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Salivary 8-hydroxyguanosine levels in smokers and non-smokers with chronic periodontitis

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Abstract

This case-controlled clinical trial was performed on the salivary 8-hydroxyguanosine (8-OHdG) levels in smokers and non-smokers with chronic periodontitis after non-surgical periodontal therapy. Subjects ($N = 40$) with periodontitis (smokers and non-smokers) and with clinically healthy conditions (smokers and non-smokers) were assigned to this study. At baseline, clinical periodontal parameters (plaque index, gingival index, pocket probing depth and clinical attachment levels) were evaluated. Saliva samples were obtained pre- and post-treatment to quantify the 8-OHdG levels using Elisa technique. Subjects diagnosed with chronic periodontitis with smoking habit (CPs) and non-smokers (CPns) received scaling and root planing. In clinically healthy subjects with smoking habit (CHs) and non-smokers (CHns), only oral hygiene tutoring was performed. All clinical measurements and salivary collection were repeated in a 3-month recall interval. Data were analyzed using Anova, Tukey post hoc test and Mann–Whitney 'U' tests ($P < 0.05$). At baseline, CPs and CPns groups showed significantly higher PI, GI, PD and CAL values than those of CHns and CHs ($P < 0.001$). Baseline salivary levels of 8-OHdG were significantly higher in CPs group (14.775 pg/mL) ($P < 0.001$) compared to the other groups. All clinical parameters in chronic periodontitis group improved at the 3rd month recall interval, however, with regards to 8-OHdG values, the CP smoker category still had a higher level compared to CP non-smoker. This study reflects an on-going periodontal destructive status in smokers and salivary 8-OHdG levels could be recognized as an oxidative biomarker for determining periodontal tissue destruction.

Keywords 8-Hydroxyguanosine · Chronic periodontitis · Salivary biomarker · Smoking · Oxidative stress

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Introduction

Periodontitis is an inflammatory disorder wherein complex interactions between the microorganisms, host response mechanisms and environmental factors result in tissue damage. Among the environmental factors, smoking has been linked with increased morbidity and mortality. Over the last decade, there have been a number of reviews that have considered the biological mechanisms underlying susceptibility of smokers to periodontitis in [1, 2]. As per demographic records, smokers demonstrate a 2.6 to sixfold increased incidence of periodontitis compared to non-smokers [3] and decreased response to periodontal therapy [4, 5].

Despite the fact that a clear dose–response relationship between chronic periodontitis and smoking was reported [6], the mechanisms by which smoking contributes to the pathogenesis of periodontitis are not yet clearly understood. However, smokers are more likely to harbour a higher prevalence of potential periodontal pathogens, which could influence host-cytokine levels [7, 8].

The past few decades have gathered strong evidence which implicates oxidative stress in the pathogenesis of periodontal disease [9, 10]. Free radicals and reactive oxygen species (ROS) at normal levels are essential for normal biological processes [11] that could also have deleterious effects at higher concentrations leading to oxidative damage. Generally, the homeostatic balance between ROS and free radicals is disturbed by various factors, one which is smoking that may enhance oxidative stress not only through the production of reactive oxygen radicals in smoke but also through weakening of the antioxidant defence systems [12, 13]. ROS and in particular the active hydroxyl (OH) group radical, are known to be involved in the destruction of various functional macromolecules, like free and conjugated proteins, lipids and carbohydrates eventually resulting in cellular damage [14, 15]. Polymorphonuclear leukocytes (PMN) are the initial defence cells that predominantly encircle the area accumulated by bacterial pathogens in pathological conditions including periodontitis. Such an infiltration in numbers is likely to lead to an increase in ROS levels [16].

8-Hydroxyguanosine (8-OHdG) is an oxidized nucleoside that is excreted in the body fluids as a reparative consequence of DNA. A stable product that is formed as a result of enzymatic dissolution after ROS induces 8-hydroxylation of guanine base on mitochondrial and nuclear DNA [17, 18]. Studies have demonstrated that the 8-OHdG released in body fluids and tissues has been implicated in the pathogenesis of malignancy, inflammatory, autoimmune disorders and diabetes mellitus [19, 20].

