Prevalence of Reactive Oxygen Metabolite Levels in Plasma, GCF and Saliva in Chronic Periodontitis, Chronic Gingivitis and Healthy Periodontium: A Biochemical study

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Chronic Periodontitis, an inflammatory disease caused by oral bacteria stimulates the host cells, neutrophils which release Reactive Oxygen Species (ROS) as a part of immune response. Excess ROS is one of the pathological features in the periodontal lesion. Recently, Reactive Oxygen Metabolites (ROM) were recognized as a useful measure of blood ROS. The aim of this study is to estimate the prevalence of ROM in Plasma, saliva and Gingival crevicular fluid in Chronic Periodontitis, chronic gingivitis and healthy controls. The study population consisted of 45 subjects belonging to both the sexes were randomly selected. Subjects were divided into three groups, Healthy periodontium (HP) (Group I) and Chronic Gingivitis (CG) (Group II), Chronic Periodontitis (CP) (Group III). GCF, saliva and plasma were collected in all the three groups to estimate the Reactive oxygen metabolite levels. ROM levels in plasma concentration were almost the same in all the groups [pvalue 0.13]. The values obtained in saliva and GCF were significantly higher in Chronic Periodontitis group compared to Chronic Gingivitis and Healthy Periodontium. [pvalue < 0.001]. The results of our study suggested that a significant oxidative stress may occur in Periodontitis. The findings also suggest that it might play an important role in the pathology of Periodontitis and the associated tissue damage.

Key words: ROM; plasma; saliva; GCF; Chronic Periodontitis.

Periodontitis is regarded as an inflammatory lesion, mediated by complex-host-parasite interactions, that leads to the loss of connective tissue attachment to the root surface cementum and adjacent alveolar bone. An emerging body of evidence is associating oxidative stress to the pathogenesis of Periodontal tissue destruction. Oxidative stress occurs when prooxidant and antioxidant balance shifts in favour of the former, leading to potential damage. Even though the primary etiological agent for Periodontitis is predominantly gram negative bacteria, which initiate the tissue destruction, majority of tissue damage in Periodontitis is caused by an inappropriate host response to these microorganisms and their products. When stimulated by pathogens, host cells (e.g. PMNs) release Reactive Oxygen Species (ROS) as a part...
of immune response. ROS include molecules like hydrogen peroxide, hypochlorous acid and singlet oxygen. Their excessive production by PMNs is one of the pathologic feature in periodontal lesion. ROS can cause tissue destruction by oxidizing DNA, proteins, lipids and important enzymes such as antiproteases, stimulating proinflammatory cytokines release through depletion of intracellular thiol compounds and activating nuclear factor kappa (NFκα).

With the progression, ROS produced in the Periodontal lesion may diffuse into the bloodstream and cause systemic oxidative stress. Numerous studies have suggested a positive association between Periodontitis and blood ROS levels. It is also known that oxidative stress makes a significant contribution to a variety of human diseases such as diabetes, arthritis, heart disease, stroke, liver disease, AIDS and Parkinson’s disease.

Reactive oxygen metabolites (ROMs) were recognized as a usual measure of ROS. This analysis measures the level of generic peroxide present, which in turn reflects the level of free radicals from which it is formed. The test is based on the reaction of samples with transition metal ions to form alkoxyl and peroxyl radicals.

In this study, levels of ROS were investigated in plasma, saliva and Gingival crevicular fluid in Chronic Periodontitis patients. The data obtained were compared with those from healthy control and gingivitis.

**MATERIALS AND METHODS**

**Study groups**

A total of 45 individuals were included in this study, 15 subjects in each group. [Chronic Periodontitis (CP), Chronic gingivitis (CG), and Health Perodontium (HP)]. The subjects were chosen from out patient pool of Department of Periodontics, Thaimoogambigai Dental College and Hospital. The patients were clinically and radiographically evaluated for CP. The patients had at least 4 teeth with one (or) more sites exhibiting probing depth of 4–1 mm, Clinical attachment level (CAL) of 4–1 mm with radiographic evidence of bone loss. The patients with chronic gingivitis had probing depth of 3–1 mm and a CAL of 1–1 mm, with clinical signs of gingival inflammation. The control group consisted of individuals with probing depth of 3–1 mm and a CAL of 1–1 mm, with no clinical signs of gingival inflammation and who also maintained a good oral hygiene.

Among the criteria for being included in the study were patients having no systemic disease, having received no periodontal treatment, antibiotics, anti-inflammatory agents (or) other drugs in the last 6 months. The participants were informed about the study and their consent was obtained. The study protocol was approved by the ethical committee of Dr. M.G.R University, Chennai, India, in accordance with the Helsinki Declaration of 1975, as revised in 2000.

**Collection of Samples**

**Collection of GCF**

The subjects were asked to accumulate saliva in the floor of the mouth and then spit into a pre-weighed or graduated test tube. Laboratory method for detection of ROM The d-ROMs test, developed by world-renowned Italian biochemist (Mauro Carratelli 2001), is a photometric test for measurement of the concentration of hydroperoxides (ROOH) in biological samples. The presence of ROOH in cells indicates oxidative attack of ROS on various organic substrates such as carbohydrates, lipids, amino acids, proteins, or nucleotides.
Test principle
The d-ROMs test uses the principle of Fenton’s reaction: by mixing a biological sample with an acidic buffer (Reagent R1), the newly-created transition metal ion (iron or copper) catalyzes the breakdown of hydroperoxide, generating new radical species such as hydroxyperoxyl (ROO+) and alkoxyl (RO•). By adding a chromogen (N, N-diethyl-paraphenylendiamine, Reagent R2) having the ability to donate an electron and change color when oxidized by free radicals, and using photometric reading available with the FRAS 4 dedicated analytical equipment, it becomes possible to quantify the level of hydroperoxides available in the sample.

