Original Article

EFFECT OF NON-SURGICAL PERIODONTAL THERAPY (NSPT) ON SALIVARY GLUTATHIONE REDUCTASE (GR) IN SMOKERS AND PERIODONTITIS SUBJECTS

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https://doi.org/10.51847/WZqhL73bwK

ABSTRACT

Deranged antioxidant status may play a role in the extreme tissue breakdown in periodontitis. Apart from periodontitis, smoking also plays a major contributing role in increasing oxidative stress of periodontal tissues. Hence this study aimed to evaluate & compare the salivary Glutathione Reductase (GR) levels following non-surgical periodontal therapy (NSPT) in smokers and non-smokers with and without periodontitis. A total of 76 subjects were divided into four groups smokers with Periodontitis (n = 19), smokers without Periodontitis (n = 19), non-smokers with periodontitis (n = 19), and non-smokers without periodontitis (n = 19). Clinical parameters such as plaque index (PI), gingival index (GI) probing pocket depth (PPD), and clinical attachment level (CAL) were assessed at baseline, on the 14th day, and at 1 month. Salivary GR levels were analyzed by ELISA pre and post-NSPT. NSPT was associated with improvement in PI and GI scores. Significant reduction in PPD and gain in CAL were observed post-NSPT ($p<0.001$). On intra-group comparison, there was a significant increase in GR activities from baseline to 1 month in each study group after NSPT ($p<0.001$). On intergroup comparison, there was no significant difference in GR activities among the study groups (0<0.188). Based on the findings of this study it can be concluded that improved oral hygiene and NSPT were effective in increasing salivary GR levels and reducing oxidative stress. Hence salivary GR levels could be considered a prognostic indicator in the assessment of periodontal status among smokers and periodontitis subjects.

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

Introduction

Periodontal diseases are pathogen-induced inflammatory conditions of multifactorial nature. They are characterized by constituting the most widespread form of bone pathology in humans, destroying the tooth-supporting structures and a modifying factor of the systemic health of individuals [1, 2]. Periodontitis is categorized as mild to severe form depending on the rate of progression, clinical signs, symptoms of inflammation, and extent of periodontal breakdown [3]. Tobacco smoking plays a significant etiological role in the development of periodontal disease. The periodontal breakdown is more pronounced among current smokers than former smokers and gingival bleeding has been constantly reported to occur less in smokers due to nicotine-induced vasoconstriction [4]. Also, greater pocket depth measurements are found in smokers due to increased alveolar bone loss [5].

During periodontitis, cigarette smoking may differentially influence neutrophil function, generally preventing the abolition of periodontal pathogens but in heavy smokers. Also, it stimulates the release of oxidative stress-mediated tissue damage reactive and oxygen species [6, 7]. Tobacco smoke contains a rich source of oxidants. It has been a dilemma that, the increased production of reactive oxygen species associated with smoking may beat the capacity of the oxidant defense system, which leads to oxidative damage [8]. The potential damage that the free radicals can cause is normally minimized by combining biological antioxidant systems including non-enzymatic and enzymatic reactions [9-12].

Many reasearch correlate periodontal diseases with an imbalance between antioxidants and oxidants, thus suggesting that this imbalance can be because of an a defect in antioxidant activity and/or increase in free radical production [13, 14]. Glutathione reductase (GR) is one of the antioxidants in reductive processes that is essential for the regulation of enzymes, synthesis and degradation of proteins, and free radicals produced even in normal metabolism and protection of the cells against ROS [15, 16].

As far as we know, among various antioxidant enzymes, salivary GR is an important, but least studied enzyme. So far, there is little scientific documentation about fluctuations in smokers and GR activity in normal individuals. Furthermore, none of the previous studies have identified if the salivary levels of GR are linked with the degree of periodontal breakdown in terms of extent/severity, or neither their association with different periodontal conditions. Very few reports are available regarding the range of activity for glutathione reductase. Hence, the present study was carried out to evaluate & compare the

salivary GR levels following NSPT in smokers and non-smokers with and without periodontitis.