A correlation between the salivary levels of 8-OHdG and periodontal microbiota has been reported indicating its effectiveness as a supportive biomarker for determination of periodontal status [21, 22]. Furthermore, a recent clinical investigation has revealed that initial periodontal therapy has a beneficial effect on decreasing the substantial levels of oxidative biomarkers in smokers and non-smokers [23], thus validating its use as a signature molecule in diagnosis. However, the association between smoking and its effect on diseased periodontium at a cellular level still remains uncertain.

The primary objectives of this study, therefore, were to estimate the salivary levels of 8-OHdG in smokers and non-smokers with chronic periodontitis and also to determine its level after initial periodontal therapy.

Materials and methods

Study groups

The present case-controlled clinical trial with an intervention arm, involved a total of 40 subjects ($n = 10$ per group), comprising of group 1 = smokers with chronic periodontitis (CPs), group 2 = non-smokers with chronic periodontitis (CPns), group 3 = clinically healthy smokers (CHs) and group 4 = non-smokers with clinically healthy periodontium (CHns). Ethical approval was obtained from the institutional review board. All participants were informed regarding the study and a written informed consent was obtained on voluntary participation. Subjects were excluded if they had any systemic disease/immune-compromised condition, or recent history of intake of antibiotics, anti-inflammatory and antioxidants and dietary supplements.

Diagnosis for chronic periodontitis was based on the 1999 international world workshop for classification of periodontal diseases and conditions [24]. A clinical and radiographic assessment characterized recruitment of chronic periodontitis patients by at least 30% teeth with pockets > 5 mm. Smokers were categorized based on verbal query of ≥ 10 cigarettes/day for more than 5 years. Non-smoker groups were subjects who fulfilled the norms of not having smoked cigarettes in their lifetime. The healthy group of individuals was categorized based on systemic well-being, having teeth with pocket depth ≤ 3 mm, no attachment loss, no bleeding on gentle probing and with radiographic confirmation of no bone loss.

Clinical measurements and initial periodontal therapy

Only subjects who fulfilled the inclusion criteria were incorporated into the clinical trial. All clinical measurements were performed by a single examiner. Initially, 5 ml of unstimulated whole saliva samples were obtained in Eppendorf tubes [25] prior to clinical examination to avoid any contamination of the oral fluids which could deter the results. Then, basic periodontal parameters comprising of Plaque Index (PI) [26], Gingival Index (GI) [27], probing pocket depth (PPD) and clinical attachment level (CAL) were recorded on a standard form. PD and CAL were assessed on six sites of a tooth where the deepest probing depth was recorded using William's periodontal probe. Subsequently, all patients received scaling and root planing (SRP), which were completed in at least 2 appointments. As per the routine norm, oral hygiene instructions were given to all subjects. In clinically healthy smoker and non-smoker category, only scaling and oral hygiene tutoring were performed. All clinical measurements and salivary collection were repeated in the 3-month recall interval. The collected saliva samples were centrifuged (2.000g for 10 min) and the supernatants were stored at −80 °C until they were assayed.

Detection of 8-OHdG

To detect the levels of 8-OHdG in saliva, a competitive enzyme immunoassay, Elisa kit (Cloud-Clone Corp,

Houston, Texas, USA) was used according to the manufacturer's instructions. The detection range was 74.07–6000 pg/mL.

Statistical analysis

The data collected was entered into microsoft excel spreadsheet and analyzed using IBM SPSS Statistics, Version 22 (Armonk, NY: IBM Corp). Descriptive data were presented in the form of mean, median, standard deviation and quartiles. Based on the distribution of the data, parametric or non-parametric tests were used. Comparison of the study variables at baseline was done using ANOVA with Tukey post hoc test. Post-treatment statistical data were analyzed by independent sample t test. Paired t test was used compare the study variables between baseline and post-treatment. Mann–Whitney 'U' test was used to compare the change in the clinical parameters and 8-OHdG levels for chronic periodontitis (CP and CP smokers) post-periodontal therapy. Pearson's and Spearman's correlation test were used to test the correlation between the study variables and 8-OHdG. P value less than 0.05 was considered to be statistically significant in all tests.

Results

All patients involved in this clinical trial returned for the follow-up visits. With regards to gender distribution, only males had volunteered to participate. The age of subjects with chronic periodontitis (smokers and non-smokers) was 40–65 years, while the age of the healthy controls was between 25 and 35 years.

