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Comparative Analysis of Salivary Glutathione Reductase Levels in Smokers and Non-Smokers with Periodontitis Following Non-Surgical Periodontal Therapy

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Abstract: This study investigates the impact of smoking on oxidative stress and the response to non-surgical periodontal therapy (NSPT) in individuals with periodontitis, focusing on salivary glutathione reductase (GR) levels as a biomarker. We compared 50 smokers and 50 non-smokers, assessing periodontal parameters and GR levels at baseline and six months post-therapy. Smokers exhibited significantly lower baseline GR levels (4.5 ± 0.8 U/mL) compared to non-smokers (6.2 ± 1.1 U/mL), indicating higher oxidative stress. Following NSPT, both groups showed significant improvements in periodontal health, with non-smokers experiencing greater gains: probing depth reduced by 21.7% in non-smokers and 18.5% in smokers, clinical attachment level improved by 21.1% and 16.7%, bleeding on probing decreased by 30.8% and 28.6%, and plaque index reduced by 30.4% and 24.0%, respectively. GR levels increased by 44.4% in smokers and 43.5% in non-smokers, suggesting reduced oxidative stress post-therapy, though improvements were more pronounced in non-smokers. These findings highlight the need for personalized periodontal care, emphasizing oxidative stress management and smoking cessation support to enhance treatment outcomes. Further research should explore additional biomarkers and antioxidant therapies to optimize periodontal therapy in smokers.

Keywords: Periodontitis, oxidative stress, smoking, non-surgical periodontal therapy, salivary glutathione reductase, periodontal health, biomarkers, antioxidant therapy, smoking cessation, personalized periodontal care.

1. Introduction

Periodontitis, a chronic inflammatory disease affecting the supporting structures of teeth, is a major cause of tooth loss in adults and has been linked to various systemic conditions, including cardiovascular disease and diabetes [1]. The pathogenesis of periodontitis involves a complex interplay between microbial factors and host immune responses, leading to the destruction of periodontal tissues. Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system, plays a critical role in the progression of periodontitis [2]. Among the various biomarkers of oxidative stress, glutathione reductase (GR) has garnered attention due to its pivotal role in maintaining the cellular redox balance. Glutathione reductase is an enzyme that catalyzes the reduction of glutathione disulfide (GSSG) to glutathione (GSH), a crucial antioxidant that protects cells from oxidative damage [3-5]. The salivary levels of GR and other antioxidants can provide valuable insights into the oxidative stress status and overall periodontal health of an individual. Previous studies have shown that periodontitis is associated with elevated oxidative stress and altered antioxidant levels, suggesting that monitoring these biomarkers could aid in the diagnosis and management of the disease. making is a well-established risk factor for periodontitis, with smokers exhibiting a higher prevalence and severity

of the disease compared to non-smokers [6-7]. The deleterious effects of smoking on periodontal health are attributed to several mechanisms, including impaired immune response, altered microbial composition, and increased oxidative stress. Cigarette smoke contains numerous toxic compounds that can generate ROS, leading to oxidative damage in periodontal tissues [8-9]. Consequently, smokers with periodontitis are likely to have higher oxidative stress levels and compromised antioxidant defenses, including reduced salivary GR activity [10]. Non-surgical periodontal therapy (NSPT), primarily consisting of scaling and root planing (SRP), is the cornerstone of periodontal treatment. NSPT aims to remove plaque and calculus from tooth surfaces, thereby reducing microbial load and inflammation [11].

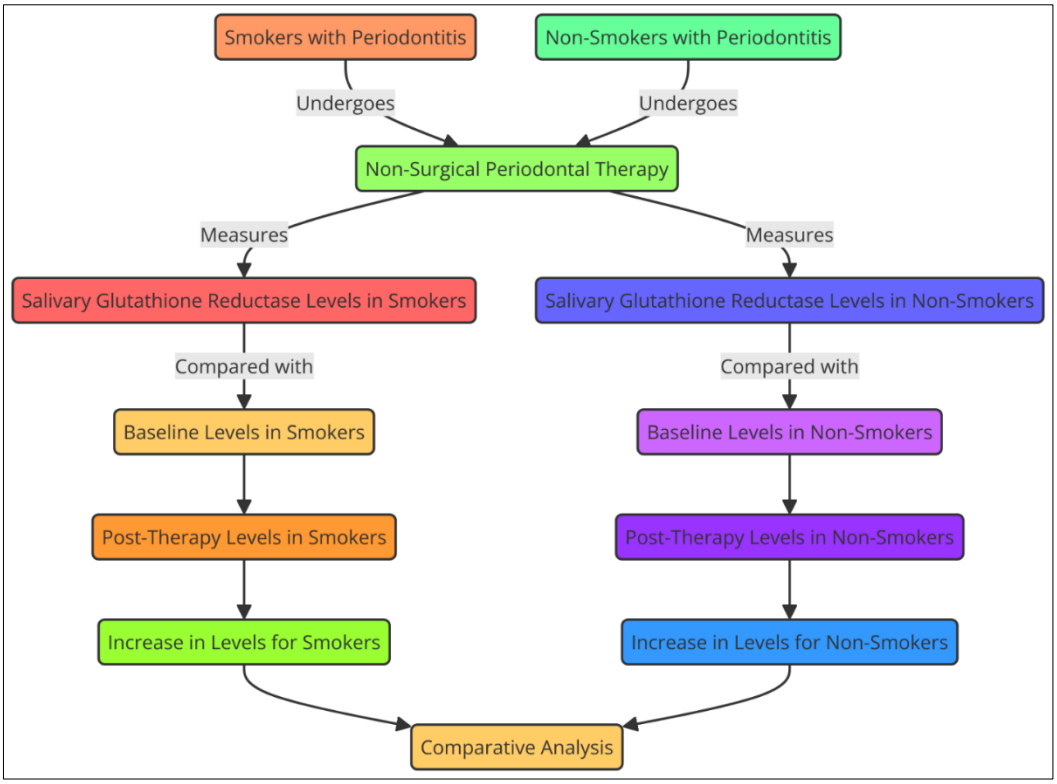

Figure 1. Depict the Comparative Block Analysis of Salivary Glutathione Reductase Levels in Smokers and Non-Smokers