Materials and Methods

Study design, study population, and inclusion/exclusion criteria

This cross-sectional study was carried out in the Department of Periodontology, School of Dental Sciences, Krishna Institute of Medical Sciences, Karad. Ethical committee approval (0328/2018-2019) from Krishna Institute of Medical Sciences Deemed University was obtained. The purpose and nature of the study were explained to the subjects and written consent was obtained before commencing the study. A total of 76 patients reporting to the Department of Periodontology, SDS, KIMSDU, and Karad were included in the study. According to the power analysis, the minimum number of study subjects required in each group was 19. This minimum number is determined on basis of 95% confidence and 80% power. Based on the status of smoking and periodontal parameters patients were categorized into 4 groups. 1] Group I: smokers with periodontitis, 2] Group II: smokers without periodontitis, 3] Group III: non-smokers' with periodontitis. 4] Group IV: non-smokers without periodontitis. Subjects were considered non-smokers if they had never smoked, or had stopped smoking for more than one year before the date of examination. Chronic generalized gingivitis subjects are considered a non-periodontitis group. All clinical periodontal parameters were based on data derived from the full-mouth examination and performed by a single trained and calibrated observer. Clinical periodontal status was determined by measuring both the probing depth (PD) and clinical attachment level (CAL) on six sites of teeth (midbuccally, midlingually, and proximally both buccally and lingually) using a calibrated periodontal probe (HU-FRIEDY UNC 15). Subsequently, these measurements were used to calculate the extent and severity according to the new classification of periodontal diseases which is given by the American Academy of Periodontology and European Federation of Periodontology 2017. All the clinical parameters including the Plaque index (PI) and Gingival index (GI) were recorded at the baseline, 14th day, and after 1 month.

Patients with other systemic diseases or conditions (e.g. uncontrolled Diabetes Mellitus) that are known as risk factors for periodontitis and also patients with connective tissue diseases (such as systemic lupus erythematosus, Sjögren's syndrome) were excluded from the study. Subjects with a history of periodontal therapy within the past 6 months, pregnant and lactating women were also excluded from study groups.

Saliva collection method

The whole saliva was collected using the Spitting method by Navazesh *et al*. (1993) [17]. At Baseline all samples were collected within 48 hours of clinical measurement in the morning following an overnight fast. Before the non-surgical periodontal treatment, 1ml of unstimulated whole saliva was collected in a centrifuge tube before breakfast intake and any dental hygiene procedure. After collection samples were centrifuged for 10 min at 2500-3000 rpm to remove cell debris, and supernatants were collected in the Eppendorf tubes and stored at -20ºC until processed. On the 14th day and at the 1st month, the same procedure of saliva sample collection was carried out for evaluation of salivary glutathione reductase level measurement and clinical periodontal parameters.

Non-surgical periodontal treatment

At the baseline, after the collection of unstimulated whole saliva, patients were treated with complete full-mouth ultrasonic scaling and root planning. After 14th day and 1 month, only saliva collection and periodontal parameter evaluation were repeated.

Glutathione reductase analysis

The GR level was estimated in saliva by using a glutathione reductase assay commercial kit (abcam, ab83461) according to the manufacturer's protocol and was analyzed by spectrophotometry at the baseline, at 14th day and 1 month in the Department of Biochemistry, KIMSDU, Karad.

Statistical methods and data management

All the collected data were statistically analyzed using SPSS software version 20. Results were reported as mean ± standard deviation, or number (percentage). The continuous variables between the inter groups and between subgroups were compared using the ordinary one-way ANOVA test and repeated measures ANOVA respectively. The P value <0.05 was considered statistically significant. The correlation between periodontal parameters and GR disease activity was analyzed by Pearson Correlation Coefficient. The correlation coefficient (r) was also said to be significant if p was <0.05 in addition to this it was categorized as 'Poor' ($r-<0.3$), 'Small' ($r=0.3-<0.5$), 'Moderate' ($r=0.5-<0.7$) and 'High' ($r=\geq 0.7$).

Results and Discussion

Demographic, clinical, and biochemical characteristics of the study population

Seventy-six patients were included in the present study. The mean and standard deviation of the age range of smokers with periodontitis, smokers without periodontitis, non-smokers with periodontitis, and non-smokers without periodontitis were 40.2±8.1, 31.7±8.1, 42.5±7.5 and 25.1±6.5 respectively. Out of 76 patients, 55 were male patients and 21 were female patients. A total of 19 current smokers, 19 former smokers, and 38 never-smokers were included in this study.

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

In non-smokers without periodontitis, the mean and standard deviation of PI and GI were 0.89±0.30 and 1.22±0.32 respectively at baseline. Their values were significantly less than other groups **(Table 1)**. On the 14th day post-NSPT and 1 month follow up PI (p‹0.001) was significantly less in non-smokers without periodontitis as compared to other groups, but GI values were not significantly different among all the study groups (p‹0.065) **(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

Among all the study groups non smokers without periodontitis group had the least mean PPD values while smokers with periodontitis had the highest mean PPD values. Intragroup revealed that there was a significant reduction in PPD on 14th day and at 1 month as compared to baseline in each study group **(Table 2)**.