Baseline clinical findings

At baseline, chronic periodontitis (smoker and non-smoker) group presented significantly higher values of PI, GI, PD and CAL compared to clinically healthy (smoker and non-smoker) group ($P < 0.001$) (Table 1). The salivary levels of 8-OHdG exhibited significantly higher levels in CP smokers group (14.775 pg/mL) ($P < 0.001$) compared to the other test groups (Table 2). Comparison of clinical variables between the groups at baseline revealed, notable statistical significance in all groups, except in PI values in the clinically healthy smokers and non-smokers and the PPD and CAL values between the chronic periodontitis and clinically healthy smokers and non-smokers (Table 2).

Table 1 Comparison of the variables between the study groups at baseline

	Study groups	N	Mean	Std. deviation	ANOVA	
					F	P value
Baseline PI	1	10	2.17	0.18	343.59	<0.001*
	2	10	1.88	0.25		
	3	10	0.33	0.05		
	4	10	0.34	0.12		
Baseline GI	1	10	1.59	0.11	1408.92	<0.001*
	2	10	1.80	0.06		
	3	10	0.12	0.05		
	4	10	0.22	0.05		
Baseline PPD	1	10	6.40	1.35	62.96	<0.001*
	2	10	5.90	0.88		
	3	10	2.10	0.57		
	4	10	1.90	0.88		
Baseline CAL	1	10	7.00	1.56	82.65	<0.001*
	2	10	7.50	2.46		
	3	10	0	0		
	4	10	0	0		
Baseline 8-OHdG levels (pg/ml)	1	10	14.78	2.39	155.73	<0.001*
	2	10	5.11	1.10		
	3	10	7.06	1.25		
	4	10	1.09	0.08		

NS non-significant

*$P < 0.05$ statistically significant, $P > 0.05$

Table 2 Pairwise comparison of the variables between the study groups at baseline

Dependent variable	(I) Group	(J) Groups	Mean difference (I − s)	Std. error	P value	95% Confidence interval	
						Lower bound	Upper bound
Baseline PI	1	2	0.29	0.08	0.002*	0.09	0.49
		3	1.84	0.08	<0.001*	1.64	2.04
		4	1.83	0.08	<0.001*	1.63	2.04
	2	3	1.55	0.08	<0.001*	1.35	1.75
		4	1.54	0.08	<0.001*	1.34	1.74
	3	4	− 0.01	0.08	0.99(NS)	− 0.21	0.19
Baseline GI	1	2	− 0.21	0.03	<0.001*	− 0.30	− 0.12
		3	1.47	0.03	<0.001*	1.38	1.55
		4	1.37	0.03	<0.001*	1.28	1.46
	2	3	1.68	0.03	<0.001*	1.59	1.77
		4	1.58	0.03	<0.001*	1.49	1.67
	3	4	− 0.10	0.03	0.03*	− 0.19	− 0.01
Baseline PPD	1	2	0.50	0.43	0.65(NS)	− 0.65	1.65
		3	4.30	0.43	<0.001*	3.15	5.45
		4	4.50	0.43	<0.001*	3.35	5.65
	2	3	3.80	0.43	<0.001*	2.65	4.95
		4	4.00	0.43	<0.001*	2.85	5.15
	3	4	0.20	0.43	0.97(NS)	− 0.95	1.35
Baseline CAL	1	2	− 0.50	0.65	0.87(NS)	− 2.26	1.26
		3	7.00	0.65	<0.001*	5.24	8.76
		4	7.00	0.65	<0.001*	5.24	8.76
	2	3	7.50	0.65	<0.001*	5.74	9.26
		4	7.50	0.65	<0.001*	5.74	9.26
	3	4	0.00	0.65	1.00(NS)	− 1.76	1.76
Baseline 8-OHdG levels (pg/mL)	1	2	9.66	0.65	<0.001*	7.91	11.41
		3	7.71	0.65	<0.001*	5.96	9.46
		4	13.69	0.65	<0.001*	11.93	15.44
	2	3	− 1.95	0.65	0.02*	− 3.70	− 0.20
		4	4.02	0.65	<0.001*	2.27	5.78
	3	4	5.97	0.65	<0.001*	4.22	7.73

