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Original Article

Impact of Non-Surgical Periodontal Therapy on Oxidative Stress Markers in Smokers and Periodontitis Patients

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Abstract

The severe tissue destruction associated with periodontitis may be caused by a deranged antioxidant state. Smoking contributes significantly to the oxidative stress of periodontal tissues in addition to periodontitis. Therefore, this study aimed to investigate and compare the levels of glutathione reductase (GR) in the saliva of smokers and non-smokers with and without periodontitis after non-surgical periodontal treatment (NSPT). Smokers with periodontitis (n = 19), smokers without periodontitis (n = 19), non-smokers with periodontitis (n = 19), and non-smokers without periodontitis (n = 19) comprised the four groups of 76 participants. Clinical measures were measured at baseline, on the 14 day, and one month later. These included plaque index (PI), gingival index (GI), probing pocket depth (PPD), and clinical attachment level (CAL). ELISA was used to measure salivary GR levels before and after NSPT. Improvements in PI and GI scores were associated with NSPT. Following NSPT, there was a significant decrease in PPD and an increase in CAL ($P < 0.001$). According to intra-group comparison, the GR activity of each study group increased significantly from baseline to one month following NSPT ($P < 0.001$). The GR activities of the study groups did not differ significantly in the between-group comparison ($P < 0.188$). The results of this study suggest that NSPT and better dental hygiene were successful in increasing salivary GR levels and reducing oxidative stress. Therefore, salivary GR levels may be regarded as a predictive indication when assessing the periodontal health of smokers and patients with periodontitis.

Key words: Antioxidant, Biomarker, Periodontitis, Oxidative stress, Glutathione reductase, Saliva

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Introduction

Multifactorial inflammatory disorders caused by pathogens are known as periodontal diseases. They are distinguished by being the most prevalent type of bone disease in humans, altering an individual's systemic health, and eroding the structures that support teeth [1, 2]. Depending on the degree of periodontal disintegration, clinical indications, inflammation symptoms, and rate of advancement, periodontitis is classified as mild to severe [3]. A major contributing factor to the etiology of periodontal disease is tobacco use. Due to nicotine-induced vasoconstriction, smokers have consistently been found to

experience less gingival bleeding and greater periodontal disintegration than non-smokers [4]. Additionally, smokers have higher pocket depth measures because of increased alveolar bone loss [5].

Cigarette smoking may have a varied effect on neutrophil function during periodontitis, which normally prevents the removal of periodontal infections, although not always in heavy smokers. Additionally, it promotes the release of reactive oxygen species and oxidative stress-mediated tissue damage [6, 7]. The oxidants found in tobacco smoke are abundant. Smoking has been linked to an increased formation of reactive oxygen species, which may surpass the oxidant defense system's capabilities and result in oxidative damage [8]. Biological antioxidant systems that include enzymatic and non-enzymatic processes typically reduce the possible harm that free radicals can do [9-12].

One of the antioxidants in reductive processes, glutathione reductase (GR) is essential for controlling enzymes, protein synthesis and degradation, and free radicals generated even during regular metabolism. It also helps shield cells from ROS.

Numerous studies link periodontal diseases to an imbalance between antioxidants and oxidants, indicating that this imbalance may be caused by a defect in antioxidant activity and/or an increase in the production of free radicals [9, 13-15].

Salivary GR is, as far as we know, one of the most significant but least researched antioxidant enzymes. There is currently a dearth of scientific research on variations in smokers' and healthy people's GR activity. Moreover, no prior research has determined whether salivary GR levels are associated with the extent or severity of periodontal breakdown or with various periodontal diseases specifically. There are very few publications on the range of glutathione reductase activity. To assess and compare the salivary GR levels after NSPT in smokers and non-smokers with and without periodontitis, the current study was conducted.

