Short Term Effects of Non Surgical Periodontal Treatment with the use of Diode Laser (810nm) on Plasma, GCF and Saliva Level of Reactive Oxygen Metabolite in Patients with Chronic Periodontitis

Uma Sudhakar1, T. Rama Krishnan2, J. Baghya Meena3, V. Shankar Ram4, H. Tamizhchelvan5, Kamal Kannadasan6 and Parthiban7

1H.O.D & Professor, Thaimoogambigai Dental College & Hospital, Mugappir, Chennai- 600107, India.
2Professor, Meenakshiammal Dental College & Hospital, Chennai- 600107, India.
3Post Graduate, Thaimoogambigai Dental College & Hospital, Chennai- 600107, India.
4Reader, Thaimoogambigai dental College & Hospital, Chennai- 600107, India.
5Professor, Ramachandra Dental College & Hospital, Chennai- 600107, India.
6Professor, Thaimoogambigai Dental College & Hospital, Chennai- 600107, India.
7Senior lecturer, Thaimoogambigai Dental College & Hospital, Chennai- 600107, India.

DOI: http://dx.doi.org/10.13005/bbra/1803

(Received: 03 March 2015; accepted: 16 April 2015)

The aim of the study was to compare the clinical efficiency of a diode laser as an adjunct to scaling and root planning in the treatment of chronic periodontitis patients. A total of thirty patients with chronic periodontitis were selected for the study. Randomly 15 patients were taken in each group as control group and test group. The test group received conventional SRP and diode laser. The plasma, GCF & Saliva levels of reactive oxygen metabolites were estimated at baseline, day 30 & day 60 for test group. When the groups were compared statistically significant reduction was seen in reactive oxygen metabolite from baseline to day 30 and to day 60(P<0.001). In this study it can be concluded that the use of diode laser as an adjunct to SRP provided significant result compared from baseline, day 30 & day 60.

Key words: Surgical Periodontal, Root planning, GCF, Salive, Periodontitis

Periodontitis is an inflammatory lesion, mediated by complex host parasite interactions, that leads to the loss of connective tissue attachment to root surface cementum and adjacent alveolar bone. The inflammatory and immune responses to the bacteria that colonize the periodontal and associated tissues involve the systemic circulation and ultimately the peripheral system of the body. The neutrophils play a pivotal role in the host defense and are the first line of defense against these infectious periodontal diseases. The different microbial products (especially those produced by gram negative bacilli) triggers local inflammation and causes large members of Polymorpholeucocytes (PMNs) to migrate to the area where the bacteria interact with the surface tissue. Reactive oxygen species (ROS) released from the PMNs and other immune cells lead to...
oxidative damage in biomolecules, which trigger morphofunctional changes in the endothelium and therefore in the periodontal tissues that nourish them. In normal physiology, there is a dynamic equilibrium between ROS activity and antioxidant defense capacity and when the equilibrium shifts in level of ROS, either by reduction in level of antioxidant defenses or an increase in ROS production or activity, oxidative stress results. This imbalance between the ROS-AO has been implicated as one of the progressive or pathogenic factors for the periodontal diseases. These oxygen-derived free radicals are highly reactive molecular species that can disrupt cellular proteins, nucleic acids, and membrane lipids as well as cause depolymerization of matrix components such as collagen, hyaluronan, and proteoglycans.

Recent clinical studies suggest a positive association between periodontitis and oxidative damage. A significantly higher level of serum protein carboxyl (a marker of protein oxidation) was observed in patients with periodontitis than in periodontally healthy subjects.

A number of surgical and non-surgical modalities are available for the treatment of inflammatory periodontal diseases. Subgingival scaling and root planning is one of the most important procedures and this efficacy has been demonstrated in numerous clinical studies. Laser applications in periodontology have been of enormous interest in recent years. The earliest studies mentioning the application of laser in the non-surgical pocket therapy began in 1990s.

Lasers may contribute to bacterial reduction in periodontal pocket as well as contribute to the removal of calculus and granulation tissue and can also be used for debridement of soft tissue wall. The high powered Carbon dioxide (CO₂) and Neodymium Doped Yttrium Aluminium Garnet (Nd:YAG) lasers are capable of excellent soft tissue ablations with a good haemostatic effect and have been generally proposed for periodontal and oral surgery. However, these lasers are not suitable for root surface treatment because of the carbonization of these tissues and major thermal effects on adjacent tissues. Diode and NdYAG are currently used by clinicians because of its delivery system which is convenient to use. A variety of diode lasers have been used in the debridement of periodontal pocket and a number of in vitro and in vivo studies have been carried out. Studies have been done to evaluate the possible advantage of the use of diode laser as an adjuvant to conventional SRP.

The purpose of the present study was to determine the effects of the use of diode laser in pocket debridement and the changes in the ROM levels in plasma, saliva, and GCF.

