

## Effect of non-surgical periodontal therapy on oxidative stress markers in saliva and serum in smokers and non-smokers with chronic periodontitis

Ramanarayana Boyapati<sup>1,\*</sup>, Srikanth C<sup>2</sup>, N. Kiran Kumar<sup>3</sup>, Shyam Sunder<sup>4</sup>, Radhika Ramachandran<sup>5</sup>

<sup>1</sup>Senior Lecturer, <sup>2</sup>Professor & HOD, <sup>3</sup>Professor, <sup>4</sup>Reader, <sup>5</sup>PG Student, Dept. of Periodontics, Mamata Dental College, Khammam, Telangana

**\*Corresponding Author:**

Email: dr.ramanarayana@gmail.com

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### Abstract

**Aim:** The main objective of this case-control study is to determine the effect of initial periodontal treatment of scaling and root planing on oxidative stress biomarkers in smokers and non-smokers with chronic periodontitis (CP).

**Materials and Method:** The study included 114 patients with CP (57 smokers and 57 non-smokers). Serum and saliva samples were collected and clinical periodontal measurements were recorded at baseline, first and third months after periodontal therapy. 8-hydroxydeoxyguanosine (OHdG) and enzyme activity of glutathione peroxidase (GSH-Px) were analyzed with enzyme-linked immunosorbent assay.

**Results:** The level of 8-OHdG, and GSH-Px was found to be significantly higher in saliva and serum of both periodontitis groups. Levels of these ROS were significantly higher in smokers than in non-smokers groups. After initial periodontal treatment, levels of 8-OHdG in serum and saliva were significantly decreased in both periodontitis groups.

**Conclusion:** Initial periodontal therapy with Scaling and Root planing will be helpful in diminishing oxidative stress in periodontitis.

**Keywords:** Periodontitis, Smoking, Reactive oxygen species, 8 hydroxydeoxyguanosine, Glutathione reductase.

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### Introduction

Reactive oxygen species (ROS) generated as a result of normal metabolism of oxygen plays major roles in cell signaling. Extreme high reactivity of ROS when produced in large amounts have proven to have destructive effects. ROS have been implicated in tissue damage mechanisms including protein damage, lipid peroxidation, DNA damage, oxidation of important enzymes, and stimulation of pro-inflammatory cytokine release. Oxidative stress is the disequilibrium between formation of free radicals and antioxidant defense mechanisms via damaging cellular macromolecules like DNA, protein, lipid, etc. 8-hydroxydeoxyguanosine(OHdG) is best evaluates oxidative damage, especially DNA damage.<sup>(4,6)</sup> High levels of saliva 8-OHdG and low levels of saliva antioxidants show increased oxygen radical activity during periodontal inflammation. Hence, the determination of 8-OHdG levels in saliva is a useful biomarker in the evaluation of periodontal state and effectiveness of periodontal treatment. Formation of reactive aldehydes is almost all associated with lipid peroxidation. Antioxidant enzymes protect tissues against oxidative injury from free oxygen radicals generated by various metabolic processes example being Glutathione peroxidase (GSH-Px) seen in plasma, saliva, gingival crevicular fluid (GCF), erythrocytes, and gingival tissues of patients with periodontitis.<sup>(8)</sup> Smoking is a major risk factor that affects the prevalence, degree, and severity of periodontal diseases. In-vitro and in-vivo studies have shown the effects of cigarette constituents on periodontal tissues. The two major phases of Cigarette smoke i.e. tar and

gas, are very rich in ROS and reactive nitrogen species. Cigarette smoking induces production of endogen oxidant and reactive species in smoking-induced inflammatory response. Smoke containing volatile aldehydes, phenols, hydrocarbons, and nitric oxide, creates a source of oxidative stress leading to the formation of oxygen-derived free radicals.<sup>(21)</sup> Therefore, primary objective of this study is to evaluate the effects of initial periodontal therapy on markers affecting oxidative balance in serum and saliva samples in smokers and non-smokers with CP. The hypothesis of this study is that periodontal therapy may be beneficial to diminish oxidative stress markers in different body fluids in patients with CP.

### Materials and Method

**Study Groups:** A total of 119 individuals (66 males and 53 females) participated in this case-control study, of which five female patients reported to the department during baseline investigations but failed to come during further recalls. The two groups, consisting of 57 smokers with CP, 57 non-smokers with CP were screened from July 2016 to March 2017. All participants were selected from Out-patients wing of the Department Of Periodontics, Mamata Dental College and Hospitals, Khammam, Telangana. The study was approved by the ethical committee. The participants were informed about the aim and methods of study, and written informed consent was obtained.

