Effects of Non-Surgical Periodontal Therapy on Plasma Level of Reactive Oxygen Metabolites and Glycemic Status in Type-2 Diabetic Patients with Chronic Periodontitis

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http://dx.doi.org/10.13005/bbra/2453

(Received: 20 March 2017; accepted: 24 March 2017)

The aim of this study is to find out the effects of non-surgical periodontal therapy on Plasma level of Reactive oxygen metabolites (ROM) and glycemic status in Type-2 diabetic patients with chronic periodontitis. One hundred and twenty patients were included in this study which was further divided into three groups. Group 1 consisted of 40 patients with chronic periodontitis, group 2 consisted of 40 patients with chronic periodontitis and controlled diabetes and group 3 consisted of 40 patients with chronic periodontitis and uncontrolled diabetes. Periodontal parameters like plaque index, gingival index, bleeding on probing, pocket depth and clinical attachment levels were evaluated. Blood samples were collected to assess the fasting blood sugar (FBS), glycosylated haemoglobin (Hba1C) and Plasma reactive oxygen metabolites (ROM) levels. All parameters were evaluated at baseline, one month and three months after non-surgical periodontal therapy. Intragroup comparison of clinical parameters showed significance at baseline, one month and three months in all the three groups. (P value<0.001) Inter group comparison of FBS and Hba1C showed significance in all the three groups. When Intragroup comparison was done for FBS and Hba1C, there was significance seen at baseline, one month and three months for FBS in all the three groups. (P value<0.001) whereas for Hba1C, there was significance observed only in group 3. Intragroup comparison of ROM showed significance at baseline, one month and three months after treatment (P value<0.001). Non-surgical periodontal treatment was effective at improving glycemic and periodontal status by reduction in the plasma ROM levels. This improvement might offer clinical benefits by decreasing the blood ROS.

Keywords: Chronic generalised periodontitis, Hba1c, Reactive oxygen metabolites (ROM), Type 2 diabetes mellitus.
aerobic organisms causing PMN’s to release a large number of ROS influencing systemic oxidative status\textsuperscript{2} and resulting in oxidative damage\textsuperscript{3}. The level of circulating ROMs is influenced by a reduction in periodontal inflammation and ROM is reported to be a useful indicator of ROS in blood.\textsuperscript{4, 5}

In normal physiology, there is a dynamic equilibrium between ROS activity and antioxidant defense capacity and when there is an imbalance in the ROS level, either by reduction in level of antioxidant defenses or an increase in ROS production or activity, oxidative stress results. With the progression of periodontitis, ROS produced in the periodontal lesion may diffuse in to the blood stream. Excessive production of ROS by polymorphonuclear leukocytes, is one of the pathologic feature in the periodontal lesion\textsuperscript{6}, and it leads to damage of the periodontal tissue by oxidizing DNA, lipids, and proteins\textsuperscript{7}. Several studies have suggested a positive correlation between periodontitis and blood ROS level or oxidative damage\textsuperscript{8}. Measuring this ROM level in blood is recognised to be useful for the evaluation of oxidative stress in the body\textsuperscript{9, 10}. Systemic increase in oxidative stress may have a detrimental effect on periodontal condition and also contribute to the development of complication in diabetes.

Diabetes is a metabolic disorder and most of the complications of diabetes are due to hyperglycemia. Critical pathogenic consequences of hyperglycemia in diabetes is a deficit in detoxification of reactive carbonyl compounds. The increase in reactive carbonyls is derived from both oxidative and nonoxidative reactions leading to increased chemical modification of proteins by carbohydrates and lipids and then at a late stage to oxidative stress and tissue damage. The AGE (Advanced glycation end products) hypothesis proposes that chronic accelerated chemical modification of proteins by reducing sugars in diabetes alters the structure and function of tissue proteins, contributing and precipitating the development of diabetic complications.\textsuperscript{10} The aim of the present study was to evaluate and compare the level of plasma ROM and glycemic status using FBS and Hba1C levels before and after nonsurgical periodontal therapy.

**MATERIALS AND METHODS**

The subjects were selected from the department of periodontics, Thai Moogambigai Dental College, Maduravoyal, Chennai. Written consent was taken from each subject. All the participants completed the study. The study protocol was approved by the ethical committee of Dr.M.G.R university, Maduravoyal, Chennai, India. This was in accordance with the declaration of 1975, as revised in 2000.