Materials and Methods

Study design, study population, and inclusion/exclusion criteria

The Department of Periodontology of the School of Dental Sciences, Krishna Institute of Medical Sciences, Karad, conducted this cross-sectional study. The clearance of the Krishna Institute of Medical Sciences Deemed University's ethical committee (0328/2018-2019) was acquired. Before the study started, the participants were informed of its nature and goal, and their written agreement was acquired. The study included 76 individuals who were seen at SDS, KIMSDU, Karad, and the Department of Periodontology. The power analysis indicated that each group needed a minimum of 19 study participants. This minimum value is established using 80% power and 95% confidence. Four groups were formed from the patient's smoking status and periodontal characteristics. Group I comprises smokers who have periodontitis, Group II consists of smokers who do not have periodontitis, and Group III consists of non-smokers who have periodontitis [4]. Group IV: those without periodontitis who do not smoke. If the subjects had never smoked or had quit smoking more than a year before the examination date, they were deemed non-smokers. Subjects with chronic generalized gingivitis are regarded as non-periodontitis. Data from the full-mouth examination served as the basis for all clinical periodontal measures, which were assessed by a single, qualified observer. Using a calibrated periodontal probe (HU-FRIEDY UNC 15), the clinical periodontal state was assessed by measuring the clinical attachment level (CAL) and probing depth (PD) on six tooth sites (proximally both buccally and lingually, midbuccally, and midlingually). The extent and severity of periodontal disorders were then determined using these data following the 2017 categorization provided by the European Federation of Periodontology and the American Academy of Periodontology. Every clinical metric, including the gingival index (GI) and plaque index (PI), was measured at baseline, on day 14, and one month later.

The study excluded patients with connective tissue illnesses (such as Sjögren's syndrome and systemic lupus erythematosus) and other systemic diseases or conditions (like uncontrolled diabetes mellitus) that are known to be risk factors for periodontitis. Pregnant and nursing women, as well as subjects who had received periodontal therapy during the previous six months, were not included in the study groups.

Saliva collection method

Researchers [16] used the Spitting method to gather the entire saliva. All samples were taken at baseline in the morning after an overnight fast, within 48 hours of the clinical assessment. Before any dental hygiene procedures or breakfast consumption, 1 milliliter of unstimulated whole saliva was collected in a centrifuge tube before the non-surgical periodontal treatment.

Following sample collection, cell debris was removed by centrifuging the samples for 10 minutes at 2500–3000 rpm. The supernatants were then collected in Eppendorf tubes and kept at -20 ºC until processing. The identical process of collecting saliva samples was done on the fourteenth day and the first month to assess clinical periodontal parameters and the assessment of salivary glutathione reductase levels.

Non-surgical periodontal treatment

At baseline, patients received full-mouth ultrasonic scaling and root planing following the collection of unstimulated whole saliva. Only the collection of saliva and the assessment of periodontal parameters were repeated after the fourteenth day and one month.

Glutathione reductase analysis

A glutathione reductase assay commercial kit (abcam, ab83461) was used to quantify the GR level in saliva following the manufacturer's instructions. Spectrophotometry was used to analyze the results at baseline, 14 days, and 1 month in the Department of Biochemistry, KIMSDU, Karad.

Statistical methods and data management

SPSS software version 20 was used to statistically analyze all of the data that was gathered. The findings were presented as a number (%) or mean ± standard deviation. The standard one-way ANOVA test and repeated measures ANOVA were used to evaluate the continuous variables between the subgroups and between the groups, respectively. A P value of less than 0.05 was deemed statistically significant. The Pearson Correlation Coefficient was used to examine the relationship between periodontal markers and GR disease activity. The correlation coefficient (r) was classified as “Poor” ($r < 0.3$), “Small” ($r = 0.3- < 0.5$), “Moderate” ($r = 0.5- < 0.7$), and “High” ($r \geq 0.7$). It was also considered significant if P was less than 0.05.

