($p = 0.98$). Overall, PD values were highest in Group IV, followed by Groups III, II, I, and V (Table 8) (Graph 5).

Comparison of mean clinical attachment loss and comparison of mean difference in clinical attachment loss between group

The mean clinical attachment level (CAL) demonstrated statistically significant differences among the study groups ($p < 0.001$). The highest mean CAL was observed in Group IV (8.168 ± 0.506 mm), followed by Group III (5.557 ± 0.066 mm) and Group II (5.010 ± 0.107 mm), whereas markedly lower CAL values were recorded in Group I (1.565 ± 0.053 mm) and Group V (1.434 ± 0.151 mm) (Table 9). Tukey's post hoc analysis demonstrated that Group IV exhibited significantly greater CAL values compared with all other groups ($p < 0.001$). Group III showed significantly higher CAL values than Groups I and V ($p < 0.001$), while Group II also demonstrated significantly elevated CAL values in comparison with Groups I and V ($p < 0.001$). However, no statistically significant differences were observed between Groups II and III ($p = 0.08$) or between Groups I and V ($p = 0.50$). Overall, CAL values were highest in Group IV, followed by Groups III, II, I, and V (Table 10) (Graph 6).

Comparison of mean Salivary 8-hydroxyguanosine levels (pg/ml) and comparison of mean difference in Salivary 8- hydroxyguanosine levels b/w groups

Salivary 8-OHdG concentrations showed marked intergroup variation and were significantly elevated in tobacco-associated chronic periodontitis groups ($p < 0.001$). Group III demonstrated the highest salivary 8-OHdG levels (12.296 ± 2.137 pg/mL), followed by Group IV (10.126 ± 2.265 pg/mL), Group II (3.479 ± 0.620 pg/mL), Group V (2.044 ± 0.694 pg/mL), and Group I (1.669 ± 0.392 pg/mL) (Table 11). Dunn's post hoc analysis demonstrated significantly higher 8-OHdG levels in Groups III and IV compared with all other groups ($p < 0.001$). No statistically significant difference was identified between Group I and Group V ($p = 0.94$) (Table 12) (Graph 7).

Determination of relationship between clinical parameters and salivary 8-hydroxyguanosine levels in each group

Spearman's correlation analysis demonstrated significant positive correlations between salivary 8-hydroxyguanosine (8-OHdG) levels and periodontal clinical parameters in Groups II, III, and IV. In Group II, salivary 8-OHdG levels exhibited a moderate positive correlation with plaque index (PI) scores ($\rho = 0.51$; $p = 0.03$) and strong positive correlations with gingival index (GI), probing depth (PD), and clinical attachment level (CAL) ($\rho = 0.63$, 0.66, and 0.65, respectively; $p < 0.01$). Similarly, in Group III, salivary 8-OHdG levels demonstrated moderate positive correlations with PI and GI scores ($\rho = 0.56$ and 0.59, respectively), along with strong positive correlations with PD and CAL ($\rho = 0.71$ and 0.68, respectively). These associations were statistically significant ($p = 0.02$, $p = 0.01$, $p = 0.001$, and $p = 0.002$, respectively). In Group IV, salivary 8-OHdG levels showed strong positive correlations with all evaluated periodontal parameters, including PI ($\rho = 0.61$), GI ($\rho = 0.66$), PD ($\rho = 0.75$), and CAL ($\rho = 0.72$), with all correlations attaining statistical significance ($p \leq 0.007$). However, no statistically significant correlations were observed between salivary 8-OHdG levels and periodontal clinical parameters in Groups I and V (Table 13).

Stepwise Multiple Linear Regression Analysis to predict Salivary 8- hydroxyguanosine levels in each group

Stepwise multiple linear regression analysis identified probing depth as a significant predictor of salivary 8-OHdG levels in Groups II, III, and IV. In Group II, both PD and CAL significantly predicted salivary 8-OHdG

concentrations, accounting for 57% of the observed variability ($R^2 = 0.57$). In Group III, PD independently predicted salivary 8-OHdG levels and explained 39% of the variability ($R^2 = 0.39$). Similarly, in Group IV, PD was identified as a significant predictor, accounting for 46% of the variability in salivary 8-OHdG levels ($R^2 = 0.46$). Collectively, these findings suggest that salivary 8-OHdG levels are significantly elevated in chronic periodontitis patients, particularly among tobacco users, and are positively associated with the severity of periodontal destruction and inflammation (Table 14).