This study consists of 120 subjects which is further divided in to

- Group 1: 40 subjects with chronic periodontitis
- Group 2: 40 subjects with chronic periodontitis with controlled type 2 Diabetes mellitus
- Group 3: 40 subjects with chronic periodontitis with uncontrolled type 2 Diabetes mellitus.

The patients were clinically and radiographically evaluated for chronic periodontitis. All the clinical parameters, blood samples were obtained from these subjects at baseline, one month and three months after non-surgical periodontal therapy.

**Inclusion criteria**

Subjects who were included in this study should have chronic periodontitis, with or without Type 2 diabetes mellitus. They presented at least four teeth with one or more sites with probing depth (PD) $\leq$ 5mm, clinical attachment level (CAL) $\leq$4mm and bleeding on probing (BOP).

**Exclusion criteria**

Patients who had undergone periodontal treatment in the past six months, Those with a history of antibiotic administration with in the last three months, Those with less than 20 remaining natural teeth, subjects who are pregnant and subjects with a history of smoking and tobacco consumption were excluded in the study.

**Periodontal Treatment and Clinical Measurements**

All patients were subjected to a periodontal examination performed in six sites per tooth excluding third molar. Periodontal parameters like plaque index (Silness and loe 1964), gingival index (Loe and silness 1963), bleeding on probing (Muhlemann and son 1971), pocket depth and clinical attachment level were evaluated. Blood
samples were collected after a minimum of 8 hour of overnight fasting for all individuals at baseline, one month and three months after treatment. After recording the periodontal status, patients received oral hygiene instructions and underwent full mouth non-surgical periodontal treatment comprising scaling and root planing under local anaesthesia. After the periodontal treatment, a professional plaque control programme was performed twice a month for 3 months consisting of supragingival plaque removal and reconstruction of oral hygiene procedures. During this experimental period patients were questioned about changes in medications related to diabetes therapy, use of anti-inflammatory or antibiotic and alteration of lifestyle, including exercise and diet.

**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 hydroperoxy (ROO+) and aikoyl (RO+). By adding a chromogen (N, N- dimethylphenylendiamine, 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.

**Sample preparation**

Venous blood samples were collected in the morning after an overnight fast, from the patients of three groups (Group1, 2 and 3) (Fig-1) and placed in plasma test tubes for centrifugation. (Fig-2,3) After centrifugation the plasma was separated and placed in a Ependex tubes. (Fig-4,5) The separated plasma is then mixed with the reagents like acetate buffer and N-Dimethyl-phenylendiamine (Fig-6,7). The prepared tubes are placed in UV spectrophotometry. (Fig-8) and the readings are collected. The carratelli unit (CARRU) was used for measurement. It was established that 1 CARRU corresponds to 0.08mg/dl hydrogen peroxide.

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PI- Plaque index; GI- Gingival index; BOP- Bleeding on probing; PD- Pocket depth; CAL- Clinical attachment level
Statistical analysis

Statistical analyses were performed using a software program (SPSSV 16, IL). Comparison of variables with in the groups was calculated by paired T- Test and between the groups were analyzed by means of using one-way analysis of variance (ANOVA).

RESULTS

When intergroup comparison was done for clinical parameters, there was statistical significance seen for Plaque index (PI), gingival index(GI) and bleeding on probing (BOP) at one month and three months after treatment. For Pocket depth (PD), there was significance seen at baseline and after one month after treatment, whereas for clinical attachment level (CAL), significance was observed only at one month after treatment (Table-1). When intragroup comparison was done for clinical parameters at baseline, one month and three months after treatment there was statistical significance observed in all the three groups (Table-2). When intergroup comparison was done for

Table 2. Intragroup comparison of clinical parameters

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<th>Clinical parameters</th>
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PI- Plaque index; GI- Gingival index; BOP- Bleeding on probing; PD- Pocket depth; CAL- Clinical attachment level

Table 3. Inter Group Comparisons Of Fbs And Hba1c

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FBS- Fasting blood sugar
Hba1c- Glycosylated haemoglobin
glycemic status using FBS and Hba1C, there was statistically significant reduction seen in all the three groups (Table-3). When intragroup comparison was done individually for FBS and Hba1C at baseline, one month and three months after treatment, there was statistically significant reduction observed for FBS in all the three groups, whereas there was significant reduction observed for Hba1c only in group 3 (Table 4). When intergroup comparison was done for ROM both in mg/dl and CARRU units, there was no statistical significance (Table-5). When intragroup comparison was done for ROM at baseline, one month and three months after non-surgical periodontal treatment, there was statistically significant reduction observed in all the three groups (Table-6).