Patients excluded were those who 1) had any systemic disease; 2) were chronic alcoholics; 3) were treated for periodontal illness in the previous six months; 4) took any antibiotics, anti-inflammatory

drugs, or antioxidants in the previous six months, and 5) were pregnant or lactating.

The patients with CP were clinically evaluated according to the criteria proposed by the 1999 International World Workshop for Classification of Periodontal Disease and Conditions.

Patients with CP had teeth with 30% periodontal bone loss and non-adjacent sites per quadrant with probing depth (PD) 5 mm and bleeding on probing.

Inclusion criteria for smokers were those who had been smoking for ten years; ten cigarettes a day. Patients included in non-smoker group were never smokers. Former smokers were not recruited.

**Clinical Measurements and Periodontal Therapy:** Periodontal status was determined by measuring full-mouth plaque index (PI), gingival index (GI), PD, and clinical attachment level (CAL). All clinical measurements were performed by a single investigator. Total number of teeth for each participant was 20. Patients with CP received initial periodontal therapy, including scaling and root planning and polishing within 14 days, and oral hygiene education. All periodontal clinical measurements and samples were recorded at three time points (baseline, first month, and third month after periodontal treatment).

**Collection and Preparation of Samples:** All samples, before and after periodontal therapy, were collected within 48 hours of clinical measurements in the morning, following an overnight fast. Participants were instructed not to eat or drink anything on the day of evaluation. Unstimulated whole saliva samples were obtained for 5 minutes in polypropylenetubes. The saliva samples were centrifuged to remove cell debris at 6,000 rpm for 10 minutes. The supernatant phase was transferred to Eppendorf tubes.

Venous blood samples were carefully obtained in biochemical tubes and was subsequently centrifuged at 5,000 rpm for 10 minutes and then transferred to the Eppendorf tubes. Saliva and serum 8-OHdG and GSH-Px enzyme activity were measured by enzyme-linked immunosorbent assay (ELISA) using commercial kits according to the manufacturer's instructions. The sensitivities for 8-OHdG and GSH-Px were 0.25 ng/mL and 1.12 U/mL, respectively. The assay ranges were 0.5 to 100 ng/mL, and 2 to 600 U/mL, respectively.

**Statistical Analysis:** Data was analysed using SPSS version 22, Descriptive statistics, non-parametric chi square test for intra group comparison (Baseline – 3

months), Independent T test was done for intergroup comparison.

#### Formulation of Hypothesis:

Null Hypothesis:  $H_0$  = There is no difference in the clinical parameters when both the groups are compared.

Alternate Hypothesis  $H_a$  = There is difference in the clinical parameters when both the groups are compared.

P value < 0.05 is considered as statistically significant.

If P value < 0.05 we can reject the null hypothesis and consider the alternate hypothesis

#### Results

Initial periodontal therapy was performed on 47 individuals with CP.

All clinical parameter scores in both CP groups were significantly higher than in both periodontally healthy individuals (P < 0.001). No marked difference of clinical parameters was found between CP groups.

**Clinical Findings After Initial Periodontal Therapy and after 1 and 3 months:** Intragroup comparison at various time periods was done using chi square test showed there is statistically significant differences present between the mean values of plaque index, gingival index, probing pocket depth and clinical attachment levels at baseline when compared with that of the mean values at 1, 3 months. (P < 0.01)

Intergroup comparison at various time periods is done by Independent T test showed statistically significant differences at base line and 3 months periods and highest reduction in group 1 when compared to group 2.

**Laboratory Findings at Baseline and after 1 and 3 months:** Intragroup comparison at various time periods was done using chi square test showed there is statistically significant differences present between the mean values of 8-OHdG levels at baseline when compared with that of the mean values at 1, 3 months. (P < 0.01) (Table 5 & 6) and (Graph 5 and 6).

Intergroup comparison at various time periods is done by Independent T test showed statistically significant differences at 1, month periods. With highest reduction in group 2.

8-OHdG: 8-OHdG level in saliva was found significantly higher in CP with smoking group than CP with non-smokers (P = 0.003).

GSH-Px: GSH-Px enzyme activity in saliva was significantly higher in both CP with smokers than in non-smokers groups.