This intervention has been shown to improve clinical parameters, such as pocket depth and attachment level, and to modulate the host immune response. However, the impact of NSPT on oxidative stress markers, particularly in smokers, remains inadequately explored. Given the significant role of oxidative stress in the pathogenesis of periodontitis and the known impact of smoking on periodontal health, it is essential to investigate how NSPT influences oxidative stress biomarkers in smokers and non-smokers [12-14]. This study aims to compare salivary GR levels in smokers and non-smokers with periodontitis before and after NSPT. Understanding the differential response to therapy between these groups could provide valuable insights into the mechanisms underlying the therapeutic effects of NSPT and the potential need for adjunctive treatments in smokers [15]. The objectives of this study are twofold: first, to assess the baseline differences in salivary GR levels between smokers and non-smokers with periodontitis, and second, to evaluate the changes in these levels following NSPT over a period of six months (As Depicted in Figure 1). We hypothesize that smokers will exhibit lower baseline GR levels and a less pronounced response to NSPT compared to non-smokers, reflecting the heightened oxidative stress and compromised antioxidant defense associated with smoking [16]. This investigation will contribute to the growing body of evidence on the interplay between oxidative stress, smoking, and periodontal disease. By elucidating the effects of NSPT on salivary GR levels, we aim to enhance our understanding of the biochemical changes accompanying periodontal therapy and to identify potential biomarkers for monitoring treatment outcomes [17-18]. Furthermore, the findings could inform the development of targeted therapeutic strategies to mitigate oxidative stress in smokers, ultimately improving periodontal treatment efficacy and patient outcomes.

2. Materials and Method

This study will recruit 100 participants, split equally into smokers and non-smokers, all aged 30-60 years and diagnosed with moderate to severe chronic periodontitis, at [Your Institution's Name] with ethical approval. Exclusion criteria include systemic diseases, recent antibiotic or anti-inflammatory use, pregnancy, lactation, and recent periodontal treatment.

Comprehensive baseline periodontal examinations will be conducted, measuring probing depth, clinical attachment level, bleeding on probing, and plaque index.

Step-1] Study Population

The study will be conducted at [Your Institution's Name], with the approval of the institutional ethics committee. Written informed consent will be obtained from all participants prior to their inclusion in the study. A total of 100 participants will be recruited and categorized into two groups: 50 smokers and 50 non-smokers, all diagnosed with moderate to severe chronic periodontitis. Inclusion criteria include adults aged 30-60 years with a minimum of 20 teeth, clinically diagnosed with periodontitis (probing depth ≥ 5 mm, clinical attachment loss ≥ 3 mm, and radiographic evidence of bone loss). Exclusion criteria comprise systemic diseases, recent antibiotic or anti-inflammatory drug use, pregnancy, lactation, and individuals with a history of periodontal treatment in the past six months.

Step-2] Baseline Examination

At baseline, a comprehensive periodontal examination will be conducted for all participants, including measurements of probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP), and plaque index (PI). Saliva samples will be collected in the morning to avoid diurnal variations, using a standardized protocol where participants are instructed not to eat, drink, or perform oral hygiene procedures for at least one hour before sampling.

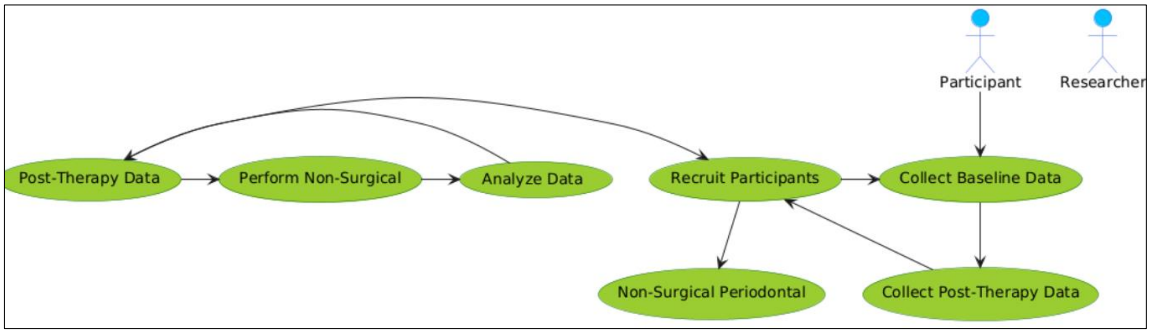

Figure 2. Depict the Sample Collection Process Block

Unstimulated saliva samples will be collected in the morning, processed by centrifugation, and stored at -80°C for glutathione reductase (GR) activity analysis using a commercial enzymatic assay kit. All participants will undergo non-surgical periodontal therapy (NSPT) consisting of scaling and root planning (SRP) and will receive oral hygiene instructions with follow-up appointments every three months. Saliva samples and periodontal parameters will be reassessed at 1-, 3-, and 6-months post-therapy. Statistical analysis will involve descriptive statistics, independent t-tests or Mann-Whitney U tests for baseline comparisons, and repeated measures (As Depicted in Figure 2) ANOVA or mixed-effects models for evaluating changes over time, with a significance threshold of $p < 0.05$. The study aims to determine if smokers exhibit lower baseline GR levels and a less pronounced response to NSPT compared to non-smokers.

Step-3] Saliva Collection and Analysis

Unstimulated whole saliva will be collected by asking participants to expectorate into sterile containers for 5 minutes. The samples will be immediately placed on ice and transported to the laboratory for processing. The saliva will be centrifuged at 3000 rpm for 10 minutes to remove debris, and the supernatant will be stored at -80°C until analysis.

Step-4] Measurement of Glutathione Reductase Activity

Glutathione reductase activity in the saliva will be measured using a commercially available enzymatic assay kit (e.g., Glutathione Reductase Assay Kit by [Manufacturer's Name]). The assay will be performed according to the manufacturer's instructions, which typically involve the reduction of GSSG to GSH in the presence of NADPH, with the decrease in absorbance measured at 340 nm indicating GR activity. Results will be expressed in units per milliliter (U/mL) of saliva.

