Total Salivary Glutathione Levels: Periodontitis in Smoker and non-Smoker
B Rai, R Jain, S Anand, S Kharb

Citation

Abstract
Objective: To determine the association between Total glutathione levels, in saliva and presence of periodontitis. Subjects and Methods: Forty subject samples, as healthy control (n=16), periodontitis in smokers (n=11) and periodontitis in non-smokers (n=13) were recruited for the study. The total glutathione levels were analyzed in the saliva of smoking and non-smoking patients of periodontitis before and after scaling and root planning and healthy control. Results and Conclusions: Total glutathione of saliva were statistically significantly higher in subject of periodontitis in smoker as compared to non-smoker and salivary total glutathione concentrations were significant reduced following scaling and root planning in smoker as well as in nonsmoker. Total glutathione of saliva was affected by periodontal disease. Thus smoking and periodontitis compromised the antioxidant capacity of saliva in systemically healthy patients.

INTRODUCTION
The human inflammatory periodontal diseases are amongst the most common chronic diseases to affect adults. Antioxidants are present in all body fluids and tissues and protecting against endogenously formed free radicals. Antioxidant (AOX) enzymes such as superoxide dimutase and glutathione peroxidase provide protection within cells whilst low molecular weight scavenging antioxidants and AOX vitamins are present in extra cellular fluid. In addition, dietary driven components such as uric acid non protein thiols and glutathione also act as antioxidants. Total antioxidant activity has been reported to be reduced in saliva of patients with periodontitis relative to that in non-periodontitis subjects. Diagnosis of periodontal disease has been primarily based upon clinical and radiographic measures of periodontal tissue destruction. These parameters (clinical examination and radiographically) provide a measure of past destruction and are of limited use in early diagnosis. Smokers are more susceptable to advanced and aggressive forms of periodontal disease than non-smokers. It has been reported that smokers tend to respond less favourably to periodontal treatment. Reactive oxygen species play an important role in cell signaling and metabolic processes and contribute to pathogenic processes in a variety of inflammatory disorders. All organisms possess a range of enzymatic and non-enzymatic antioxidant systems to protect them against harmful oxidative reactions. Under certain conditions, an increase in oxidants and a decrease in antioxidants cannot be prevented, and the oxidant or antioxidant balance shifts towards the oxidative state. There is increasing evidence that oxidative stress is an important contributing factor in the pathogenesis of periodontal disease. Antioxidant molecules are present in all body fluids and tissues. Saliva possesses a variety of defense mechanisms responsible for the protection of oral cavity from oxidative attacks, including uric acid, vitamin C, glutathione, and others. Together with uric acid and albumin, ascorbic acid is among the major antioxidants in saliva.

Hence, the present study was planned to determine the relationship between total glutathione levels in saliva in cases of healthy periodontium, periodontitis in smokers and non-smokers, and effect of scaling and root planning on salivary glutathione levels.

MATERIALS AND METHODS
The patients of periodontitis in smokers (11), periodontitis in non-smokers (13) and healthy periodontium (16), without any systemic disease, aged 30-50 years attending Jain Diagnostic clinic, New Delhi, India were selected. The clinical examination, involed measurement of both probing depths and bleeding on probing, performed by two
experienced examiners who measured the same clinical parameters throughout the study. During the examination and one week after scaling and root planing, paraffin wax stimulated whole saliva was collected, and samples were stored at -20 °C until analyzed. Saliva were centrifuged at 8000g for 9 minutes, and levels of total salivary glutathione was determined by using a kinetic enzymatic recycling assay, according to the manufacturers instructions (Oxford Biomedical research, Oxford, MI, USA)

Differences in Total glutathione of saliva levels between groups at baseline were analyzed by the Mann-Whitney’s U-test. Differences in Total glutathione of saliva samples, probing depths and bleeding on probing were analyzed using a student’s t-test. All statistical analyses were performed using SPSS.