Intergroup comparison demonstrated that after the 14th day and 1-month post NSPT significant PPD reduction was observed in smokers with periodontitis group as compared to other groups (p‹0.001) **(Table 2)**.

Table 2. Inter and Intra Group Comparison of PPD at Baseline, on 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	

Among all the study groups non-smokers without periodontitis, had the least mean CAL values while smokers with periodontitis had the highest mean CAL values. Intra group revealed that there was a significant gain in CAL on the 14th day and at 1 month as compared to baseline in each study group **(Table 3)**.

Intergroup comparison demonstrated that after the 14th day and 1-month post NSPT significant CAL gain was observed in smokers with periodontitis group as compared to other groups (p‹0.001) **(Table 3)**.

There was no significant correlation of Glutathione reductase (GR) activity with PI and GI score at baseline, on the 14th day, and at 1 month **(Table 4)**.

Table 3. Inter and Intra Group Comparison of CAL at Baseline, at 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 on basis of OD value. It was determined by applying the cubic regression approach as follows.

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

Using this equation the GR (concentration) activities for four study groups at baseline, at 14 days, and at 1 month was computed.

These GR activities were calculated and are depicted in **Table 5**. However, on the intragroup comparison, there was a significant increase in GR activities from baseline to 1 month in each study group after non-surgical periodontal treatment (NSPT) ($p<0.001$). On intergroup comparison, there was no significant difference in GR activities among the study groups. Maximum improvement in GR concentration was found in non-smokers without periodontitis group followed by smoking without periodontitis group and non-smokers with periodontitis group at 14thday and 1 month follow up post-NSPT. Among the entire study group, the least improvement in GR activity was observed in smokers with the periodontitis group **(Table 5)**. The standard concentration of GR and its respective OD is graphically presented in **Figure 1**.

Table 5. Comparison of Mean and Standard Deviation of GR activities at Baseline, on 14th Day, and at 1 Month pre and post NSPT

GROUP		BASELINE	AT 14TH DAY	AT 1 MONTH	REPEATED ANOVA F VAL (P VAL)
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	

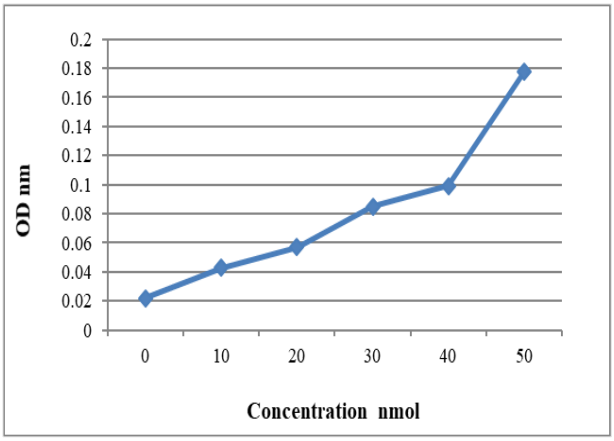

Figure 1. Standard Curve of GR Activity

Backgrounds of various periodontal disease biomarkers in saliva have been described to originate from host response or the periodontopathic bacteria [18, 19]. These biologically active substances involved in the process might build a pool of possible biomarkers for the periodontopathogenic process. Salivary biomarkers of disease in succession has an essential role in life sciences and have begun to assume a greater role in diagnosis, drug discovery, and monitoring of therapy outcomes [20, 21]. The relation to the mechanism of disease progression and therapeutic intervention must be more fully understood for biomarkers to assume their rightful role in routine practice [22]. The severity of periodontitis is associated with increases in IL-1β, TNF-α, and prostaglandins such as prostaglandin E2 and MMPs. Advanced stages of periodontal lesions are populated by a large proportion of B lymphocytes and plasma cells and increased levels of immunoglobulins in GCF [23]. Periodontitis is a multifactorial disease that involves the production of reactive oxygen species (ROS). The strong evidence linking ROS to the pathological destruction of the connective tissue during periodontal disease depends on the presence of neutrophil infiltration as the main event in the host's response to bacterial invasion [23]. Moreover, saliva contains various antioxidants, including glutathione reductase (GR). The ubiquitous tripeptide glutathione (GSH) and other amino thiols are also involved in the production of volatile sulphur compounds responsible for bad breath in periodontopathic patients and generally, salivary GSH levels decrease in periodontal diseases [24]. Glutathione metabolism is an important mechanism for cellular protection against agents that cause oxidative stress and lipid peroxidation. Glutathione and glutathione-dependent enzymes play a critical role in cellular defense against toxic environmental agents, according to genetic and biochemical evidence.