Tukey post hoc test

*$P < 0.05$ statistically significant, $P > 0.05$

NS non-significant

Post-treatment (3 month) recall period

Initial periodontal therapy was provided only for CP (smoker and non-smoker) group. The other two groups belonged to the healthy category and hence only oral health measures were instructed. All clinical parameters demonstrated reduction in measurements at the 3rd month recall interval (Table 3). Considering comparisons between groups 1 and 2, the PI ranges did display reduction, but were not statistically significant among the groups. However, the CP non-smoker category display marked significant reduction in GI values compared to CP smoker category ($P < 0.001$). Likewise, the post-treatment 8-OHdG levels were seen to reduce in both groups, with predisposition in the CP non-smoker group ($P < 0.001$) (Table 3).

Baseline post-periodontal therapy comparison

Intergroup comparison within CP groups demonstrated significant reduction in all parameters, except in PPD levels in CP non-smokers category (Table 4). Notable changes were examined in the variables during the study period between the CP groups using the Mann–Whitney U test, which showed all significant figures except in variables of CAL and 8-OHdG, wherein no statistical changes were observed (Table 5).

Table 3 Comparison of the variables between the study groups at post-treatment

	Group	N	Mean	SD	Mean difference	95% confidence interval of the difference		t	df	P value
						Lower	Upper			
Post Pl	1	10	0.72	0.12	0.05	− 0.05	0.15	1.00	18	0.33(NS)
	2	10	0.67	0.10						
Post GI	1	10	0.69	0.10	0.35	0.27	0.43	9.46	18	<0.001*
	2	10	0.33	0.06						
Post PPD	1	10	5.30	1.70	− 0.30	− 1.60	1.00	− 0.49	18	0.63 (NS)
	2	10	5.60	0.97						
Post CAL	1	10	5.50	2.42	− 0.60	− 2.98	1.78	− 0.53	18	0.60 (NS)
	2	10	6.10	2.64						
Post 8-OHdG levels (pg/ml)	1	10	13.33	2.32	9.12	7.48	10.76	11.68	18	<0.001*
	2	10	4.22	0.84						

Independent sample *t* test

NS non-significant

*$P<0.05$ statistically significant, $P>0.05$

Table 4 Comparison of the variables between baseline and post treatment in chronic periodontitis groups

Groups			N	Mean	SD	Mean difference	95%		t	df	P value
							Lower	Upper			
1	PI	Baseline	10	2.17	0.18	1.45	1.32	1.58	25.41	9	<0.001*
		Post	10	0.72	0.12						
	GI	Baseline	10	1.59	0.11	0.90	0.78	1.02	16.74	9	<0.001*
		Post	10	0.69	0.10						
	PPD	Baseline	10	6.40	1.35	1.10	0.47	1.73	3.97	9	0.003*
		Post	10	5.30	1.70						
	CAL	Baseline	10	7.00	1.56	1.50	0.53	2.47	3.50	9	0.007*
		Post	10	5.50	2.42						
	8-OHdG levels(pg/ml)	Baseline	10	14.78	2.39	1.44	0.68	2.21	4.27	9	0.002*
		Post	10	13.33	2.32						
2	PI	Baseline	10	1.88	0.25	1.21	1.03	1.39	15.50	9	<0.001*
		Post	10	0.67	0.10						
	GI	Baseline	10	1.80	0.06	1.47	1.43	1.50	91.12	9	<0.001*
		Post	10	0.33	0.06						
	PPD	Baseline	10	5.90	0.88	0.30	− 0.38	0.98	1.00	9	0.34(NS)
		Post	10	5.60	0.97						
	CAL	Baseline	10	7.50	2.46	1.40	0.90	1.90	6.33	9	<0.001*
		Post	10	6.10	2.64						
	8-OHdG levels (pg/ml)	Baseline	10	5.11	1.10	0.90	0.48	1.31	4.92	9	0.001*
		Post	10	4.22	0.84						

Paired *t* test

NS non-significant

*$P<0.05$ statistically significant, $P>0.05$

Table 5 Comparison of change in the variables during study period between chronic periodontitis groups