Statistical Analysis
All statistical analyses were performed using a software program (MSPS 15 version). Changes in the ROM levels in GCF, plasma and saliva in Health Periodontium, Chronic gingivitis and Chronic Periodontitis were analysed using one way Anova.

RESULTS
The mean value of ROM levels in plasma, saliva and GCF in the Chronic Periodontitis, Chronic Gingivitis and Healthy Periodontium are given in Table 1.

While the ROM levels in Plasma concentration were almost the same in all groups [Table 1.

Comparison between groups

<table>
<thead>
<tr>
<th></th>
<th>HP</th>
<th>CG</th>
<th>CP</th>
<th>HP</th>
<th>CG</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCF</td>
<td>274.35</td>
<td>326.88</td>
<td>532.53</td>
<td>375.53</td>
<td>375.70</td>
<td>592.07</td>
</tr>
<tr>
<td>Plasma</td>
<td>287.04</td>
<td>375.53</td>
<td>577.53</td>
<td>577.53</td>
<td>577.53</td>
<td>592.07</td>
</tr>
<tr>
<td>Saliva</td>
<td>299.17</td>
<td>375.53</td>
<td>577.53</td>
<td>577.53</td>
<td>577.53</td>
<td>592.07</td>
</tr>
</tbody>
</table>

*onewayANOVA
*p value<0.005-significant.
p-value 0.13] the values obtained in saliva and GCF were significantly higher in Chronic Periodontitis group compared with Chronic Gingivitis and Healthy Periodontium. [p-value < 0.001].

There was no significant difference in ROM values in GCF, plasma and saliva in Healthy Periodontium and chronic gingivitis [p-value 0.39, 0.29] respectively. However there was a significant difference in ROM levels in Chronic Periodontitis. [p-value .02].

**DISCUSSION**

To the best of our knowledge, this is one of the few studies performed to estimate the level of ROM in Plasma, Saliva and GCF in Chronic Periodontitis, Chronic Gingivitis and Healthy Periodontium. Several approaches are possible to demonstrate the involvement of oxidative stress in the pathophysiologic mechanisms of diseases. One of these approaches in the assay of end products of peroxides. [Romeno et al. 1998]7.

In the present study, while the plasma ROM levels in the Chronic Periodontitis did not differ significantly compared with Chronic Gingivitis and Healthy Periodontium. Saliva and GCF values showed significant increase and the highest value was observed in GCF. Our results are in accordance with the studies done by [Sobanice H and Sobanice Lotowska 2000, Sheikhi et al. 2001, Tuter et al. 2001, Panjamoorthy et al. 2005, Tsai et al. 2005]12.

A possible association of significantly higher LPO concentrations with an increased percentage of GCF in Periodontitis patients was reported (Tsai et al. 2005)12. Increased GCF flow relates to increased PMN levels, which in turn contributes to over all peroxidise enhancement by myeloperoxidase activity (Battino et al. 2002)14. Moreover the increase level of ROM in saliva, in Chronic Periodontitis in the present study could be partly due to an increased action of ROS in the saliva itself against the increasing amount of bacteria and their products and partly due to an increased leakage of ROS to saliva from plasma and GCF.

Disturbances of balance between ROS and antioxidants contributes significantly to the development of inflammatory oral diseases (Chapple 199713, Battino et al. 2002)14.

ROM levels in GCF, which were higher than those in plasma and saliva in the present study showed that a local increase in ROS level is more prominent in the Periodontal pocket in Chronic Periodontitis and was more significant than the systemic increase in terms of periodontal disease pathology. This is similar to the study by Akalin etal 2007.

While unstimulated saliva was collected in this study (Swalley and Langly Evans 2003) as it represents the major intraoral condition regarding the saliva rate and composition. It also contains some elements of GCF and tissue metabolites that may be useful in the determination of tissue degradation (Kaufman and Lamster 2000)16. In addition, stimulating Saliva flow has been demonstrated to increase saliva volume and disrupt the concentration (Moore et al. 1994)17.

**CONCLUSION**

In conclusion, the results of our study suggest that a significant oxidative stress may occur in Periodontitis. The findings also suggest that it might play an important role in the pathology of Periodontitis and the associated tissue damage. Oxidative stress plays an important role in the pathologies related to smoking and diabetes which are among the risk factors of Periodontitis. [Loe 199317, Toretti and Claffey 2005].

Further longitudinal studies in a larger sample size could be helpful in depicting the role of ROS metabolites in periodontal health, which would be beneficial in treating the multifactorial disease.

**REFERENCES**

4. Akalins FA, Balcacoglu E, Alver A. Lipid Peroxidation levels and total oxidant status in serum, saliva and GCF in Patients with Chronic Periodontitis. *Journal of Clinical Periodontology*