**DISCUSSION**

At baseline, plasma ROM level was higher in all the three groups. Since ROM is
reported to be a useful indicator of ROS in blood, the findings from this study indicated that chronic periodontitis increased plasma ROS. Excessive ROS oxidizes DNA, lipids and proteins thereby contributing to tissue damage. In a study conducted by Matthews and Wright, peripheral blood neutrophils from patients with periodontitis exhibited a hyperactive phenotype in terms of production of ROS\textsuperscript{11}. Periodontal treatment may also affect plasma ROS levels by improving a hyperactive phenotype in blood neutrophils.

In our study, when intergroup comparison was done for clinical parameters, there was statistical significance seen for Plaque index (PI) gingival index(GI) and bleeding on probing (BOP) at one month and three months after treatment. For pocket depth (PD) there was significance seen at baseline and after one month, whereas for clinical attachment level (CAL), significance was observed only at one month after treatment. When intragroup comparison was done for clinical parameters at baseline, one month and three months after treatment there was statistical significance observed in all the three groups. similar to a study done by Al-shammari and Aldredge\textsuperscript{12}. In another study, Periodontal treatment significantly reduced the serum levels of IL-6 and increased HDL cholesterol in chronic periodontitis and Type-2 diabetes patients.\textsuperscript{13} The reduction in plasma ROM level and oxidative stress after non-surgical periodontal treatment in the present study was consistent with the previous study. The results obtained in this study appear to demonstrate a strong, statistically significant, association between clinical improvement in the periodontal condition and non-surgical periodontal treatment.

In our study, when intergroup comparison was done for glycemic status using FBS and Hba1C, there was statistically significant reduction seen in all the three groups. When
intragroup comparison was done individually for FBS and Hba1C at baseline, one month and three months after non surgical periodontal treatment, there was statistical significance observed for FBS in all the three groups, whereas there was significance observed for Hba1c only in group 3. The results of this study suggests that following periodontal therapy there is a statistically significant improvement in glycemic control. In a patient with diagnosed diabetes, the HbA1C level is used to monitor the patient’s overall glycemic control. HbA1C reflects the mean glucose level over the preceding 2–3 months. Periodontal treatment that reduces periodontal inflammation may help to restore insulin sensitivity, thereby improving glycemic control.14 Studies done by Janket and Grossi et al suggested that an improvement in a subject’s periodontal health can positively affect his glycemic metabolic control.15,16

In our study, when intergroup comparison was done for ROM both in mg/dl and CARRU units, there was no statistical significance. When intragroup comparison was done for ROM at baseline, one month and three months after non-surgical periodontal treatment, there was statistical significance observed in all the three groups. There was a positive association between reduction in ROM level and BOP, which was significant in our study. This was in accordance with the study done by Naofumi tamaki etal.17 Since BOP reflects the present disease activity in the periodontium, the decrease in the plasma ROM level might be due to the reduction in disease activity in the periodontium.18

Various studies done by Ekuni and Tomofuji et al revealed that periodontal inflammation induces oxidative tissue damage in the aorta19 and liver with increasing serum ROS20.
Periodontitis may affect various organs through increased ROS in the blood. Oxidative damage is induced by an imbalance between the production of ROS and antioxidant defence. Therefore, it may be preferable to consider that reduction in circulating ROS by non-surgical periodontal treatment can offer clinical benefits in terms of reducing the risk for future systemic disease. In addition, the resolution of periodontal inflammation after non-surgical periodontal therapy resulted in decreases in the plasma ROM level. It is conceivable that the non-surgical periodontal treatment reduces periodontal inflammation as well as plasma ROS in all the three groups.

CONCLUSION

In this study, there was improvement in all the clinical parameters compared to the baseline values. There is statistically significant reduction in FBS and Hba1C values after non-surgical periodontal treatment. There is also reduction in plasma ROM level compared to the baseline values. Non-surgical periodontal treatment was effective in improving clinical parameters, glycemic status and also in reducing plasma ROM level after one month and three months of non-surgical periodontal treatment. Longitudinal studies are needed to examine the causal relationship between plasma ROM level and periodontal condition.

REFERENCES