	Groups	N	Mean (SD)	Range	Median (Q1–Q3)	Mann–Whitney U test	
						U statistic	P value
PI	1	10	1.45 (0.18)	1.20–1.70	1.44 (1.27–1.62)	18.5	0.02*
	2	10	1.21 (0.25)	0.72–1.74	1.21 (1.14–1.26)		
GI	1	10	0.90 (0.17)	0.70–1.22	0.87 (0.77–1.07)	0	<0.001*
	2	10	1.47 (0.05)	1.42–1.60	1.45 (1.44–1.48)		
PPD	1	10	1.10 (0.88)	0.00–3.00	1.00 (0.75–1.25)	18.5	0.008*
	2	10	0.30 (0.95)	0.00–3.00	0.00 (0.00–0.00)		
CAL	1	10	1.50 (1.35)	0.00–4.00	1.00 (0.75–3.00)	46.5	0.77 (NS)
	2	10	1.40 (0.70)	1.00–3.00	1.00 (1.00–2.00)		
8-OHdG levels (pg/ml)	1	10	1.44 (1.07)	0.15–3.67	1.24 (0.52–2.04)	36	0.29 (NS)
	2	10	0.90 (0.58)	0.13–2.11	0.87 (0.40–1.29)		

Mann–Whitney U test

NS non-significant

*$P < 0.05$ statistically significant, $P > 0.05$

Correlation between 8-OHdG levels and clinical variables

A positive correlation was observed post-treatment between 8-OHdG and PPD levels in Group 2 ($r = 0.63$), which exhibited a statistical significant effect ($P = 0.04$) (Table 6).

Discussion

The present clinical trial has been conducted to relate the relevance of oxidative damage induced by ROS that are liberated during periodontal disease process and the damage caused by the influence of smoking on the periodontium. For this purpose, 8-OHdG was chosen as a biomarker for estimation of oxidative DNA damage. The deleterious effects of oxidative stress generally follows after exposure to a high concentration of ROS and/or inadequate functioning of antioxidant defence system within the host. Lately, this has been linked to various systemic diseases including periodontitis [9, 23].

In this study, at baseline clinical parameters (PI, GI, PD and CAL) of the chronic periodontitis group (smokers and non-smokers) showed higher values compared to the healthy category (smokers and non-smokers) which validates the periodontal disease process. Saliva is a naturally available biofluid that can be collected with ease from the patient without any need of sophisticated tool. It contains various constituents which reflect the relationship between periodontal disease and diseases of other systemic tissues/organs. Literature review has revealed that unstimulated saliva contains some GCF elements and tissue metabolites which are beneficial for estimation of periodontal disease [28, 29]. In advanced periodontal disease with hopeless prognosis,

salivary 8-OHdG levels were found to be high, suggesting its role as a biomarker [22, 30, 31]. In this present study, the levels of 8-OHdG in saliva were significantly higher in the chronic periodontitis and smoker groups compared to clinically healthy category. Similar results were observed previously where higher levels of this oxidative biomarker were correlated to the clinical parameters [31]. The findings in this study also supported its useful role as an oxidative indicator. In comparison, in the smokers category, (chronic periodontitis smokers and clinically healthy smokers) higher mean values of 8-OHdG were observed. This could be explained by the fact that cigarette smoke contains large quantities of ROS [32] and 8-OHdG, being the main by-product of hydroxyl radical attack on DNA that could be regarded as a dependable biomarker for oxidative damage since it can be ascertained with high sensitivity [33].

Long-term exposure to cigarette smoke was reported to increase ROS levels, which in turn decrease the antioxidant status and hindered the DNA restorative capacity that finally led to oxidative DNA damage. Hence, this mechanism led to determining 8-OHdG levels in monitoring oxidative damage in smokers [34].

Salivary flow rate is another factor, found to increase with periodontitis [35], which could be causal for the higher levels of 8-OHdG levels in CP subjects. However, literature related to long-term effects of smoking shows hypofunction of the salivary glands and hence decreased quantity and quality of salivary flow rate [36, 37]. However, our findings indicated higher levels of 8-OHDG levels in chronic periodontitis and smoker category, reflecting the 8-OHDG as a convincible biomarker of inflammatory destruction. Additionally, age criterion is also a factor that needs to be considered while quantifying this biomarker. A study conducted by Gan W et al. [38] observed an age-dependant rise in the

Table 6 Correlation between post-treatment 8-OHdG levels and chronic periodontitis smoker and non-smoker group