Table 1: Comparison of mean Age (in yrs.) between 5 groups using Kruskal Wallis Test

Comparison of mean Age (in yrs.) between 5 groups using Kruskal Wallis Test						
Groups	N	Mean	SD	Min	Max	p-value
Group I	18	52.44	11.991	32	69	0.26
Group II	18	58.39	5.436	45	66	
Group III	18	57.67	8.381	38	68	
Group IV	18	59.28	9.015	40	71	
Group V	18	55.56	6.119	47	66	

Table 2: Comparison of gender distribution between 5 groups using Chi square Test

Comparison of gender distribution between 5 groups using Chi square Test					
Group	Males		Females		p-value
	n	%	n	%	
Group I	12	66.7%	6	33.3%	0.61
Group II	13	72.2%	5	27.8%	
Group III	16	88.9%	2	11.1%	
Group IV	13	72.2%	5	27.8%	
Group V	13	72.2%	5	27.8%	

Table 3: Comparison of mean PI Scores between 5 groups using One-way ANOVA Test

Comparison of mean PI Scores between 5 groups using One-way ANOVA Test						
Groups	N	Mean	SD	Min	Max	p-value
Group I	18	0.647	0.066	0.55	0.75	<0.001*
Group II	18	1.620	0.214	1.28	1.96	
Group III	18	1.345	0.053	1.26	1.43	
Group IV	18	2.360	0.214	2.02	2.70	
Group V	18	0.580	0.158	0.31	0.80	

* - Statistically Significant

Table 4: Multiple comparison of mean difference in PI Scores b/w groups using Tukey's post hoc Test

Multiple comparison of mean difference in PI Scores b/w groups using Tukey's post hoc Test					
(I) Group	(J) Group	Mean Diff. (I-J)	95% CI for the Diff.		p-value
			Lower	Upper	
Group I	Group II	-0.973	-1.119	-0.827	<0.001*
	Group III	-0.698	-0.844	-0.552	<0.001*
	Group IV	-1.713	-1.859	-1.567	<0.001*
	Group V	0.067	-0.079	0.213	0.70
Group II	Group III	0.275	0.129	0.421	<0.001*
	Group IV	-0.740	-0.886	-0.594	<0.001*
	Group V	1.040	0.894	1.186	<0.001*
Group III	Group IV	-1.015	-1.161	-0.869	<0.001*
	Group V	0.765	0.619	0.911	<0.001*
Group IV	Group V	1.780	1.634	1.926	<0.001*

* - Statistically Significant

Table 5: Comparison of mean GI Scores between 5 groups using One-way ANOVA Test

Comparison of mean GI Scores between 5 groups using One-way ANOVA Test						
Groups	N	Mean	SD	Min	Max	p-value
Group I	18	0.403	0.081	0.26	0.56	
Group II	18	0.735	0.220	0.35	1.04	
Group III	18	1.649	0.156	1.33	1.81	
Group IV	18	2.268	0.183	1.97	2.65	
Group V	18	0.924	0.099	0.79	1.08	<0.001*

* - Statistically Significant

Table 6: Multiple comparison of mean difference in GI Scores b/w groups using Tukey's post hoc Test

Multiple comparison of mean difference in GI Scores b/w groups using Tukey's post hoc Test					
(I) Group	(J) Group	Mean Diff. (I-J)	95% CI for the Diff.		p-value
			Lower	Upper	
Group I	Group II	-0.332	-0.477	-0.186	<0.001*
	Group III	-1.246	-1.391	-1.100	<0.001*
	Group IV	-1.864	-2.010	-1.719	<0.001*
	Group V	-0.521	-0.666	-0.375	<0.001*
Group II	Group III	-0.914	-1.059	-0.768	<0.001*
	Group IV	-1.533	-1.678	-1.387	<0.001*
	Group V	-0.189	-0.334	-0.043	0.004*

Group III	Group IV	-0.619	-0.764	-0.473	<0.001*
	Group V	0.725	0.580	0.871	<0.001*
Group IV	Group V	1.344	1.198	1.489	<0.001*

* - Statistically Significant

Table 7: Comparison of mean PD levels between 4 groups using One-way ANOVA Test

Comparison of mean PD levels between 4 groups using One-way ANOVA Test						
Groups	N	Mean	SD	Min	Max	p-value
Group I	18	1.545	0.053	1.46	1.63	
Group II	18	4.065	0.053	3.98	4.15	
Group III	18	4.205	0.053	4.12	4.29	
Group IV	18	7.270	0.360	6.65	7.88	
Group V	18	1.511	0.127	1.20	1.69	<0.001*

*Statistically Significant

Table 8: Multiple comparison of mean difference in PD levels b/w groups using Tukey's post hoc Test