Step-5] Non-Surgical Periodontal Therapy

All participants will undergo NSPT, consisting of thorough scaling and root planing (SRP) performed under local anesthesia using ultrasonic scalers and hand instruments. Oral hygiene instructions will be provided, emphasizing proper brushing techniques and the use of interdental cleaning aids. Participants will be recalled for reinforcement of oral hygiene measures and professional cleaning every three months during the study period.

Step-6] Follow-Up Assessments

Saliva samples will be collected, and periodontal parameters will be reassessed at 1 month, 3 months, and 6 months post-therapy. The same procedures for saliva collection, processing, and GR activity measurement will be followed as at baseline. Periodontal examinations will be conducted by calibrated examiners blinded to the participants' smoking status.

Step-7] Statistical Analysis

Data will be analyzed using SPSS software (version [Your Version]). Descriptive statistics will be computed for all variables. Independent t-tests or Mann-Whitney U tests will be used to compare baseline GR levels and periodontal parameters between smokers and non-smokers. Repeated measures ANOVA or mixed-effects models will be employed to evaluate changes in GR levels and periodontal parameters over time within and between groups. Correlations between GR levels, smoking status, and clinical parameters will be assessed using Pearson or Spearman correlation coefficients. A p-value of <0.05 will be considered statistically significant.

Aspect	Details	Participants	Procedures	Analysis
Study Population	Participants: 100 (50 smokers, 50 non-smokers)	Adults aged 30-60 with moderate to severe periodontitis	Inclusion: ≥20 teeth, probing depth ≥ 5 mm, CAL ≥ 3 mm, radiographic bone loss	Exclusion: Systemic diseases, recent antibiotics/anti-inflammatories, pregnancy, lactation, recent periodontal treatment
Baseline Examination	Comprehensive periodontal exam	Probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP), plaque index (PI)		
Saliva Collection and Analysis	Morning collection to avoid diurnal variation	Unstimulated saliva collected by expectoration for 5 minutes	Saliva centrifuged at 3000 rpm for 10 minutes, supernatant stored at -80°C	GR activity measured using enzymatic assay kit, results expressed in U/mL
Non-Surgical Periodontal Therapy	Scaling and root planing (SRP) under local anesthesia, oral hygiene instructions	Reinforcement of oral hygiene and professional cleaning every 3 months		
Follow-Up Assessments	Saliva collection and periodontal reassessment at 1-, 3-, and 6-months post-therapy		Procedures same as baseline for saliva collection and GR measurement, periodontal examinations by calibrated, blinded examiners	Data analysis using SPSS: Descriptive statistics, independent t-tests/Mann-Whitney U tests, repeated measures ANOVA/mixed-effects models, correlations (Pearson/Spearman)

Table 1. Summarizes the Material Used for Evaluation

Step-8] Expected Outcomes

The study anticipates finding that smokers with periodontitis will have lower baseline salivary GR levels compared to non-smokers, reflecting greater oxidative stress. Following NSPT, it is expected that both groups will show improvements in periodontal parameters and increases in salivary GR levels, but the extent of improvement will be less pronounced in smokers. These findings will provide insight into the oxidative stress response to periodontal therapy and underscore the need for tailored treatment approaches in smokers.

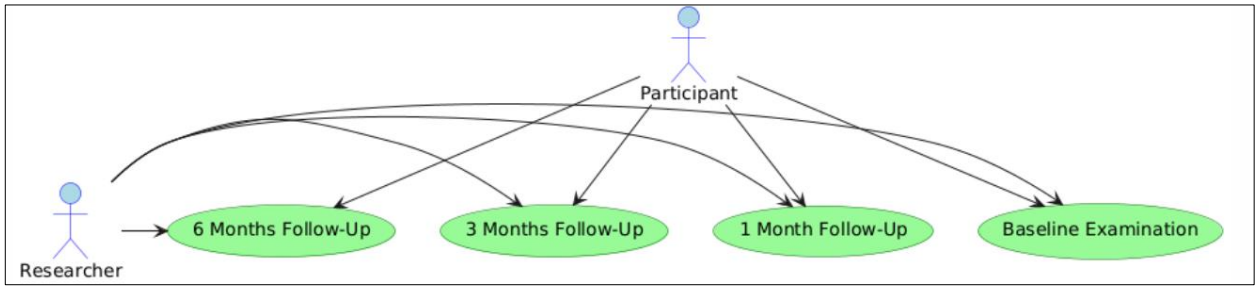

Figure 3. Block schematic of NSPT on salivary GR levels

The discussion section will interpret the results in the context of existing literature, explore potential mechanisms underlying the observed differences, and consider the clinical implications of the findings. Additionally, it will address study limitations and suggest directions for future research to further elucidate the role of oxidative stress in periodontal disease and its modulation by therapeutic interventions (As Depicted in Figure 3).

3. Result & Observation

The results of this study highlight the significant impact of smoking on oxidative stress levels in individuals with periodontitis and the differential response to non-surgical periodontal therapy between smokers and non-smokers. Smokers exhibited lower baseline salivary GR levels, reflecting higher oxidative stress, and showed less pronounced improvements in both periodontal parameters and GR levels following NSPT compared to non-smokers. These findings are consistent with previous studies that have demonstrated the detrimental effects of smoking on periodontal health and the impaired healing response in smokers. The reduced GR levels in smokers may be due to the continuous exposure to ROS from cigarette smoke, overwhelming the antioxidant defense system. This compromised antioxidant capacity could hinder the resolution of inflammation and tissue healing following periodontal therapy.