The results of the present study demonstrated that GR levels differed significantly among all four groups at baseline. Similar results are reported by several authors among various study groups. Preianò *et al*. (2020) found less GSSG (oxidized glutathione) levels in gingival crevicular fluid with chronic periodontitis as compared to periodontally healthy subjects [25]. Kluknavská *et al*. (2020) also observed significantly less salivary and plasma GR activity in a chronic and aggressive form of periodontitis as compared to gingivitis subjects [26]. Panjamurthy *et al*. (2005) found a decreased level of plasma GSH levels but increased GSH levels in the gingival tissue of periodontitis patients [27]. In a study done by T. Satishkumar *et al*. (2010) a drastic decrease in the GR activity among the smoker group than normal healthy subjects was observed [28]. Possible explanations for this discrepancy in GR levels could be attributed to the sensitivity and the specificity of the detection techniques used, the differences in the types of samples used for GR assessment, study sample size, sampling method, smoking status, and varying stages of the periodontal disease among the study subjects.

The present study is the first study where the effects of NSPT were assessed on salivary GR levels among smokers and non-smoker patients with and without periodontitis. In the present study, there was a significant increase in GR activities from baseline to 1 month in each study group after NSPT. Maximum improvement in GR activities was observed in the non-smokers without periodontitis group followed by smokers without periodontitis group on the 14th day and at 1-month post NSPT. These results are contradictory to the finding reported by Ivan Borges *et al*. (2007). These authors observed no differences in GR activities in gingival tissue samples after NSPT in the gingivitis and chronic periodontitis groups [29]. In the current study, all study group's demonstrated a significant increase in GR activity after NSPT compared to baseline. Similar findings were observed by Tsai *et al*. (2005) [30].

In the present study, the GR salivary levels were increased with the improvement in Plaque Index, and Gingival Index scores after NSPT. These results are in agreement with Tsai *et al*. 2005 who reported improved salivary and GCF GR levels with improved GI and PI levels post-NSPT [30]. The current study demonstrated increased GR activity with decreased probing pocket depth (PPD) and increased clinical attachment level (CAL) among all the study groups after NSPT. These results are in agreement with studies by Villa-Correa *et al*. (2016), and Tsai *et al*. (2005) [24, 30]. However, the small sample size in association with a narrow variability of patterns of disease among the patients might have influenced the results of the present study.

These findings may suggest that the immune system needs a large amount of GSH for protection of the periodontal tissues and GSH is consumed during the inflammatory defense. The extent of the disease and the severity of periodontal breakdown might influence the production of GR [31]. The GR values were inversely propositional to the smoking and oxidatively stressed-induced periodontitis in the present study. Hence, it could be considered a prognostic indicator in the assessment of periodontal status among smokers and periodontitis subjects.

Smoking habits are an important modifiable risk factor in periodontitis subjects. Non-smokers have less prevalence and severity of periodontitis than smokers. Non-smokers respond highly favorably than smokers to non-surgical periodontal treatment. Smoking cessation exerts a positive effect on the periodontium. It is associated with greater probing depth reduction after non-surgical treatment. As primary care providers, dentists and general physicians should inform their patients about the harmful effects of smoking habits and the favorable effect of smoking cessation therapy on health [32].

Finally, there are certain limitations associated with the present study that need to be taken into account during future investigations. The cross-sectional design makes it difficult to evaluate the effect of periodontal treatment on the salivary GR activity and the association of GR salivary levels with the progression of periodontal disease but also. Hence, further longitudinal monitoring is needed to establish possible associations between salivary GR activity and periodontal health.

Conclusion

It can be concluded that NSPT can alter salivary GR levels and periodontal parameters in favor of health within the limitations of this study. Smokers with and without periodontitis responded less favorably to NSPT than non-smokers and non-periodontitis individuals. Salivary GR levels can be used as a biomarker to evaluate the status of an inflammatory condition associated with oxidative stress such as periodontitis.

Acknowledgments: We wish to acknowledge the Department of Biochemistry, Krishna Institute of Medical Sciences, Karad, and KIMS Deemed to be University for permitting us to carry out this research.

Conflict of interest: None

Financial support: None

Ethics statement: Ethical committee approval from Krishna Institute of Medical Sciences Deemed University was obtained vide letter no. *0328/2018-2019*.

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