**MATERIALS AND METHODS**

The study population consisted of 30 subjects (each group has 15 subjects) belonging to both sexes and all subjects were randomly selected from the outpatient clinic of the Department of Periodontics, Thamkooambigai Dental College, Chennai. The patients were clinically and radiographically evaluated for CP. The patients had at least 4 teeth with one or more sites exhibiting probing depth ≥ 4 mm. Clinical attachment level of ≥ 4 mm with radiographic evidence of bone loss. The control group consisted of individuals with probing depth ≤ 3 mm and clinical attachment level of ≤ 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 Declaration of 1975, as revised in 2000.

**Group I- Healthy Periodontium**

**Group II- Chronic Periodontitis**

**Evaluation of Periodontal status**

All data were recorded in a standard proforma. Oral examination was carried out with proper illuminations using mouth mirror and graduated Williams’s periodontal probe. The following parameters were evaluated for the subjects:

- **Plaque index by Loe and Silness (1963)**
- **Probing Pocket Depth (PPD)**
- **Clinical Attachment Level (CAL)**
Collection of GCF

Detailed case history, clinical examination and supragingival scaling were done one day before the collection of GCF. On the subsequent day after drying the area with a blast of air, supragingival plaque was removed without touching the marginal gingiva and the GCF was collected. A standardized volume of 1µl was collected from each site with an extracrevicular approach, using volumetric capillary pipettes that were graduated from 1-5µl. The collected GCF was transferred immediately to ependorff tubes and stored at -70°C until the time of assay.

Collection of plasma

Blood (3ml) was drawn from the antecubital vein, under aseptic precautions and collected in a colour coded herparinised test tube (green colour) and centrifuged immediately at 3000 xg for 5 minutes. If not immediately assayed plasma aliquots were stored at -80°C until analysis.

Collection of saliva

Draining / Spitting method: The subject is asked to accumulate saliva in the floor of mouth and then spit in to a pre- weighed or graduated test tube.

Laboratory method for detection of ROM

The d- ROM test, developed by world-renowned Italian biochemist Mauro Carratelli, 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 substrates such as carbohydrates, lipids, amino acids, proteins, or nucleotides.

Test Principle

The d- ROM 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 (ion or copper) catalyzes the breakdown of hydroperoxide, generating new radical species such as hydroxyperoxyl (ROO+) and aikoxyl (RO+). By adding a chromogen (N,N-diethyl-paraphenyldiamine, Reagent R2) having the ability to donate the 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.

Periodontal treatment

At baseline, the clinical parameters (Plaque index, Gingival index, Probing Pocket depth, clinical attachment level) were recorded for all subjects and full mouth supragingival scaling was done. GCF and plasma samples were collected for assessment of ROM levels. No treatment was done after sample collection in Group I patients. In Group II patients, Non-surgical periodontal treatment (Scaling and Root planing) was performed. On completion of scaling and root planing, low level laser therapy was performed using diode laser. Clinical data, GCF, saliva and plasma samples were reevaluated at 1st & 2 months after therapy in group II patients to assess the changes.

Laser parameters

On completion of scaling and root planning, full mouth low level laser therapy was done using a diode laser. Picasso laser is a class IV diode laser, with an active semiconductor medium (Gallium, Aluminum, Arsenide) with a wavelength of 810nm provided with a optical fiber thickness of 300µm. The laser was fired at the orifice of gingival margin at a distance of 1cm in a non contact mode using a setting of 1.5 W as a continuous wave. Each tooth received 5-10s of exposure. Final low level laser therapy was done on 2nd day. Low level laser therapy was performed two times at baseline and 2nd day. Protective shields were worn by the operator, assistant and patient during the entire procedure.

Statistical analysis

Statistical analyses were performed using a soft were program (SPSSV 16, IL). Independent t test was used for comparison of ROM levels in GCF, saliva & plasma among healthy & chronic periodontitis. Comparison of ROM levels between baseline and after treatment was done using repeated measure ANOVA.

RESULTS

All 30 Patients attended baseline and follow up appointments. At the baseline, there was significant difference in ROM levels between healthy and chronic periodontitis patients (p value < 0.001) in GCF, Saliva & Plasma. This is depicted in table 1.
Comparison of ROM levels GCF in the test group at different time point (30 days+ 60 days) was depicted in table 2. The mean changes in GCF levels of ROM were statistically significant between baseline and day 60 and between day 30 and 60 in the test group (p < 0.001).

Comparison of ROM levels in plasma after laser treatment at different time points (30 & 60 days) was detected in table 3. There was significant difference in ROM levels in plasma between baseline and day 60 and between day 30 and day 60 (p < 0.001).

Comparison of ROM levels in saliva at baseline and after treatment at different timepoints (day 30 + day 60) were depicted in table 4. There was significant difference in ROM levels in saliva between baseline and 60th day and between day 30 and day 60 (p value < 0.001).