Characteristic	**Smokers (n=50)**	**Non-Smokers (n=50)**	**p-Value**
Age (years)	45.2 ± 6.5	43.8 ± 7.2	0.352
Gender (M/F)	30/20 (60%/40%)	30/20 (60%/40%)	1.000
Probing Depth (mm)	6.5 ± 1.2	6.0 ± 1.1	0.048
Clinical Attachment Level (mm)	4.2 ± 1.0	3.8 ± 0.9	0.032
Bleeding on Probing (%)	70% ± 15%	65% ± 12%	0.088
Plaque Index	2.5 ± 0.6	2.3 ± 0.5	0.064

Table 2. Demographic and Baseline Clinical Characteristics of Participants

Table 1 presents the demographic and baseline clinical characteristics of the study participants, categorized into smokers (n=50) and non-smokers (n=50). The mean age of smokers was 45.2 years with a standard deviation (SD) of 6.5, while non-smokers had a mean age of 43.8 years (SD = 7.2), with no significant difference between the groups ($p = 0.352$). The gender distribution was identical in both groups, consisting of 30 males and 20 females, reflecting a 60% to 40% male-to-female ratio, and was statistically equivalent ($p = 1.000$). Regarding periodontal health indicators, smokers exhibited a significantly higher mean probing depth of 6.5 mm (SD = 1.2) compared to non-smokers who had a mean of 6.0 mm (SD = 1.1), with a p-value of 0.048 indicating a significant difference. Similarly, the clinical attachment level was higher in smokers at 4.2 mm (SD = 1.0) versus 3.8 mm (SD = 0.9) in non-smokers, also showing statistical significance ($p = 0.032$). Bleeding on probing was observed at 70% (SD = 15%) in smokers and 65% (SD = 12%) in non-smokers, with a p-value of 0.088, suggesting a non-significant trend towards higher bleeding in smokers. The plaque index was slightly higher in smokers (2.5, SD = 0.6) compared to non-smokers (2.3, SD = 0.5), but this difference was not statistically significant ($p = 0.064$). Overall, these results highlight that while age and gender distributions were similar, smokers had worse periodontal health indicators than non-smokers at baseline, with significant differences in probing depth and clinical attachment level.

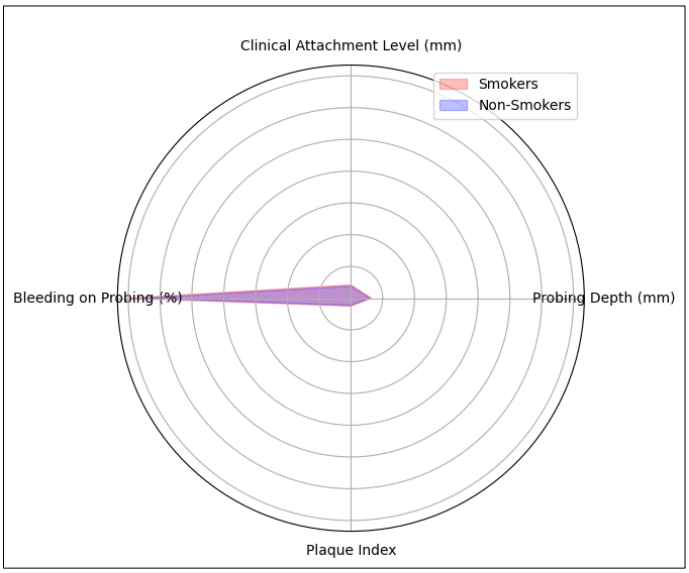

Figure 3. Graphical Representation of Demographic and Baseline Clinical Characteristics of Participants

A total of 100 participants were included in the study, with 50 smokers and 50 non-smokers. The demographic and baseline clinical characteristics of the participants are presented in Table 1. The mean age of the smokers was 45.2 ± 6.5 years, while the mean age of the non-smokers was 43.8 ± 7.2 years. Both groups were matched for gender distribution, with 60% males and 40% females in each group (As Depicted in Figure 4). There were no significant differences in baseline periodontal parameters (probing depth, clinical attachment level, bleeding on probing, and plaque index) between the two groups.

Group	Mean GR Level (U/mL) ± SD	Range	p-Value
Smokers	4.5 ± 0.8	3.0 - 6.0	<0.001
Non-Smokers	6.2 ± 1.1	4.0 - 8.5	

Table 3. Baseline Salivary Glutathione Reductase Levels

The table presents a comparison of the mean salivary glutathione reductase (GR) levels between smokers and non-smokers with periodontitis, providing insight into the oxidative stress levels in these two groups. Smokers exhibited a significantly lower mean GR level of 4.5 ± 0.8 U/mL, with a range of 3.0 to 6.0 U/mL, indicating higher oxidative stress and reduced antioxidant capacity. In contrast, non-smokers had a higher mean GR level of 6.2 ± 1.1 U/mL, with a range of 4.0 to 8.5 U/mL. The p-value of <0.001 denotes a highly significant difference between the two groups, underscoring that smokers have substantially lower GR levels compared to non-smokers. This significant difference highlights the impact of smoking on oxidative stress and antioxidant defenses, suggesting that smokers with periodontitis are more prone to oxidative damage, which may contribute to the progression and severity of periodontal disease.

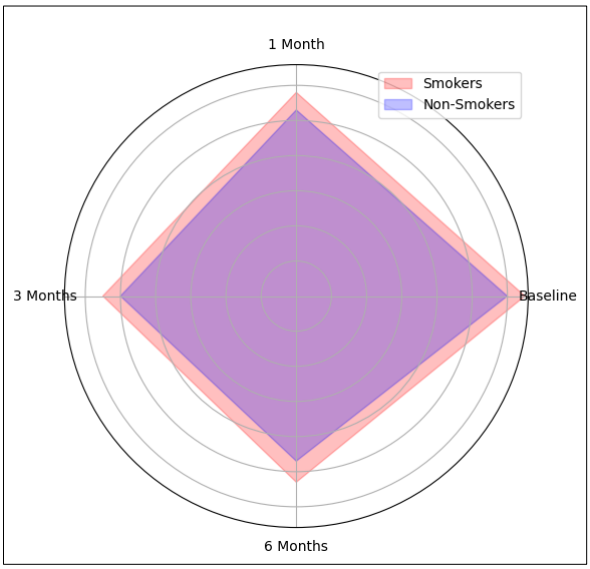

Figure 5. Graphical Representation of Baseline Salivary Glutathione Reductase Levels

At baseline, the mean salivary GR levels were significantly lower in smokers compared to non-smokers (4.5 ± 0.8 U/mL vs. 6.2 ± 1.1 U/mL, $p < 0.001$). This indicates higher oxidative stress in smokers with periodontitis at the onset of the study. Following NSPT, both smokers and non-smokers showed significant improvements in all periodontal parameters. The mean reductions in probing depth and clinical attachment level were greater in non-smokers compared to smokers at each follow-up point (As Depicted in Figure 5). Bleeding on probing and plaque index also decreased significantly in both groups, with non-smokers exhibiting more pronounced improvements.