Multiple comparison of mean difference in PD levels b/w groups using Tukey's post hoc Test					
(I) Group	(J) Group	Mean Diff. (I-J)	95% CI for the Diff.		p-value
			Lower	Upper	
Group I	Group II	-2.520	-2.683	-2.357	<0.001*
	Group III	-2.660	-2.823	-2.497	<0.001*
	Group IV	-5.725	-5.888	-5.562	<0.001*
	Group V	0.034	-0.129	0.198	0.98
Group II	Group III	-0.140	-0.303	0.023	0.13
	Group IV	-3.205	-3.368	-3.042	<0.001*
	Group V	2.554	2.391	2.718	<0.001*
Group III	Group IV	-3.065	-3.228	-2.902	<0.001*
	Group V	2.694	2.531	2.858	<0.001*
Group IV	Group V	5.759	5.596	5.923	<0.001*

* - Statistically Significant

Table 9: Comparison of mean CAL levels between different groups using One-way ANOVA Test

Comparison of mean CAL levels between 5 groups using One-way ANOVA Test						
Groups	N	Mean	SD	Min	Max	p-value
Group I	18	1.565	0.053	1.48	1.65	<0.001*
Group II	18	5.010	0.107	4.84	5.18	
Group III	18	5.557	0.066	5.43	5.65	

Group IV	18	8.168	0.506	7.50	9.00	
Group V	18	1.434	0.151	1.17	1.68	

* - Statistically Significant

Table 10: Multiple comparison of mean difference in CAL levels b/w groups using Tukey's post hoc Test

Multiple comparison of mean difference in CAL levels b/w groups using Tukey's post hoc Test					
(I) Group	(J) Group	Mean Diff. (I-J)	95% CI for the Diff.		p-value
			Lower	Upper	
Group I	Group II	-3.445	-3.672	-3.218	<0.001*
	Group III	-3.992	-4.218	-3.765	<0.001*
	Group IV	-6.603	-6.830	-6.377	<0.001*
	Group V	0.131	-0.096	0.357	0.50
Group II	Group III	-0.547	-0.773	-0.320	0.08
	Group IV	-3.158	-3.385	-2.932	<0.001*
	Group V	3.576	3.349	3.802	<0.001*
Group III	Group IV	-2.612	-2.838	-2.385	<0.001*
	Group V	4.122	3.896	4.349	<0.001*
Group IV	Group V	6.734	6.507	6.961	<0.001*

* - Statistically Significant

Table 11: Comparison of mean Salivary 8-hydroxyguanosine levels (pg/ml) between the groups using Kruskal Wallis Test

Comparison of mean Salivary 8-hydroxyguanosine levels (pg/ml) between 4 groups using Kruskal Wallis Test						
Groups	N	Mean	SD	Min	Max	p-value
Group I	18	1.669	0.392	1.07	2.18	<0.001*
Group II	18	3.479	0.620	2.42	4.60	
Group III	18	12.296	2.137	8.77	15.98	
Group IV	18	10.126	2.265	6.99	14.60	
Group V	18	2.044	0.694	0.97	3.23	

* - Statistically Significant

Table 12: Multiple comparison of mean difference in Salivary 8-hydroxyguanosine levels b/w groups using Dunn's post hoc Test

Multiple comparison of mean difference in Salivary 8-hydroxyguanosine levels b/w groups using Dunn's post hoc Test					
(I) Group	(J) Group	Mean Diff. (I-J)	95% CI for the Diff.		p-value
			Lower	Upper	
Group I	Group II	-1.810	-3.170	-0.450	0.003*
	Group III	-10.627	-11.987	-9.266	<0.001*

	Group IV	-8.457	-9.817	-7.096	<0.001*
	Group V	-0.376	-1.736	0.985	0.94
Group II	Group III	-8.817	-10.177	-7.456	<0.001*
	Group IV	-6.647	-8.007	-5.286	<0.001*
	Group V	1.434	0.074	2.795	<0.001*
Group III	Group IV	2.170	0.810	3.530	<0.001*
	Group V	10.251	8.891	11.611	<0.001*
Group IV	Group V	8.081	6.721	9.441	<0.001*

* - Statistically Significant

Table 13: Spearman's correlation test to determine the relationship b/w Clinical Parameters & Salivary 8-hydroxyguanosine levels in each group