Results and Discussion

Demographic, clinical, and biochemical characteristics of the study population

The current study included seventy-six participants. Among the age groups of smokers with periodontitis, smokers without the disease, non-smokers with the disease, and non-smokers without the disease, the mean and standard deviation were 40.2 ± 8.1, 31.7 ± 8.1, 42.5 ± 7.5, and 25.1 ± 6.5, respectively. There were 21 female patients and 55 male patients out of the 76 total. This study comprised 38 people who had never smoked, 19 people who now smoked, and 19 people who had previously smoked.

Comparison of clinical parameters pre and post-NSPT at baseline, on the 14th day, and at 1 month among study groups

At baseline, the mean and standard deviation of GI and PI in nonsmokers without periodontitis were 1.22 ± 0.32 and 0.89 ± 0.30, respectively. Compared to other groups, their values were substantially lower **(Table 1)**. GI values were not statistically different across all research groups ($P < 0.065$), while PI ($P < 0.001$) was considerably lower in non-smokers without periodontitis on the 14th day post-NSPT and one-month follow-up than in other groups **(Table 1)**.

Table 1. Comparison of mean PI and GI score pre and post-NSPT at Baseline, 14th Day, and at 1 Month

Group	PI Baseline	GI Baseline	PI 14th Day	GI 14th Day	PI 1 Month	GI 1 Month
Non-smokers without periodontitis	0.89 ± 0.30	1.22 ± 0.32	0.63 ± 0.38	1.06 ± 0.34	0.38 ± 0.32	0.94 ± 0.34
Smokers with periodontitis	1.22 ± 0.14	1.18 ± 0.08	1.06 ± 0.08	1.13 ± 0.07	0.95 ± 0.11	0.99 ± 0.10
Smokers without periodontitis	1.06 ± 0.14	1.16 ± 0.19	1.03 ± 0.10	1.08 ± 0.09	0.96 ± 0.09	1.01 ± 0.08
Non-smokers with periodontitis	2.13 ± 0.33	1.86 ± 0.81	1.06 ± 0.48	1.22 ± 1.91	0.40 ± 0.16	0.57 ± 1.08
F-value	96.011	10.795	8.506	0.100	54.246	2.512

P-value	< 0.001	< 0.001	< 0.001	0.960	< 0.001	0.065

The study found that smokers with periodontitis had the highest mean PPD values, while nonsmokers without the disease had the lowest mean PPD values. Intragroup analysis showed that, in comparison to baseline, PPD significantly decreased in each study group on the fourteenth day and at one month **(Table 2)**.
Intergroup comparison showed that smokers with periodontitis had a significantly lower PPD than other groups after the 14th day and 1 month after NSPT (P < 0.001) **(Table 2)**.

Table 2. Inter and intra-group comparison of PPD at Baseline, on the 14th day, and at 1 month

Group		PPDDB Baseline	PPDDB 14th day	PPDDB 1 month	Repeated measures ANOVA
Non-smokers without periodontitis	Mean	1.13	1.08	1.10	6.230 (0.002)
	Std. deviation	.406	.293	.295	
Smokers with periodontitis	Mean	6.39	5.50	3.12	3089.4 (< 0.001)
	Std. deviation	1.292	1.334	1.285	
smokers without periodontitis	Mean	2.67	2.55	2.28	51.942 (< 0.001)
	Std. deviation	.974	1.043	.458	
Non-smokers with periodontitis	Mean	6.12	5.04	3.44	1301.00 (< 0.001)
	Std. deviation	1.602	1.292	.944	
ANOVA	F-value	3444.829	2591.080	1055.527	
	P-value	< 0.001	< 0.001	< 0.001	

Smokers with periodontitis had the highest mean CAL levels across all research groups, while non-smokers without the disease had the lowest mean CAL values. Intragroup analysis showed that, in comparison to the baseline, each research group's CAL increased significantly on the fourteenth day and at one month **(Table 3)**.
Intergroup comparison showed that smokers with periodontitis had a substantial increase in CAL after the 14th day and 1 month after NSPT compared to other groups (P < 0.001) **(Table 3)**.
Glutathione reductase (GR) activity did not significantly correlate with PI or GI score at baseline, 14 days later, or 1 month later **(Table 4)**.