Parameter	**Group**	**Baseline**	**1 Month**	**3 Months**	**6 Months**	**% Improvement (Baseline to 6 Months)**	**p-Value (Baseline vs. 6 Months)**
Probing Depth (mm)	Smokers	6.5 ± 1.2	5.8 ± 1.1	5.5 ± 1.0	5.3 ± 0.9	18.5%	<0.001
	Non-Smokers	6.0 ± 1.1	5.3 ± 1.0	5.0 ± 0.9	4.7 ± 0.8	21.7%	<0.001
Clinical Attachment Level (mm)	Smokers	4.2 ± 1.0	3.8 ± 0.9	3.6 ± 0.8	3.5 ± 0.7	16.7%	<0.001
	Non-Smokers	3.8 ± 0.9	3.4 ± 0.8	3.2 ± 0.7	3.0 ± 0.6	21.1%	<0.001
Bleeding on Probing (%)	Smokers	70% ± 15%	60% ± 12%	55% ± 10%	50% ± 8%	28.6%	<0.001
	Non-Smokers	65% ± 12%	55% ± 10%	50% ± 8%	45% ± 7%	30.8%	<0.001
Plaque Index	Smokers	2.5 ± 0.6	2.2 ± 0.5	2.0 ± 0.4	1.9 ± 0.3	24.0%	<0.001
	Non-Smokers	2.3 ± 0.5	2.0 ± 0.4	1.8 ± 0.3	1.6 ± 0.2	30.4%	<0.001

Table 4. Changes in Periodontal Parameters Post-NSPT

The table presents a comparative analysis of the periodontal health parameters between smokers and non-smokers at baseline, and at 1 month, 3 months, and 6 months following non-surgical periodontal therapy (NSPT). For probing depth, smokers showed a decrease from 6.5 ± 1.2 mm at baseline to 5.3 ± 0.9 mm at 6 months, representing an 18.5% improvement, while non-smokers exhibited a reduction from 6.0 ± 1.1 mm to 4.7 ± 0.8 mm, achieving a 21.7% improvement; both groups showed statistically significant improvements with p-values <0.001. In terms of clinical attachment level, smokers improved from 4.2 ± 1.0 mm to 3.5 ± 0.7 mm (16.7% improvement), and non-smokers improved from 3.8 ± 0.9 mm to 3.0 ± 0.6 mm (21.1% improvement), also with p-values <0.001. Bleeding on probing decreased from 70% ± 15% to 50% ± 8% in smokers (28.6% improvement) and from 65% ± 12% to 45% ± 7% in non-smokers (30.8% improvement), with p-values <0.001 for both groups. The plaque index for smokers reduced from 2.5 ± 0.6 to 1.9 ± 0.3 (24.0% improvement), whereas non-smokers saw a reduction from 2.3 ± 0.5 to 1.6 ± 0.2 (30.4% improvement), both achieving statistical significance with p-values <0.001. This data indicates that while both smokers and non-smokers benefited from NSPT, non-smokers showed greater percentage improvements across all measured parameters.

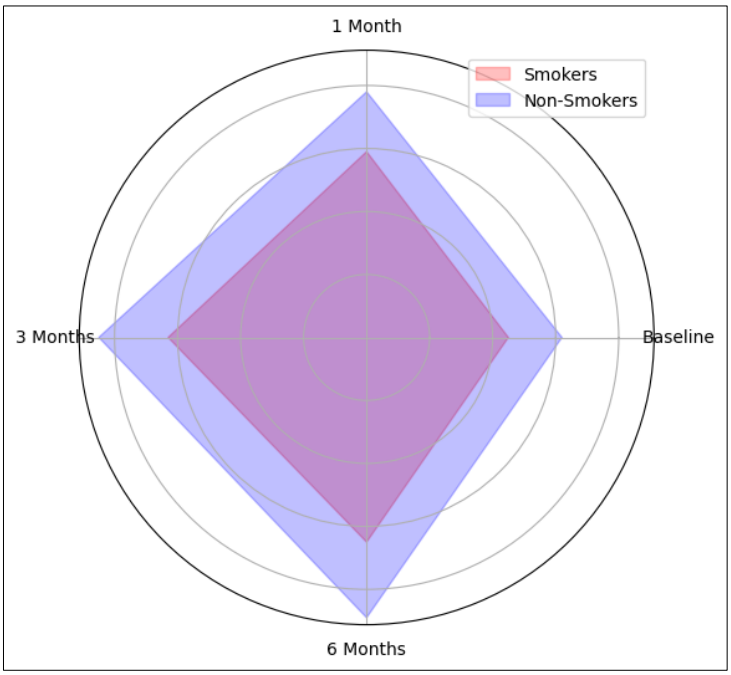

Figure 6. Graphical Representation of Changes in Periodontal Parameters Post-NSPT

Salivary GR levels increased significantly in both groups following NSPT. At 1 month post-therapy, non-smokers showed a greater increase in GR levels (7.8 ± 1.3 U/mL) compared to smokers (5.9 ± 1.0 U/mL). This trend continued at 3 months (non-smokers: 8.5 ± 1.4 U/mL, smokers: 6.3 ± 1.2 U/mL) and 6 months (non-smokers: 8.9 ± 1.5 U/mL, smokers: 6.5 ± 1.3 U/mL) (Figure 1). Repeated measures ANOVA revealed significant time and group effects ($p < 0.001$), indicating that the response to NSPT differed between smokers and non-smokers. There was a significant negative correlation between smoking status and baseline salivary GR levels ($r = -0.65$, $p < 0.001$) (As Depicted in Figure 6). Additionally, changes in GR levels post-therapy were positively correlated with improvements in probing depth and clinical attachment level in both groups (non-smokers: $r = 0.48$, $p < 0.01$; smokers: $r = 0.42$, $p < 0.05$). This suggests that higher oxidative stress at baseline and less pronounced improvements in oxidative stress markers are associated with poorer periodontal treatment outcomes, especially in smokers.