Spearman's correlation test to determine the relationship b/w Clinical Parameters & Salivary 8-hydroxyguanosine levels in each group						
Group	Marker	values	PI	GI	PPD	CAL
Group I	Sal. 8-OHdG levels	rho	0.16	0.12	0.35	0.15
		p-value	0.54	0.64	0.16	0.56
Group II	Sal. 8-OHdG levels	rho	0.51	0.63	0.66	0.65
		p-value	0.03*	0.005*	0.003*	0.004*
Group III	Sal. 8-OHdG levels	rho	0.56	0.59	0.71	0.68
		p-value	0.02*	0.01*	0.001*	0.002*
Group IV	Sal. 8-OHdG levels	rho	0.61	0.66	0.75	0.72
		p-value	0.007*	0.003*	<0.001*	0.001*
Group V	Sal. 8-OHdG levels	rho	0.37	0.40	0.43	0.44
		p-value	0.13	0.10	0.08	0.07

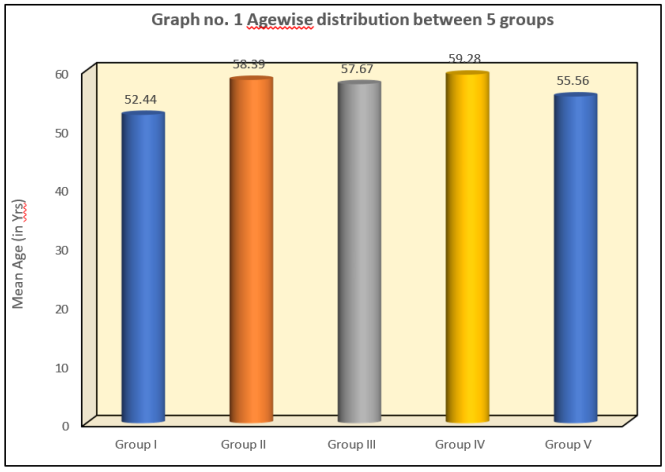
* - Statistically Significant

Table 14: Stepwise Multiple Linear Regression Analysis to predict Salivary 8 hydroxyguanosine levels in each group

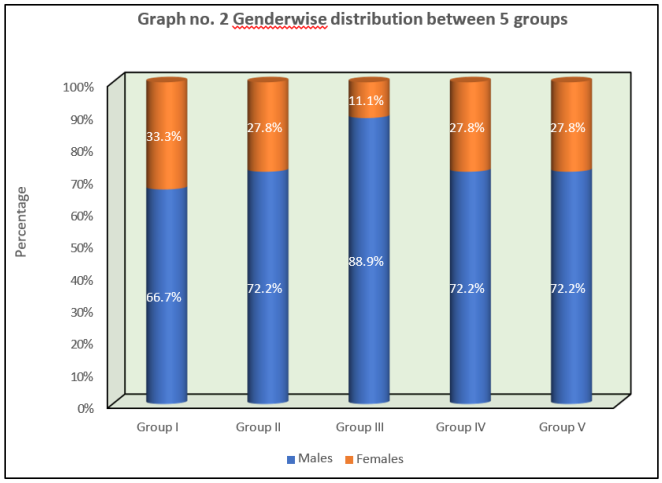
Stepwise Multiple Linear Regression Analysis to predict Salivary 8-hydroxyguanosine levels in each group						
Groups	IV	Unstd. Coefficients		t	p-value	R^2
		β	SE			
Group II	Constant	-136.091	45.475	-2.993	0.009*	
	PPD	76.910	28.902	2.661	0.02*	
	CAL	14.545	14.451	-2.39	0.03*	0.57
Group III	Constant	-97.642	31.875	-3.063	0.007*	
	PPD	26.144	7.580	3.449	0.003*	0.39

Group IV	Constant	-21.777	8.198	-2.656	0.02*	0.46
	PPD	4.388	1.126	3.896	0.001*	

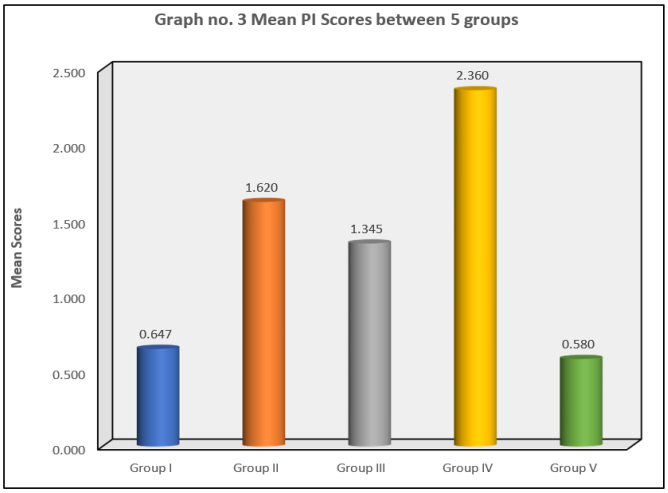
Graph 1: Age wise distribution between 5 groups