**Table 1: Comparison of Plaque index in both the groups**

| Group                                  | Baseline |        | 1 month  |       | 3 months |       | chi sq value | P value  |
|--|----------|--------|----------|-------|----------|-------|--------------|----------|
|  |          |        |          |       |          |       |              |          |
| Smokers with chronic periodontitis     | 2.3179   | .51974 | 1.975    | .4691 | 1.321    | .4267 | 114          | <0.001** |
| Non Smokers with chronic periodontitis | 2.0656   | .44730 | 1.849    | .4150 | 1.375    | .3424 | 111.02       | <0.001** |
| T value                                | 2.777    |        | 1.523    |       | 5.303    |       |              |          |
| P value                                | 0.006**  |        | 0.131 NS |       | 0.023*   |       |              |          |

\*\*= highly significant (p<0.01), \*-Statistically significant (P<0.05), NS- Not Significant (P>0.05)

**Table 2: Comparison of Gingival index in both the groups**

| Group                                  | Baseline |        | 1 month  |        | 3 months |        | chi sq value | P value      |
|--|----------|--------|----------|--------|----------|--------|--------------|--------------|
|  |          |        |          |        |          |        |              |              |
| Smokers with chronic periodontitis     | 2.6009   | .29452 | 2.3930   | .30021 | 1.3518   | .20243 | 112.03       | <0.001*<br>* |
| Non Smokers with chronic periodontitis | 2.5658   | .17376 | 2.3061   | .17730 | 1.9737   | .16640 | 112          | <0.001*<br>* |
| T value                                | 0.775    |        | 1.88     |        | 12.685   |        |              |              |
| P value                                | 0.440 NS |        | 0.063 NS |        | 0.001**  |        |              |              |

\*\*= highly significant (p<0.01), \*-Statistically significant (P<0.05), NS- Not Significant (P>0.05)

**Table 3: Comparison of Probing depth in both the groups**

| Group                                  | Baseline |        | 1 month |       | 3 months |       | chi sq value | P value  |
|--|----------|--------|---------|-------|----------|-------|--------------|----------|
|  |          |        |         |       |          |       |              |          |
| Smokers with chronic periodontitis     | 6.2125   | .88335 | 5.765   | .8370 | 4.905    | .8301 | 144          | <0.001** |
| Non Smokers with chronic periodontitis | 4.9509   | .91342 | 4.182   | .6000 | 3.228    | .5927 | 87.06        | <0.001** |
| T value                                | 7.496    |        | 10.521  |       | 8.17     |       |              |          |
| P value                                | <0.001** |        | 0.002** |       | 0.005**  |       |              |          |

\*\*= highly significant (p<0.01), \*-Statistically significant (P<0.05), NS- Not Significant (P>0.05)

**Table 4: Comparison of CAL in both the groups**

| Group                                  | Baseline |       | 1 month |        | 3 months |       | chi sq value | P value  |
|--|----------|-------|---------|--------|----------|-------|--------------|----------|
|  |          |       |         |        |          |       |              |          |
| Smokers with chronic periodontitis     | 1.065    | .6556 | .9496   | .61939 | .311     | .3310 | 105.257      | <0.001** |
| Non Smokers with chronic periodontitis | 1.065    | .6556 | .9496   | .61939 | .311     | .3310 | 105.257      | <0.001** |
| T value                                | 0        |       | 0       |        | 0        |       |              |          |
| P value                                | 1 NS     |       | 1 NS    |        | 1 NS     |       |              |          |

\*\*= highly significant (p<0.01), \*-Statistically significant (P<0.05), NS- Not Significant (P>0.05)

**Table 5: Comparison of 8-OHdG levels in both the groups**

| Group                                  | Baseline |        | 1 month |        | 3 months |        | chi sq value | P value  |
|--|----------|--------|---------|--------|----------|--------|--------------|----------|
|  |          |        |         |        |          |        |              |          |
| Smokers with chronic periodontitis     | 47.77    | 20.085 | 44.75   | 19.166 | 39.56    | 18.442 | 108.44       | <0.001** |
| Non Smokers with chronic periodontitis | 51.12    | 15.980 | 45.72   | 14.914 | 38.37    | 14.955 | 114          | <0.001** |
| T value                                | 0.986    |        | 6.779   |        | 0.379    |        |              |          |
| P value                                | 0.326 NS |        | 0.010*  |        | 0.706 NS |        |              |          |

\*\*= highly significant (p<0.01), \*-Statistically significant (P<0.05), NS- Not Significant (P>0.05)