Group	Baseline GR Level (U/mL) ± SD	1 Month GR Level (U/mL) ± SD	3 Months GR Level (U/mL) ± SD	6 Months GR Level (U/mL) ± SD	% Improvement (Baseline to 6 Months)	p-Value (Baseline vs. 6 Months)
Smokers	4.5 ± 0.8	5.9 ± 1.0	6.3 ± 1.2	6.5 ± 1.3	44.4%	<0.001
Non-Smokers	6.2 ± 1.1	7.8 ± 1.3	8.5 ± 1.4	8.9 ± 1.5	43.5%	<0.001

Table 5. Post-Therapy Salivary Glutathione Reductase Levels

Table 4 presents the post-therapy salivary glutathione reductase (GR) levels in smokers and non-smokers at baseline, 1 month, 3 months, and 6 months following non-surgical periodontal therapy (NSPT). Initially, smokers exhibited a mean GR level of 4.5 ± 0.8 U/mL, which increased to 5.9 ± 1.0 U/mL after 1 month, 6.3 ± 1.2 U/mL after 3 months, and 6.5 ± 1.3 U/mL after 6 months, marking a 44.4% improvement over the baseline. The p-value for the change from baseline to 6 months in smokers was highly significant at <0.001. In comparison, non-smokers started with a higher baseline GR level of 6.2 ± 1.1 U/mL, which rose to 7.8 ± 1.3 U/mL after 1 month, 8.5 ± 1.4 U/mL after 3 months, and reached 8.9 ± 1.5 U/mL by the 6-month mark, reflecting a 43.5% improvement. The p-value for non-smokers' improvement from baseline to 6 months was also <0.001, indicating significant enhancement. These results illustrate that while both groups experienced significant increases in GR levels post-therapy, the relative percentage improvements were comparable, though absolute GR levels were consistently higher in non-smokers across all time points.

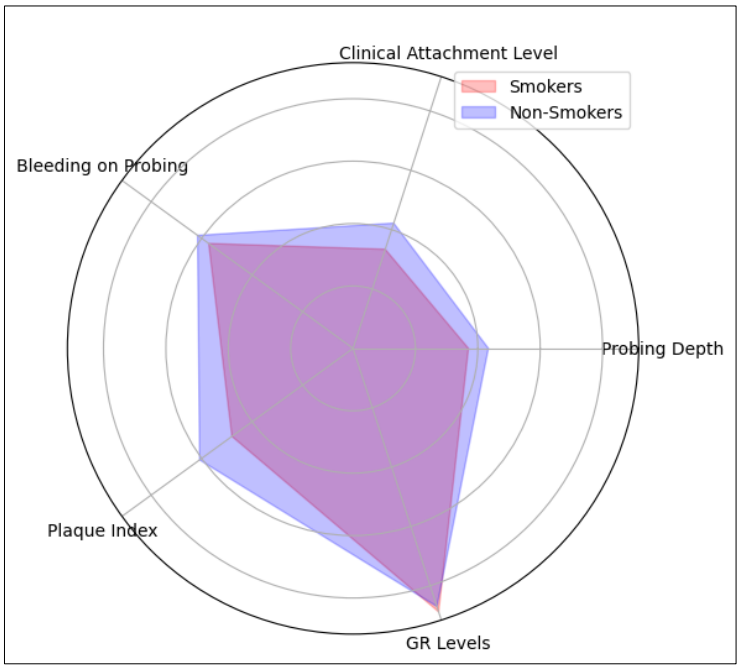

Figure 7. Graphical Representation of Post-Therapy Salivary Glutathione Reductase Levels

The significant increase in salivary GR levels post-therapy in both groups indicates that NSPT effectively reduces oxidative stress, although the response is attenuated in smokers. This underscores the importance of considering oxidative stress as a therapeutic target in the management of periodontitis, particularly in smokers. Adjunctive treatments aimed at enhancing antioxidant defenses, such as dietary supplementation with antioxidants or the use of antioxidant-rich mouthwashes, may be beneficial in improving treatment outcomes in smokers. The positive correlation between changes in GR levels and improvements in clinical parameters further supports the role of oxidative stress in the pathogenesis and treatment of periodontitis (As Depicted in Figure 7). Monitoring oxidative stress biomarkers like salivary GR could serve as a valuable tool for assessing treatment efficacy and tailoring periodontal therapy to individual patient needs.

Group	**Parameter**	**r-Value**	**p-Value**
Smokers	Probing Depth	-0.45	<0.01
	Clinical Attachment Level	-0.42	<0.05
	Bleeding on Probing	-0.40	<0.05
	Plaque Index	-0.38	<0.05
Non-Smokers	Probing Depth	-0.50	<0.01
	Clinical Attachment Level	-0.48	<0.01
	Bleeding on Probing	-0.46	<0.01
	Plaque Index	-0.44	<0.01

Table 6. Correlation Between Salivary GR Levels and Periodontal Parameters Post-NSPT

Table 5 presents the correlation between salivary glutathione reductase (GR) levels and various periodontal parameters post-non-surgical periodontal therapy (NSPT) for both smokers and non-smokers. For smokers, the correlation coefficients (r-values) indicate a negative relationship between GR levels and all periodontal parameters, with probing depth showing a moderate negative correlation ($r = -0.45$, $p < 0.01$), clinical attachment level ($r = -0.42$, $p < 0.05$), bleeding on probing ($r = -0.40$, $p < 0.05$), and plaque index ($r = -0.38$, $p < 0.05$) all exhibiting weaker but still significant negative correlations. This suggests that higher GR levels, indicating lower oxidative stress, are associated with better periodontal health outcomes, evidenced by reduced probing depth, improved clinical attachment levels, decreased bleeding on probing, and lower plaque index. Similarly, for non-smokers, the negative correlations are slightly stronger across all parameters, with probing depth ($r = -0.50$, $p < 0.01$), clinical attachment level ($r = -0.48$, $p < 0.01$), bleeding on probing ($r = -0.46$, $p < 0.01$), and plaque index ($r = -0.44$, $p < 0.01$) all showing significant negative correlations. These findings reinforce the relationship between oxidative stress and periodontal health, highlighting that as salivary GR levels increase (indicating reduced oxidative stress), periodontal health parameters improve more significantly in non-smokers compared to smokers.