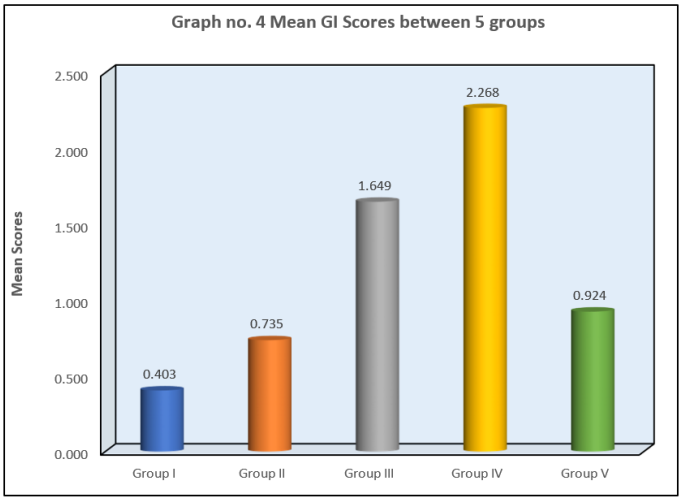
Graph 2: Gender wise distribution between 5 groups



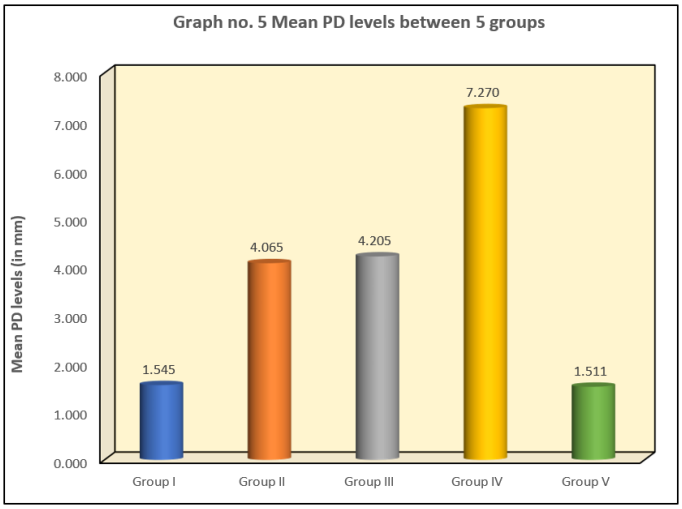
Graph 3: Mean PI scores between 5 groups



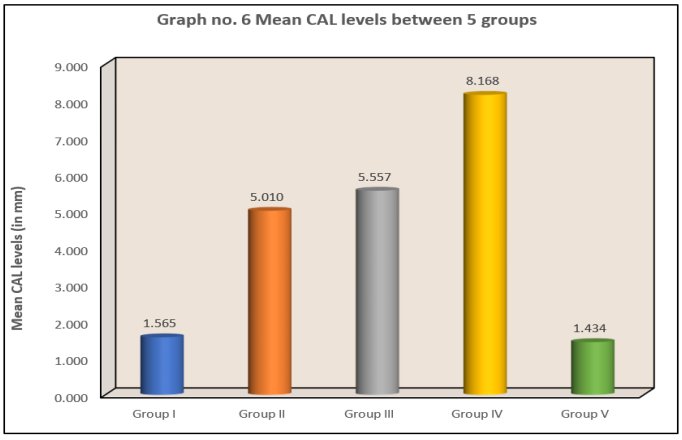
Graph 4: Mean GI scores between 5 group



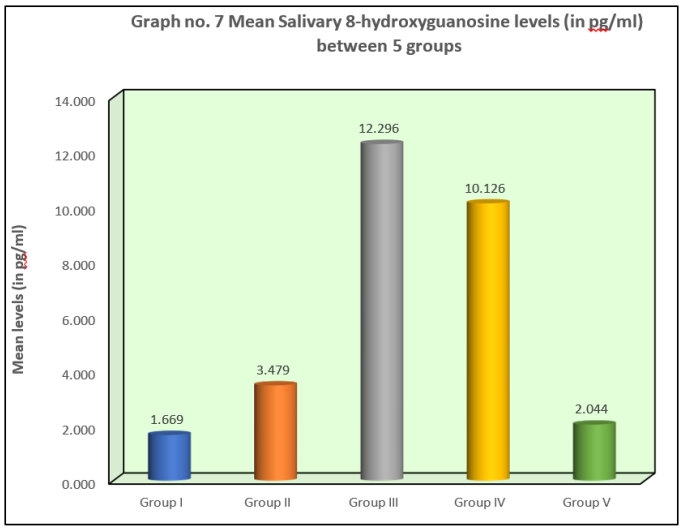
Graph 5: Mean PD levels between 5 groups