Groups	8-OHdG levels (pg/ml)		
	Baseline[#]	Post[#]	Change[##]
1			
P1			
Correlation coefficient	− 0.11	− 0.45	0.16
P value	0.77 (NS)	0.19 (NS)	0.65 (NS)
GI			
Correlation coefficient	0.21	− 0.07	0.07
P value	0.56 (NS)	0.85 (NS)	0.84 (NS)
PPD			
Correlation coefficient	0.19	0.13	0.54
P value	0.59 (NS)	0.72 (NS)	0.11 (NS)
CAL			
Correlation coefficient	− 0.45	− 0.27	0.04
P value	0.20 (NS)	0.45 (NS)	0.92 (NS)
2			
P1			
Correlation coefficient	− 0.22	0.12	0.00
P value	0.55 (NS)	0.75 (NS)	1.00 (NS)
GI			
Correlation coefficient	− 0.31	− 0.06	0.06
P value	0.39 (NS)	0.87 (NS)	0.86 (NS)
PPD			
Correlation coefficient	0.21	0.63	− 0.17
P value	0.57 (NS)	0.04*	0.63 (NS)
CAL			
Correlation coefficient	− 0.001	− 0.07	0.05
P value	1.00 (NS)	0.84 (NS)	0.90 (NS)

NS non-significant

[#]Pearson's correlation test

[##]Spearmans correlation test

*$P < 0.05$ statistically significant, $P > 0.05$

levels of 8-OHdG marker levels over a wide age group. Since the age group of CP patients in the current study, belonged to 40–65 years, it could have influenced the higher range of the biomarker. However, the high range of 8-OHdG marker in the healthy smoker category (age range 25–35 years) is perhaps the reflection of inflammatory destruction.

After initial periodontal therapy, all clinical parameters and salivary 8-OHdG levels decreased, which was in accordance with a previous study where significant decrease in 8-OHdG levels both in saliva and GCF were observed [23]. However, work carried out by Dede et al. [39] did not comply with the present results, as the authors did not observe a decrease in salivary levels. Also, the present study showed a reduction in biomarker levels in CP non-smokers group after treatment compared to the CP smoker category at the 3rd month recall. This could be attributed to the combined

effect of periodontal destruction enhanced by smoking in the CP smokers group. Literature has provided evidence that smokers tend to respond less favourable to periodontal therapy [4, 40, 41].

Considering the GI values, CP non-smokers demonstrated considerable reduction after SRP compared to CP smoker category. This was supported by the study conducted by Ah et al. [4], in which the GI scores in non-smokers were decreased after initial therapy compared to smoker group. This was explained by the decrease in wound healing process in smokers.

Considering the comparison of clinical variables from baseline to 3rd month recall visits, demonstrated significant reduction in both CP groups, except for the PPD levels in CP non-smoker group. This state cannot be mitigated with conventional periodontal therapy alone and may require further surgical intervention for pocket elimination.

With regards to changes in the variables during the study interval, almost all parameters demonstrated significant reduction, except for the clinical attachment levels. This was in agreement with previous studies [42, 43] wherein the authors have suggested the possibility of a local effect of cigarette and the high serum cotinine levels to be among the reasons for clinical attachment loss. Likewise, post-therapeutic difference in 8-OHdG biomarker levels between the two CP groups were also not statistically significant, reflecting that further invasive treatment may be required for complete healing of tissues.

The 3rd month recall interval was chosen according to the plaque control protocol described by Axelsson and Lindhe [44], which was found to be effective against recurrence of periodontitis. The clinically healthy smokers who were acquainted with the smoking habit for at least 5 year period, did not have any periodontal disease. In this study, PI scores in periodontally healthy smokers were comparatively less than the CP groups, which justifies that, well-maintained oral health and host response are responsible for disease predisposition.

In the present study, voluntary participation of both genders was intended to be recruited but no female participants joined the trial. This could probably be due to the social stigma of revealing the smoking status. The community where the study was conducted, does not completely accept habitual smoking in woman. Hence, only males were observed to be under the participant category. Future studies should also consider gender effect on the obtained results.

Conclusions

The results of the current study suggest that salivary 8-OHdG values can be utilized as an oxidative biomarker for determining periodontal tissue destruction. However, it

may not serve as a beneficial determinant to quantify the periodontal destruction occurring in chronic periodontitis smokers and non-smokers category.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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