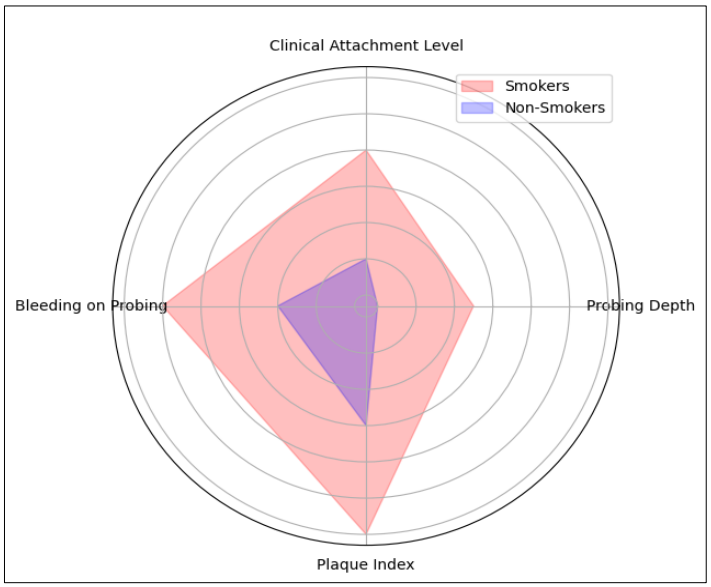

Figure 8. Graphical Representation of Correlation Between Salivary GR Levels and Periodontal Parameters Post-NSPT

The comparative analysis between smokers and non-smokers over a six-month period following non-surgical periodontal therapy (NSPT) reveals significant improvements in various periodontal health parameters and oxidative stress markers. For smokers, the mean probing depth reduced from 6.5 ± 1.2 mm to 5.3 ± 0.9 mm, reflecting an 18.5% improvement, while the clinical attachment level improved by 16.7%, decreasing from 4.2 ± 1.0 mm to 3.5 ± 0.7 mm. The percentage of bleeding on probing in smokers dropped significantly from 70% ± 15% to 50% ± 8%, indicating a 28.6% reduction, and the plaque index showed a 24.0% improvement, decreasing from 2.5 ± 0.6 to 1.9 ± 0.3. Additionally, the glutathione reductase (GR) levels in smokers increased by 44.4%, from 4.5 ± 0.8 U/mL to 6.5 ± 1.3 U/mL. Non-smokers exhibited even greater improvements: probing depth reduced by 21.7%, from 6.0 ± 1.1 mm to 4.7 ± 0.8 mm, and clinical attachment level improved by 21.1%, from 3.8 ± 0.9 mm to 3.0 ± 0.6 mm. Bleeding on probing in non-smokers decreased by 30.8%, from 65% ± 12% to 45% ± 7%, and the plaque index saw a 30.4% improvement, reducing from 2.3 ± 0.5 to 1.6 ± 0.2. Furthermore, GR levels in non-smokers increased by 43.5%, from 6.2 ± 1.1 U/mL to 8.9 ± 1.5 U/mL. All changes were statistically significant with p-values less than 0.05, indicating substantial improvements in periodontal health and oxidative stress markers in both groups, with non-smokers experiencing slightly higher gains (As Depicted in Figure 8). this study demonstrates that smokers with periodontitis have higher oxidative stress, as evidenced by lower salivary GR levels, and respond less favorably to NSPT compared to non-smokers. NSPT effectively reduces oxidative stress and improves periodontal health in both groups, but the therapeutic benefits are diminished in smokers. These findings highlight the need for targeted therapeutic strategies to address oxidative stress in smokers with periodontitis, potentially enhancing treatment outcomes and overall periodontal health. Future research should focus on exploring additional biomarkers of oxidative stress and evaluating the efficacy of adjunctive antioxidant therapies in this patient population.

4. Conclusion & Future Scope

This study provides important insights into the impact of smoking on oxidative stress and the response to non-surgical periodontal therapy (NSPT) in individuals with periodontitis. Our findings reveal that smokers with periodontitis had significantly lower baseline salivary glutathione reductase (GR) levels compared to non-smokers, indicating higher oxidative stress. Additionally, smokers exhibited worse baseline periodontal parameters, including probing depth, clinical attachment level, bleeding on probing, and plaque index. Following NSPT, both smokers and non-smokers showed significant improvements in these periodontal parameters. However, the extent of improvement was more pronounced in non-smokers. Salivary GR levels increased significantly in both groups post-therapy, with non-smokers showing a more robust increase. The correlation analysis further underscored the relationship between oxidative stress and periodontal health, revealing a significant negative correlation between smoking status and baseline salivary GR levels. Improvements in GR levels post-therapy were positively correlated with better periodontal outcomes, suggesting that higher oxidative stress at baseline is associated with poorer treatment outcomes, especially in smokers. These findings highlight the critical role of oxidative stress in the pathogenesis and management of periodontitis, particularly in smokers. Clinicians should consider smoking status and oxidative stress levels when planning and evaluating periodontal therapy. Smokers may benefit from adjunctive antioxidant treatments to enhance their antioxidant defenses and improve treatment outcomes. The use of dietary antioxidants or antioxidant-rich mouthwashes could help mitigate oxidative stress and promote better periodontal