Graph 6: Mean CAL levels between 5 groups



Graph 7: Mean Salivary 8-hydroguanosine levels (in pg/ml) between 5 groups



Discussion

Periodontitis is a chronic inflammatory disease that results in the progressive destruction of the tooth-supporting structures in response to pathogenic dental biofilm. The severity and progression of periodontal disease are significantly influenced by several modifying risk factors, among which tobacco consumption remains one of the most important environmental determinants[8]. Both smoked and smokeless forms of tobacco have been associated with increased periodontal breakdown, altered host immune response, enhanced oxidative stress, and impaired periodontal healing[9]. In view of these associations, the present study was designed to evaluate and compare the clinical periodontal parameters and salivary 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels among healthy individuals, chronic periodontitis patients without tobacco exposure, smokers with chronic periodontitis, smokeless tobacco users with chronic periodontitis, and tobacco users without chronic periodontitis. The present study demonstrated significant differences in periodontal clinical parameters and salivary 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels among the study groups. Smokeless tobacco users with chronic periodontitis exhibited significantly higher plaque index, gingival index, probing depth, and clinical attachment loss compared with smokers and non-tobacco users with periodontitis. These findings suggest that smokeless tobacco may exert a more destructive effect on the periodontal supporting tissues than smoking alone. The higher plaque accumulation observed among smokeless tobacco users may be related to poor oral hygiene practices, increased calculus deposition, and prolonged retention of tobacco products within the oral cavity[10]. Similar findings were reported by Kishore Kumar Katuri et al.,[11] where smokeless tobacco users demonstrated poorer periodontal status compared with smokers. Continuous placement of smokeless tobacco adjacent to gingival tissues may also favor plaque retention and create a local environment conducive to periodontal breakdown. Gingival inflammation was significantly greater among smokeless tobacco users than smokers in the present study. This finding may be attributed to the chronic local irritation caused by direct contact of smokeless tobacco with the gingival tissues. In contrast, smokers demonstrated comparatively lower gingival inflammation despite exhibiting periodontal destruction[12]. Similarly, Bergström J et.al.,[13] reported reduced clinical signs of gingival inflammation among smokers. This phenomenon is likely related to the vasoconstrictive effect of nicotine, which reduces gingival blood flow and suppresses overt inflammatory signs such as bleeding and erythema. The present study also demonstrated significantly greater probing depth and clinical attachment loss among smokeless tobacco users with chronic periodontitis. Similar observations were reported by Anand et al.,[14] where smokeless tobacco users exhibited greater attachment loss and gingival recession at tobacco-associated sites. The greater periodontal destruction observed in the present study may

be explained by sustained exposure of periodontal tissues to nicotine and other toxic constituents present in smokeless tobacco products[15]. Prolonged contact of tobacco quid with the gingiva may induce chronic inflammation, localized trauma, and connective tissue breakdown, ultimately leading to deeper periodontal pockets and increased attachment loss[15]. An important finding of the present study was the significantly elevated salivary 8-OHdG levels among smokers with chronic periodontitis, followed by smokeless tobacco users with chronic periodontitis. Similar findings were reported by van Zeeland et al.,[16] where tobacco users demonstrated significantly elevated oxidative stress marker levels compared with controls. Cigarette smoke contains a large number of reactive oxygen species and free radicals capable of inducing oxidative DNA damage. In addition, smoking stimulates inflammatory cell activation and oxidative burst reactions, resulting in excessive production of reactive oxygen species[17]. These mechanisms may explain the higher salivary 8-OHdG levels observed among smokers in the present study. Interestingly, although smokeless tobacco users demonstrated greater clinical periodontal destruction, smokers exhibited higher salivary 8-OHdG levels[17]. This discrepancy may be explained by differences in the pattern of tissue injury associated with the two forms of tobacco consumption. Smokeless tobacco primarily causes localized periodontal destruction through chronic direct contact with gingival tissues, whereas smoking induces a greater systemic oxidative burden because of continuous exposure to inhaled oxidants and free radicals[18]. As a result, smokers may exhibit greater oxidative DNA damage despite comparatively lower clinical periodontal destruction. Another notable finding was that tobacco users without periodontitis demonstrated relatively low salivary 8-OHdG levels, with no significant difference compared with healthy controls. Similar findings were reported by Hendek MK et al.,[19] where oxidative stress marker levels were closely associated with periodontal inflammatory status. The findings of the present study therefore suggest that tobacco exposure alone may not substantially elevate salivary oxidative stress markers in the absence of periodontal inflammation. Rather, the coexistence of periodontal inflammation and tobacco-associated oxidative stress appears to enhance oxidative DNA damage. The present study further demonstrated significant positive correlations between salivary 8-OHdG levels and periodontal clinical parameters including plaque index, gingival index, probing depth, and clinical attachment loss among chronic periodontitis groups. Similar observations were reported by Baltacıoğlu E et al.,[20] where oxidative stress marker levels positively correlated with periodontal disease severity. Increased periodontal destruction is associated with greater inflammatory burden and enhanced production of reactive oxygen species, which may contribute to elevated oxidative DNA damage and increased salivary 8-OHdG levels[21]. Stepwise multiple linear regression analysis identified probing depth as a significant predictor of salivary 8-OHdG levels among smokers and smokeless tobacco users with chronic periodontitis. Deeper periodontal pockets may harbor increased bacterial load and inflammatory infiltrate, thereby promoting excessive reactive oxygen species generation and oxidative tissue injury[22]. This may explain the positive association between probing depth and salivary oxidative stress marker levels observed in the present study. Certain limitations should be considered while interpreting the findings of the present study. Salivary samples were collected only once, despite evidence suggesting intraday variability in oxidative