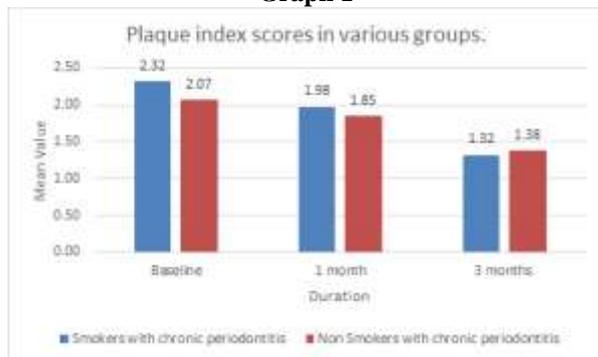
**Table 6: Comparison of GSHP-x values in both the groups**

| Group                                  | Baseline |        | 1 month |        | 3 months |        | chi sq value | P value  |
|--|----------|--------|---------|--------|----------|--------|--------------|----------|
|  |          |        |         |        |          |        |              |          |
| Smokers with chronic periodontitis     | 109.72   | 31.172 | 102.98  | 31.490 | 94.91    | 30.021 | 114          | <0.001** |
| Non Smokers with chronic periodontitis | 75.37    | 19.876 | 68.51   | 18.856 | 58.72    | 18.810 | 114          | <0.001** |

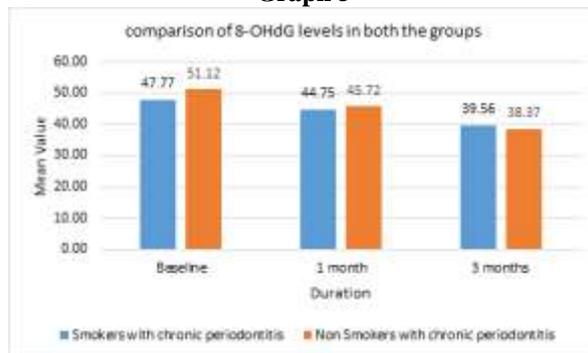
|         |          |  |          |  |          |  |
|---------|----------|--|----------|--|----------|--|
| T value | 7.221    |  | 7.091    |  | 7.713    |  |
| P value | <0.001** |  | <0.001** |  | <0.001** |  |

\*\*= highly significant (p<0.01), \*-Statistically significant (P<0.05), NS- Not Significant (P>0.05)