Table 3. Inter and intra-group comparison of CAL at baseline, on the 14th day and 1 month

Group		CAL baseline	CAL 14th day	CAL 1 month	Repeated measures ANOVA
Non-smokers without periodontitis	Mean	0.02	0.00	0.00	8.125 (< 0.001)
	Std. deviation	0.13	0.00	0.00	
Smokers with periodontitis	Mean	4.98	4.05	1.17	1244.8 (< 0.001)
	Std. deviation	1.30	1.41	1.56	
smokers without periodontitis	Mean	0.00	0.00	0.00	-
	Std. deviation	0.00	0.00	0.00	
Non-smokers with periodontitis	Mean	4.32	3.46	2.35	633.35 (< 0.001)
	Std. deviation	1.50	0.88	0.90	
ANOVA	F-value	3340.984	3151.772	712.099	
	P-value	< 0.001	< 0.001	< 0.001	

Table 4. Correlation between GI, PI, and GR activities at baseline, 14th day, and 1 month

Smokers With periodontitis		**PI B**	**GI B**	**PI 14D**	**GI 14D**	**PI 1M**	**GI 1M**
GR B	Pearson correlation	.166	-.206	.210	.143	.565*	.333
GR 14 D	Pearson correlation	-.055	-.263	.262	-.108	.199	.130
GR 1 M	Pearson correlation	.080	.156	.047	.324	.150	.203
Smokers without periodontitis		**PI B**	**GI B**	**PI 14D**	**GI 14D**	**PI 1M**	**GI 1M**
GR B	Pearson correlation	.212	.000	-.403	.278	.038	-.063
GR 14 D	Pearson correlation	-.238	-.080	-.088	.087	-.004	.094
GR 1 M	Pearson correlation	-.317	-.101	-.242	.016	.144	.239
Non-smokers with periodontitis		**PI B**	**GI B**	**PI 14D**	**GI 14D**	**PI 1M**	**GI 1M**
GR B	Pearson correlation	-.010	-.183	.009	-.054	.014	-.036
GR 14 D	Pearson correlation	-.027	-.052	-.084	.214	.269	.250
GR 1 M	Pearson correlation	.098	-.084	-.158	.071	.060	.125
Non-smokers without periodontitis		**PI B**	**GI B**	**PI 14D**	**GI 14D**	**PI 1M**	**GI 1M**
GR B	Pearson correlation	.413	.276	-.152	.063	-.052	-.106
GR 14 D	Pearson correlation	.103	-.145	-.377	-.161	.116	.106
GR 1 M	Pearson correlation	.120	-.155	-.176	-.233	-.024	-.112

* Correlation is significant at the 0.05 level (2-tailed).
PI: plaque index
GI: gingival index
GR: glutathione reductase
B: baseline
14 D: 14 day
1 M: 1 month

Comparison of glutathione reductase (GR) activity and mean GR pre and post-NSPT among study group subjects.

The regression model was developed to determine the concentration of GR based on OD value. It was determined by applying the cubic regression approach as follows.

$$\text{Concentration} = 416.186\text{XO.D} + 2276.420\ (\text{OD})^2 - 15263.941\ (\text{OD})^3 - 10.092 \quad (1)$$

Using this equation the GR (concentration) activities for four study groups at baseline, at 14 days, and 1 month was computed. **Table 5** shows these GR activities, which were computed. On the intragroup comparison, however, each study group's GR activity increased significantly from baseline to one month following non-surgical periodontal therapy (NSPT) ($P < 0.001$).

The study groups' GR activities did not differ significantly when compared between groups. At the 14-day and 1-month follow-up post-NSPT, the non-smokers without periodontitis group showed the greatest improvement in GR concentration, followed by the smoking-free group and the non-smokers with periodontitis group. Smokers in the periodontitis group showed the least improvement in GR activity out of all the research participants **(Table 5)**. **Figure 1** shows a graphic representation of the standard concentration of GR and its corresponding OD.