stress markers. In addition, systemic and lifestyle-related factors such as obesity, psychological stress, dietary habits, and undiagnosed systemic diseases were not comprehensively evaluated and may have influenced oxidative stress levels. The relatively limited sample size may also restrict generalization of the findings to larger populations. Overall, the findings of the present study suggest that tobacco-associated periodontitis is accompanied by increased oxidative DNA damage, as reflected by elevated salivary 8-OHdG levels. The significant association between salivary 8-OHdG levels and periodontal clinical parameters further supports the role of oxidative stress in periodontal disease progression and highlights the potential utility of salivary 8-OHdG as a non-invasive biomarker for assessing periodontal disease activity in tobacco users.

Conclusion

Within the limitations of the present study, salivary 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were found to be significantly associated with chronic periodontitis in tobacco users. Although smokeless tobacco users exhibited more severe periodontal destruction clinically, smokers demonstrated higher salivary 8-OHdG levels, suggesting a greater oxidative stress burden associated with cigarette smoking. The positive correlation observed between salivary 8-OHdG levels and periodontal clinical parameters further indicates that oxidative DNA damage increases with periodontal disease severity. The findings of the present study therefore support the study hypothesis that salivary 8-OHdG reflects periodontal disease activity in smokers and smokeless tobacco users with chronic periodontitis. Furthermore, the comparatively lower biomarker levels among tobacco users without periodontitis suggest that periodontal inflammation, rather than tobacco exposure alone, may play a major role in elevating salivary oxidative stress levels. Collectively, these observations highlight the potential utility of salivary 8-OHdG as a non-invasive biomarker for assessing oxidative stress and periodontal disease severity in tobacco-associated periodontitis.

Clinical significance

The findings of the present study highlight the important role of oxidative stress in tobacco-associated periodontitis and suggest that salivary 8-hydroxy-2'-deoxyguanosine (8-OHdG) may be a promising non-invasive biomarker for assessing periodontal disease activity. The significant association between salivary 8-OHdG levels and periodontal clinical parameters indicates that oxidative DNA damage may closely reflect the extent of ongoing periodontal tissue destruction. The study also reinforces the adverse effects of both smoking and smokeless tobacco on periodontal health, although the pattern of tissue damage appears to differ between the two habits. While smokeless tobacco users demonstrated more severe clinical periodontal destruction, smokers exhibited a greater oxidative stress burden. Assessment of salivary oxidative stress markers may therefore provide additional value in identifying high-risk individuals, monitoring disease progression, and improving the overall management of periodontal disease in tobacco users.

References

1. Hajishengallis, G., Chavakis, T., & Lambris, J. D. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. Periodontology 2000 2020;84(1):14–34.
2. Cardoso, E. M., Reis, C., & Manzanares - Céspedes, M. C. Chronic periodontitis, inflammatory cytokines, and interrelationship with other chronic diseases. Postgraduate Medicine 2017;130(1):98–104.
3. Almerich-Silla, J. M., Montiel-Company, J. M., Pastor, S., Serrano, F., Puig-Silla, M., & Dasí, F.

Oxidative Stress Parameters in Saliva and Its Association with Periodontal Disease and Types of Bacteria. Disease Markers 2015;1–7.