**OBSERVATIONS AND RESULTS**

The mean salivary total glutathione level in subjects with a clinically healthy periodontium was 3.6±1.8 micromole (p < 0.05). In periodontitis in smokers and non-smokers, invalid teeth of hopeless prognosis, the mean total salivary glutathione levels were 5.7±1.8 micromole (p < 0.05), 4.8±1.3 micromole (p < 0.05) respectively. The mean probing depth and bleeding on probing were 4.82±0.12 mm & 64.2±0.5 % & 3.94±0.14 mm & 54.3±0.6 % & 1.92±0.14 mm & 25.2±0.4 % in periodontitis in smokers & non-smokers and healthy periodontium. Significant reductions in clinical recordings were obtained in smoker and non-smoker patients following periodontal treatment. Salivary total glutathione conc. were reduced following therapy in smoker as well as in non-smoker (Table-1, P<0.05)

**DISCUSSION**

Stimulated saliva contains a lower concentration of antioxidants but when flow rates are taken into account, antioxidant capacity is higher than in unstimulated saliva. Stimulated saliva has been used in the analysis of antioxidant. PMN has been observed to be in greater concentration at sites of gingival inflammation. They postulated that O2⁻ produced by PMN as part of the host immune response could cause host tissue oxidative damage if it were not matched by an increase in antioxidant concentration. Number of studies have evaluated the use of various host derived factors in saliva for diagnosis of periodontal disease. In the present study, higher level of total glutathione contents were observed in periodontitis patients and level were still higher in smokers as compared to non-smokers (Table 1, p<0.05). Our results are compared with those reported in the literature. It may be due to one potential mechanism is through tissue damage mediated by oxidative species originating from tobacco smoke and

**Figure 1**

Table 1 Total Glutathione (micromole), probing depth and bleeding in probing in periodontitis in smokers, periodontitis in non-smokers and healthy periodontium before and after treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periodontitis in smokers</td>
<td></td>
</tr>
<tr>
<td>Periodontitis in non-smokers</td>
<td></td>
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<tr>
<td>Healthy periodontium</td>
<td></td>
</tr>
<tr>
<td><strong>Total salivary glutathione content</strong> (before treatment)</td>
<td></td>
</tr>
<tr>
<td>Periodontitis in smokers</td>
<td>4.1 ± 1.3</td>
</tr>
<tr>
<td>Periodontitis in non-smokers</td>
<td>3.9 ± 1.6</td>
</tr>
<tr>
<td>Healthy periodontium</td>
<td>3.6 ± 1.8</td>
</tr>
<tr>
<td><strong>Probing depth in (mm)</strong> before treatment</td>
<td></td>
</tr>
<tr>
<td>Periodontitis in smokers</td>
<td>4.94 ± 0.14</td>
</tr>
<tr>
<td>Periodontitis in non-smokers</td>
<td>3.21 ± 0.14</td>
</tr>
<tr>
<td>Healthy periodontium</td>
<td>1.92 ± 0.14</td>
</tr>
<tr>
<td><strong>Bleeding on probing (in %)</strong> before treatment</td>
<td></td>
</tr>
<tr>
<td>Periodontitis in smokers</td>
<td>34.6 ± 0.7</td>
</tr>
<tr>
<td>Periodontitis in non-smokers</td>
<td>54.3 ± 0.6</td>
</tr>
<tr>
<td>Healthy periodontium</td>
<td>25.3 ± 0.4</td>
</tr>
</tbody>
</table>

*p<0.05, as compared to healthy control. *p<0.05, as compared to periodontitis in non-smokers.
tobacco induced inflammation, in addition to the direct cigarette smoke–mediated depletion of antioxidants. Significant reductions in clinical recordings were obtained in smoker and non-smoker patients following periodontal treatment. Salivary total glutathione conc. were significantly reduced following therapy in smoker as well as in non-smoker (Table-1, P<0.05) while in previous study only no statistically reduction of glutathione levels in periodontal disease patients. The acute influence of smoking a single cigarette on concentrations of glutathione, uric acid, and total antioxidant activity measured in saliva has been addressed previously.

It has been reported that the possible effects of smoking and gingival inflammation on salivary antioxidants in gingivitis patients. They reported that no statistically significant difference was found in any of antioxidant indices between any of the groups. Few authors determined that GCF antioxidant concentration was significantly lower in periodontitis subjects compared to healthy controls. Thus, periodontal disease has been suggested to be associated with reduced salivary antioxidant status and increased oxidative damage within oral cavity. Since, saliva can be easily collected, measurement of Total salivary antioxidant capacity levels may prove to useful in identifying patients at risk of tooth loss. Moreover, a salivary analysis for periodontal diagnosis may prove a cost effective method for screening large populations. Further, studies involving larger group sizes and analysis of GCF total glutathione are required to address these questions.

References
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