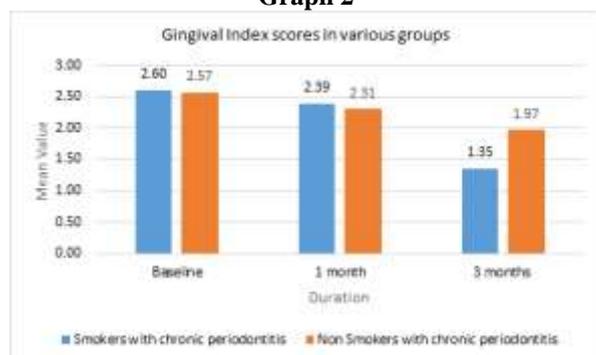
**Graph 1**



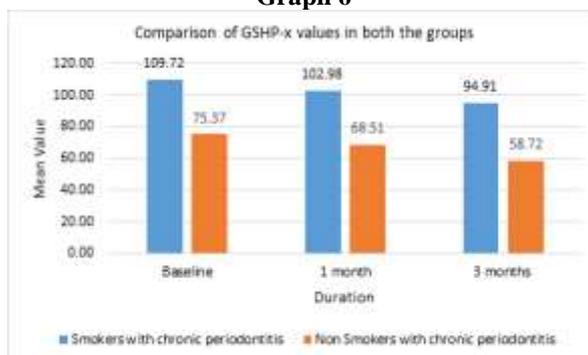
**Graph 5**



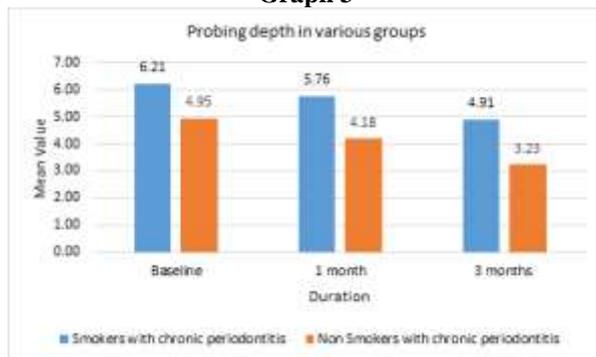
**Graph 2**



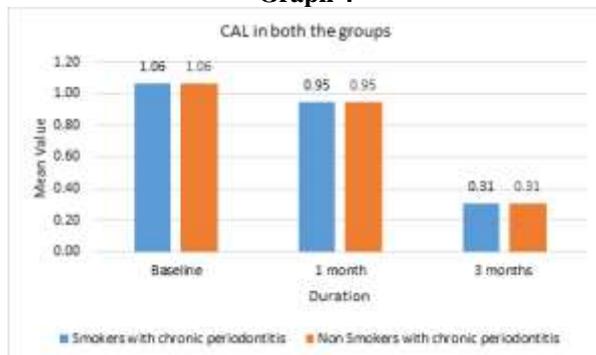
**Graph 6**



**Graph 3**



**Graph 4**



**Discussion**

Oxidative stress, is an imbalance between free oxygen radicals and antioxidant defense system, and is capable of causing damage to various cellular and extracellular components. 8-OHdG is one of the major products of nucleotide oxidation in DNA. Salivary 8-OHdG levels are often studied in oral pathology, including periodontitis, Sjogren syndrome, and oral cancers.

It was found that salivary 8-OHdG levels were very high in patients with periodontally hopeless teeth and teeth with advanced periodontal breakdown. Canakci et al. reported that high salivary 8-OHdG levels and low antioxidant activity increased oxygen radical activity. Mechanical stimulation of periodontally involved gingiva reduced 8-hydroxydeoxyguanosine in plasma and may contribute to a reduction in circulating oxidative stress associated molecules.<sup>(5)</sup>

Similarly, in the present study, it was shown that 8-OHdG levels in saliva were significantly higher in both CP groups compared with periodontally healthy non-smokers. Dede et al. evaluated the effects of initial periodontal treatment on GCF and salivary levels of 8-OHdG in periodontitis, and it was found that after periodontal treatment, 8-OHdG levels in serum and saliva significantly decreased in both periodontitis groups.

The link between cellular production of such important mediators of inflammation and the antioxidant (AO) thiols, cysteine and reduced glutathione (GSH), is discussed and it is hypothesised that NF-kappa B antagonists may offer important therapeutic benefits.<sup>(2)</sup>

Saliva is easily obtained and contains microbial and host response mediators that are produced locally. During the inflammatory response, the GCF flow rate increases, and inflammatory response components, such as, lipid peroxidation products can be found in saliva. In the present study, after periodontal therapy, 8-OHdG levels in serum and saliva decreased from baseline to the first and the third months in both CP groups. All of these findings have supported that 8-OHdG level in serum and saliva reflect oxidative DNA damage in presence of periodontal disease.<sup>(4,6)</sup> There are several approaches to involvement of oxidative stress in pathophysiologic mechanism. One of these approaches is to evaluate the end-products of lipid peroxidation.<sup>(9)</sup> Lipid peroxidation causes an increase in the quantity of products, especially aldehydes. Also, since ROS have a very short life, it is not very easy to find the presence of ROS. Therefore, tissue breakdown associated with ROS has been measured with end-products of lipid peroxidation.<sup>(6)</sup> Tsai et al. reported that lipid peroxidation in GCF and saliva increased in disease areas compared to healthy areas and that the balance between oxidative stress and antioxidant levels was impaired in periodontitis.

GSH-Px, using glutathione as a reducing agent, catalyzes reduction of hydrogen peroxide and various hydroperoxides. Glutathione metabolism is one of the most important antioxidant defense mechanisms, and GSH-Px is one of the major sources of protection against oxidative stress.<sup>(11)</sup> Tongucx et al. showed that gingival superoxide dismutase, catalase and GSH-Px activity increased in smokers compared with non-smokers and former smokers. Similarly, in the present study, there was a significant increase in the activity of saliva GSH-Px in smokers than in non-smokers with CP, explained as a result of tissue protective and adaptive mechanisms.<sup>(21)</sup>

Increased antioxidant activity of GSH-Px in individuals with periodontitis can be explained as consisting of inflamed periodontal tissues in response to oxidative stress. In the present study, increased GSHPx enzyme activity was observed in saliva samples in both CP groups compared with non-smokers.<sup>(8)</sup>

A limitation of this study is that smoking status was recorded based on self-reporting by participants. It has been suggested that the estimation of serum and/or saliva cotinine assays is more reliable for evaluation of smoking status.<sup>(21)</sup>

## Conclusions

Results of this study suggest that significant oxidative stress may occur in periodontitis.

Additionally, 8-OHdG levels in serum and saliva may be used as a biomarker to detect DNA damage, and GSH-Px enzyme activities can be used to determine protective mechanisms against oxidative stress, and initial periodontal treatment on oxidative stress-induced tissue damage may be regarded as an effective treatment approach.

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