4. Arunachalam, R., Reshma, A. P., Rajeev, V., Kurra, S. B., Prince, M. R. J., & Syam, N. Salivary 8-Hydroxydeoxyguanosine – a valuable indicator for oxidative DNA damage in periodontal disease.
5. Silva, H. Tobacco Use and Periodontal Disease—The Role of Microvascular Dysfunction. Biology 2021;10(5):441.
6. Chaffee, B. W., Couch, E. T., Vora, M. V., & Holliday, R. S. Oral and periodontal implications of tobacco and nicotine products. Periodontology 2000 2021;87(1):241–253.
7. Al Kawas, S., Al-Marzooq, F., Rahman, B., Shearston, J. A., Saad, H., Benzina, D., & Weitzman, M. The impact of smoking different tobacco types on the subgingival microbiome and periodontal health: a pilot study. Scientific Reports 2021;11(1).
8. Alwithanani N. Periodontal disease and smoking: systematic review. Journal of Pharmacy and Bioallied Sciences. 2023 Jul 1;15(Suppl 1):S64-71.
9. Amarasena N, Ekanayaka AN, Herath L, Miyazaki H. Tobacco use and oral hygiene as risk indicators for periodontitis. Community dentistry and oral epidemiology. 2002 Apr;30(2):115-23.
10. Kulkarni V, Uttamani JR, Bhatavadekar NB. Comparison of clinical periodontal status among habitual smokeless-tobacco users and cigarette smokers. International dental journal. 2016 Feb 1;66(1):29-35.
11. Katuri KK, Alluri JK, Chintagunta C, Tadiboina N, Borugadda R, Loya M, Marella Y, Bollepalli AC. Assessment of periodontal health status in smokers and smokeless tobacco users: a cross-sectional study. Journal of Clinical and Diagnostic Research: JCDR. 2016 Oct 1;10(10):ZC143.
12. Scott DA, Singer DL. Suppression of overt gingival inflammation in tobacco smokers–clinical and mechanistic considerations. International journal of dental hygiene. 2004 Aug;2(3):104-10.
13. Bergström J. Tobacco smoking and chronic destructive periodontal disease. Odontology. 2004 Sep;92(1):1-8.
14. Anand PS, Kamath KP, Bansal A, Dwivedi S, Anil S. Comparison of periodontal destruction patterns among patients with and without the habit of smokeless tobacco use–a retrospective study. Journal of periodontal research. 2013 Oct;48(5):623-31.
15. Ojima M, Hanioka T. Destructive effects of smoking on molecular and genetic factors of periodontal disease. Tobacco Induced Diseases. 2010 Feb 20;8(1):4.
16. van Zeeland AA, de Groot AJ, Hall J, Donato F. 8-Hydroxydeoxyguanosine in DNA from leukocytes of healthy adults: relationship with cigarette smoking, environmental tobacco smoke, alcohol and coffee consumption. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 1999 Feb 19;439(2):249-57.
17. Burke A, FitzGerald GA. Oxidative stress and smoking-induced vascular injury. Progress in cardiovascular diseases. 2003 Jul 1;46(1):79-90.
18. Caliri AW, Tommasi S, Besaratinia A. Relationships among smoking, oxidative stress, inflammation, macromolecular damage, and cancer. Mutation Research/Reviews in Mutation Research. 2021 Jan 1;787:108365.
19. Hendek MK, Erdemir EO, Kisa U, Ozcan G. Effect of initial periodontal therapy on oxidative stress markers in gingival crevicular fluid, saliva, and serum in

smokers and non-smokers with chronic periodontitis. Journal of periodontology. 2015 Feb;86(2):273-82.

20. Baltacıoğlu E, Yuva P, Aydın G, Alver A, Kahraman C, Karabulut E, Akalın FA. Lipid peroxidation levels and total oxidant/antioxidant status in serum and saliva from patients with chronic and aggressive periodontitis. Oxidative stress index: a new biomarker for periodontal disease?. Journal of periodontology. 2014 Oct;85(10):1432-41.
21. Çanakçi CF, Çanakçi V, Tatar A, Eltas A, Sezer U, Çiçek Y, Oztas S. Increased salivary level of 8-hydroxydeoxyguanosine is a marker of premature oxidative mitochondrial DNA damage in gingival tissue of patients with periodontitis. Archivum immunologiae et therapiae experimentalis. 2009 Jun;57(3):205-11.
22. Konopka T, Król K, Kopeć W, Gerber H. Total antioxidant status and 8-hydroxy-2′-deoxyguanosine levels in gingival and peripheral blood of periodontitis patients. Archivum immunologiae et therapiae experimentalis. 2007 Dec;55(6):417-25.