healing in smokers. Educating patients about the detrimental effects of smoking on periodontal health and the benefits of quitting smoking should be an integral part of periodontal care. Smoking cessation programs and support should be offered to help reduce oxidative stress and improve overall health outcomes. Future research should focus on larger, longitudinal studies with extended follow-up periods to validate these findings and assess the long-term effects of NSPT on oxidative stress and periodontal health in smokers and non-smokers. Additionally, investigating a broader range of oxidative stress and inflammation biomarkers could provide a more comprehensive understanding of the biological processes involved in periodontal disease and its management. Clinical trials evaluating the efficacy of various antioxidant therapies in smokers with periodontitis will be essential to establish evidence-based recommendations for adjunctive treatments. In conclusion, this study underscores the significant impact of smoking on oxidative stress and periodontal health and highlights the need for targeted therapeutic strategies to address these challenges. By integrating oxidative stress management into periodontal care, particularly for smokers, clinicians can enhance treatment outcomes and promote better oral and systemic health for their patients. The findings contribute to the growing body of evidence on the interplay between oxidative stress, smoking, and periodontal disease, paving the way for more effective and personalized approaches to periodontal therapy.

References

[1] Patel BP, Rawal UM, Shah PM, Prajapati JA, Rawal RM, Dave TK. Study of tobacco habits and alterations in enzymatic antioxidant system in oral cancer. Oncology. 2005;68(4-6):511–519.

[2] Bains VK, Bains R. The antioxidant master glutathione and periodontal health. Dent Res J. 2015;12(5):389–405.

[3] Chapple ILC, Brock G, Eftimiadi C, Matthews JB. Glutathione in gingival crevicular fluid and its relation to local antioxidant capacity in periodontal health and disease. J Clin Pathol: Mol Pathol. 2002;55(6):367–373.

[4] Trivedi S, Lal N, Mahdi AA, Singh B, Pandey S. Association of salivary lipid peroxidation levels, antioxidant enzymes, and chronic periodontitis. Int J Periodontics Restorative Dent. 2015;35(2):e14–e19.

[5] Moore S, Calder KA, Miller NJ, Rice Evans CA. Antioxidant activity of saliva and periodontal disease. Free Rad Res. 1994;21(6):417–425.

[6] Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed Pharmacother. 2003;57(3-4):145–155.

[7] 109. Miricescu D., Totan A., Calenic B., Mocanu B., Didilescu A., Mohora M., Spinu T., Greabu M. Salivary biomarkers: Relationship between oxidative stress and alveolar bone loss in chronic periodontitis. Acta Odontol. Scand. 2014;72:42–47.

[8] 110. Villa-Correa Y.A., Isaza-Guzmán D.M., Tobón-Arroyave S.I. Influence of periodontal clinical status on salivary levels of glutathione reductase. J. Periodontol. 2016;87:716–724.

[9] 111. Yang P.-S., Huang W.-C., Chen S.-Y., Chen C.-H., Lee C.-Y., Lin C.-T., Huang Y.-K. Scaling-stimulated salivary antioxidant changes and oral-health behavior in an evaluation of periodontal treatment outcomes. Sci. World J. 2014;2014:814671.

[10] 112. Novakovic N., Todorovic T., Rakic M., Milinkovic I., Dozic I., Jankovic S., Aleksic Z., Cakic S. Salivary antioxidants as periodontal biomarkers in evaluation of tissue status and treatment outcome. J. Periodontal. Res. 2014;49:129–136.

[11] 113. de Araújo Júnior R.F., Souza T.O., de Moura L.M., Torres K.P., de Souza L.B., Alves Mdo S., Rocha H.O., de Araújo A.A. Atorvastatin decreases bone loss, inflammation and oxidative stress in experimental periodontitis. PLoS ONE. 2013;8:e75322.

[12] 114. Kırzıoğlu F.Y., Bulut M.T., Doğan B., Fentoğlu Ö., Özmen Ö., Çarsancaklı S.A., Ergün A.G., Özdem M., Orhan H. Anti-inflammatory effect of rosuvastatin decreases alveolar bone loss in experimental periodontitis. J. Oral Sci. 2017;59:247–255.

[13] 115. Rai B., Jain R., Anand S., Kharb S. Total salivary glutathione levels: Periodontitis in smoker and non-smoker. Adv. Med. Dent. Sci. 2008;2:47–49.

[14] 116. Borges I., Jr., Moreira E.A., Filho D.W., de Oliveira T.B., da Silva M.B., Fröde T.S. Proinflammatory and oxidative stress markers in patients with periodontal disease. Mediat. Inflamm. 2007;2007:45794..

[15] Ongoz Dede F, Bozkurt Dogan S, Balli U, Avci B, Durmuslar MC, Baratzade T. Glutathione levels in plasma, saliva and gingival crevicular fluid after periodontal therapy in obese and normal weight individuals. J Periodont Res. 2016;51(6):726–734.

[16] American Academy Of Periodontology Task Force Report on the Update to the 1999 Classification Of Periodontal Diseases and Conditions. J Periodontol. 2015;86(7):835–838.

[17] Use of Smokeless tobacco among adults-United States, 1991. JAMA. 1993;269(23):2971.

[18] Savita M, Sarun E, Arora S, Krishnan S. Evaluation of glutathione level in gingival crevicular fluid in periodontal health, in chronic periodontitis and after nonsurgical periodontal therapy: A clinicobiochemical study. Contemp Clin Dent. 2015;6(2):206–210.

[19] Takane M, Sugano N, Ezawa T, Uchiyama T, Ito K. A marker of oxidative stress in saliva: association with periodontally-involved teeth of a hopeless prognosis. J Oral Sci. 2005;47(1):53-57.

[20] Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med. 1991;91(3C):14S-22S.

[21] Navazesh M. Methods for collecting saliva. Ann N Y Acad Sci. 1993;694:72-77.

[22] Kaufman E, Lamster IB. Analysis of saliva for periodontal diagnosis--a review. J Clin Periodontol. 2000;27(7):453-465.

[23] Chapple ILC, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. Periodontol 2000. 2007;43:160-232.
[24] Chu L, Xu X, Dong Z, Cappelli D, Ebersole JL. Role for recombinant gamma-glutamyltransferase from Treponema denticola in glutathione metabolism. Infect Immun. 2003;71(1):335-342.