Table 5. Comparison of mean and standard deviation of GR activities at baseline, on the 14th day, and at 1-month pre and post-NSPT

GROUP		Baseline	At 14TH day	At 1 month	Repeated Anova F-value (P-value)
Non-smokers without periodontitis	Mean	18.21	33.99	47.57	31.909 (< 0.001)
	Std. deviation	16.77	22.25	14.22	
Smokers with periodontitis	Mean	18.67	26.72	34.27	4.765 (0.0146)
	Std. deviation	16.87	22.99	27.39	
Smokers without periodontitis	Mean	25.80	29.04	52.78	8.571 (< 0.001)
	Std. deviation	19.50	20.16	46.38	
Non-smokers with periodontitis	Mean	32.09	40.09	55.35	17.520 (< 0.001)
	Std. deviation	22.07	21.36	31.42	
ANOVA	F-value	2.301	1.413	1.638	
	P-value	0.084	0.246	0.188	

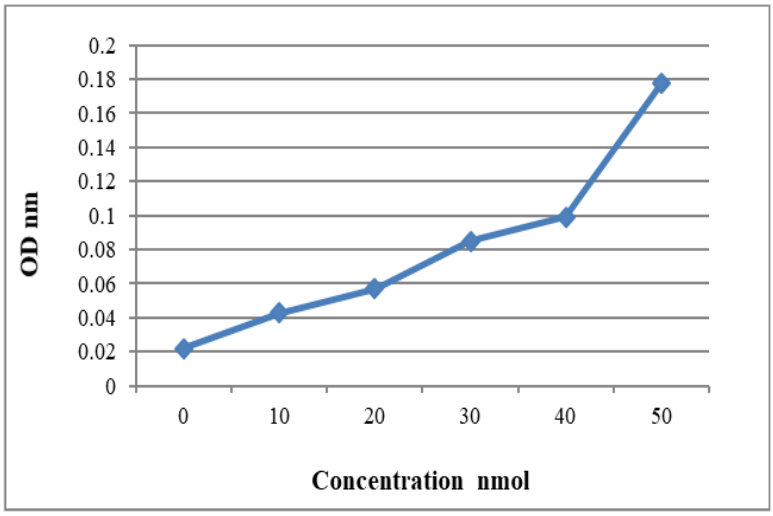

Figure 1. Standard curve of GR activity

Salivary indicators of different periodontal diseases have been reported to have origins in either the host response or the periodontopathic bacteria [17, 18]. A collection of potential biomarkers for the periodontopathogenic process may be created by these physiologically active ingredients. Salivary indicators of disease are crucial to the life sciences and are starting to play a bigger part in drug discovery, diagnosis, and therapy outcome monitoring [19, 20]. For biomarkers to take their proper place in standard practice, their relationship to the mechanism of disease progression and therapeutic action needs to be better understood [21]. Increases in IL-1β, TNF-α, and prostaglandins such as prostaglandin E2 and MMPs are linked to the severity of periodontitis. Higher levels of immunoglobulins in GCF and a significant percentage of B lymphocytes and plasma cells are found in advanced stages of periodontal diseases [22]. In the multifactorial disease known as periodontitis, reactive oxygen

species (ROS) are produced. The existence of neutrophil infiltration as the primary event in the host's response to bacterial invasion is necessary for the compelling evidence that links ROS to the pathological degradation of connective tissue during periodontal disease [22]. Saliva also contains glutathione reductase (GR) and other antioxidants. Salivary GSH levels typically fall in periodontal disorders, and the ubiquitous tripeptide glutathione (GSH) and other amino thiols are also implicated in the synthesis of volatile sulfur compounds that cause bad breath in periodontopathic patients [23]. Glutathione metabolism is a crucial defense mechanism for cells against substances that lead to lipid peroxidation and oxidative stress. Genetic and molecular data suggest that glutathione and glutathione-dependent enzymes are essential for cellular defense against harmful environmental chemicals.