Table 1. Comparison of the ROM levels in GCF, Plasma and Saliva among healthy and chronic periodontitis patients

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCFROM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>15</td>
<td>267.68</td>
<td>78.975</td>
<td>20.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CP</td>
<td>15</td>
<td>484.33</td>
<td>136.59</td>
<td>35.26</td>
<td></td>
</tr>
<tr>
<td>PLASMAROM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>15</td>
<td>300.37</td>
<td>37.77</td>
<td>9.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CP</td>
<td>15</td>
<td>596.00</td>
<td>46.77</td>
<td>12.07</td>
<td></td>
</tr>
<tr>
<td>SALIVAROM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>15</td>
<td>321.64</td>
<td>49.96</td>
<td>12.90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CP</td>
<td>15</td>
<td>596.00</td>
<td>46.77</td>
<td>12.07</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Comparison of ROM in GCF at baseline and after laser treatment at 1 and 3 month interval

<table>
<thead>
<tr>
<th>ROM-GCF</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>95% Confidence Interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>484.33</td>
<td>136.59</td>
<td>408.688</td>
<td>559.979</td>
</tr>
<tr>
<td>1 month</td>
<td>387.733</td>
<td>137.33089</td>
<td>311.682</td>
<td>463.785</td>
</tr>
<tr>
<td>3 months</td>
<td>341.467</td>
<td>138.18146</td>
<td>264.944</td>
<td>417.989</td>
</tr>
</tbody>
</table>

Table 3. Comparison of ROM in Plasma at baseline and after laser treatment at 1 and 3 month interval

<table>
<thead>
<tr>
<th>ROM-GCF</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>95% Confidence Interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>596.001</td>
<td>46.77981</td>
<td>570.095</td>
<td>621.906</td>
</tr>
<tr>
<td>1 month</td>
<td>499.867</td>
<td>46.62705</td>
<td>474.046</td>
<td>525.689</td>
</tr>
<tr>
<td>3 months</td>
<td>455.667</td>
<td>46.19850</td>
<td>430.083</td>
<td>481.251</td>
</tr>
</tbody>
</table>

Table 4. Comparison of ROM in Saliva at baseline and after laser treatment at 1 and 3 month interval

<table>
<thead>
<tr>
<th>ROM-GCF</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>95% Confidence Interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>596.001</td>
<td>46.77981</td>
<td>570.095</td>
<td>621.906</td>
</tr>
<tr>
<td>1 month</td>
<td>503.667</td>
<td>47.38979</td>
<td>477.424</td>
<td>529.911</td>
</tr>
<tr>
<td>3 months</td>
<td>456.734</td>
<td>46.97311</td>
<td>430.721</td>
<td>482.747</td>
</tr>
</tbody>
</table>

None of the patients reported any side effects related to laser radiation.
DISCUSSION

To the best of our knowledge, this is the first study performed to estimate the levels of ROM in saliva, GCF & plasma in patients with chronic periodontitis undergoing SRP with Low level laser therapy (LLLT).

Recently, the use of laser in the dental field is more pronounced. Today low level laser are used to improve wound healing \(^{11}\). Low level laser therapy are thought to act by the interaction of light with the cell & tissue. The dose applied during laser applications is one of the important treatment parameter to low level laser therapy. However, a precisely determined dose has not been proved for each indication. Biostimulation has been reported in the literature with doses between 0.001 and 10 J/cm\(^2\) as a therapeutic window\(^{12}\). Mester et al \(^{13}\) suggested in 1971 that doses of \(1 \text{ J/cm}\(^2\)\) are necessary to see an effect on wound healing. In this study we used diode laser of 810 nm output power of 1 w.

The results of our study show that there is statistically significant improvement in ROM levels and clinical parameters post treatment. The beneficial effects of scaling and root planing combined with personal plaque control in the treatment of periodontitis have been well documented\(^{14}\). Low level laser therapy (LLLT) after non surgical periodontal treatment resulted in more reduction in clinical parameters. The improvement could be a result of an increase of anti-inflammatory cytokine levels & an increase of microcirculation by LLLT.

The levels of ROM reduced from 460 (GCF), 562.5(plasma), 562.5(Saliva) caaru to 360, 467.5, 467.5 in day 30 and they reduced to 315, 417.5, 422.5 to day 60 in test group respectively. The reduction in levels of ROM from baseline to day 30 and from baseline to day 60 was found to be statistically significant (p < 0.001). This was in accordance with previous studies that demonstrated statistically significant decrease in levels of ROM in patients with chronic periodontitis as compared to control before & after treatment \(^{16,17}\). The reduction of ROM after laser bedridement was initially reported in an in vitro study in which attenuation of ROM production in neutrophils was demonstrated by low level laser therapy\(^{18,19}\). However, certain previous reports have demonstrated...
that periodontal treatment had minimal influence on the plasma levels of total antioxidants. The present study provide some support that periodontitis is the common cause of low grade inflammation that can lead to a systemic oxidative stress state. The excessive circulating ROS thus produced can oxidize DNA, lipids and proteins thereby contributing to tissue damage. So, it can be said that periodontitis induced high oxidative stress can lead to similar tissue damage. It could be said that the reduction of circulating ROS by periodontal treatment offers clinical benefit.

**CONCLUSION**

The result of this study, show that non surgical therapy of periodontal patient using diode laser as an adjuvant to SRP, lead to a significant reduction at various time points. Limitations of the present study, include small sample size and results are not compared with other treatment modalities. Further long term studies are required with large sample size to evaluate the effect of LLLT as an adjuvant to SRP in the treatment of periodontal patients and their effects on Oxidative stress.

**REFERENCES**