The current study's findings showed that there were substantial baseline differences in GR levels across all four groups. Several writers report similar findings across different study groups. According to Preianò *et al*. [24], gingival crevicular fluid from patients with chronic periodontitis had lower levels of GSSG (oxidized glutathione) than that of subjects in good periodontal health. In a chronic and aggressive form of periodontitis, Kluknavská *et al*. [25] similarly found significantly lower salivary and plasma GR activity compared to gingivitis individuals. Narendra *et al*. [26] discovered that patients with periodontitis had higher GSH levels in their gingival tissue but lower plasma GSH levels. A significant drop in GR activity compared to normal healthy people was noted in a study conducted by Sathishkumar *et al*. [27]. The sensitivity and specificity of the detection techniques employed, variations in the types of samples used for GR assessment, study sample size, sampling technique, smoking status, and different stages of periodontal disease among the study subjects are some possible explanations for this discrepancy in GR levels.

The effects of NSPT on salivary GR levels in smokers and non-smokers with and without periodontitis were evaluated for the first time in this study. In the current study, each study group's GR activity significantly increased from baseline to one month following NSPT. On the fourteenth day and one month after NSPT, the non-smokers without periodontitis group showed the greatest improvement in GR activities, followed by the smokers without periodontitis group. These findings run counter to the findings of Borges *et al*. [28]. Following NSPT in the gingivitis and chronic periodontitis groups, these investigators found no variations in GR activity in gingival tissue samples [28]. All research groups showed a significant increase in GR activity following NSPT in comparison to the baseline in the current investigation. Similar results were noted by Veljovic *et al*. [29].

In the current study, the improvement in Plaque Index and Gingival Index scores following NSPT was associated with an increase in GR salivary levels. These findings concur with those of Veljovic *et al*. [29], who found that after NSPT, there was an improvement in GI and PI levels along with improved salivary and GCF GR levels. Following NSPT, the current investigation showed that all study groups had higher clinical attachment levels (CAL) and enhanced GR activity with decreased probing pocket depth (PPD). These findings concur with research conducted by Villa-Correa *et al*. [23] and Veljovic *et al*. [29]. The outcomes of the current investigation, however, may have been impacted by the small sample size combined with the limited variety of disease patterns across the patients.

These results may indicate that GSH is utilized during the inflammatory defense and that the immune system requires a significant amount of GSH to protect the periodontal tissues. The generation of GR may be influenced by the degree of periodontal disintegration and the extent of the illness [30]. In the current investigation, the GR levels were inversely proportional to the periodontitis caused by smoking and oxidative stress. In evaluating the periodontal health of smokers and patients with periodontitis, it may therefore be regarded as a prognostic indication.

In individuals with periodontitis, smoking is a significant modifiable risk factor. Compared to smokers, nonsmokers had a lower prevalence and severity of periodontitis. When it comes to non-surgical periodontal care, non-smokers fare much better than smokers. Quitting smoking has a beneficial impact on the periodontium. Following non-surgical treatment, it is linked to a larger reduction in probing depth. Dentists and general practitioners should educate their patients about the negative health impacts of smoking and the benefits of smoking cessation therapy as primary care providers [31].

Lastly, there are certain restrictions related to this study that should be considered in future research. Assessing the impact of periodontal therapy on salivary GR activity and the correlation between GR salivary levels and the advancement of periodontal disease is challenging due to the cross-sectional methodology. Therefore, additional longitudinal monitoring is required to determine any potential correlations between periodontal health and salivary GR activity.

Conclusion

Within the constraints of this study, it can be said that NSPT can change periodontal parameters and salivary GR levels in a way that is beneficial to health. NSPT was less well received by smokers with and without periodontitis than by those who did not smoke or have periodontitis. Periodontitis and other inflammatory conditions linked to oxidative stress can be assessed using salivary GR levels as a biomarker.

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