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Determination of Oxidative Stress Parameters and Tissue Factor Activity in the Saliva of Patients with Periodontitis

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ABSTRACT

Objective: Periodontitis, an inflammatory disease, leads to the destruction of the periodontium and results in tooth loss. Reactive oxygen species are involved in the destruction of periodontal tissues and systemic inflammation. The aim of the study was to evaluate and compare the oxidative status in different kinds of periodontal disease and whether the treatment amends these effects.

Materials and Methods: Whole saliva was collected from 30 patients with chronic marginal gingivitis, chronic periodontitis and generalized aggressive periodontitis at baseline and after non-surgical periodontal therapy and 10 healthy control subjects. Lipid peroxidation (LPO) and glutathione (GSH) levels and glutathione-s-transferase, superoxide dismutase catalase and tissue factor (TF) activities were determined in the whole saliva.

Results: Antioxidant enzyme activities were significantly higher in periodontitis groups before non-surgical periodontal therapy and also increased LPO levels and decreased TF activities were found in these groups. Significant decreases in antioxidant enzymes and LPO levels, and increases in TF activities were detected after treatment. GSH levels increased after treatment.

Conclusion: Increased antioxidant enzyme activities and LPO levels indicate destruction of periodontal tissues due to excessive radical production. Treatment of periodontitis and restoring the balance of oxidant-antioxidants ameliorates tissue damage caused by oxygen species and inflammation.

Keywords: Oxidative stress, periodontitis, saliva

INTRODUCTION

Gingivitis and periodontitis, which are called periodontal diseases are among the most common chronic conditions affecting world's populations (1,2). Periodontal disease is a polymicrobial subgingival inflammatory disease of the periodontal tissues (3). Chronic marginal gingivitis (CMG) is a non-destructive periodontal disease and characterized by inflammation of the gums due to the accumulation of plaque at or near the gingival sulcus (1). Chronic periodontitis (CP) is a widespread periodontal disease of the oral cavity involving of chronic inflammation of the surrounding and supporting structures of the teeth which is caused by aggregation of plenty of dental plaque (4). Generalized aggressive periodontitis (GAgP) which is characterized by rapid loss of attachment and bone destruction and familial aggregation, is mostly affects younger patients (5).

Periodontitis is a multifactor phenomenon and it has a strong relationship with generation of reactive

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oxygen species (ROS) and the destruction of the connective tissues during periodontal disease (6). In all organisms, there is a balance between antioxidants and oxidants. Antioxidants, consisting of enzymatic and non-enzymatic, prevent, limit or intercept oxidative tissue injury caused by ROS. If the balance is disrupted by physical-chemical, environmental or pathological agents, the level of oxidants outweigh the level of antioxidants and the cell will be under oxidative stress (7). During periodontitis, oxidative stress is enhanced as a result of excessive production of ROS and if immune system cannot defence enough, connective tissue and bone damage occurs. This damage leads to the formation of other systemic diseases and spreads to other organs from the mouth (8,9). Thus, early detection and control of periodontal disease is critical for the prevention of diseases that may occur in other organs.

Whole saliva is a mixture of gingival fluids and secretions of the salivary glands. Determination of the saliva components levels reflect the microbial condition and severity of periodontitis (10). Because of saliva's ready availability it is suitable for study. Thus, saliva may be used as a cost effective and noninvasive sample in determination of oxidative stress and analysis for periodontal diagnosis. We therefore evaluated and compared some oxidative stress and coagulation parameters in whole saliva of patients with CMG, CP and GAgP baseline and after non-surgical periodontal therapy.

MATERIALS AND METHODS

Study Groups

The study was approved by the Ethical Committee of Marmara University (No. MAR-YC-2009-0282).Written informed assent was obtained from patients who participated in this study.

A total of 30 adult patients, who referred to Marmara University Faculty of Dentistry, Department of Periodontology and systemically and periodontally healthy 10 person were included in the study. The study groups consisted of: CMG; non-surgical periodontal therapy applied with CMG diagnose, CP; non-surgical periodontal therapy applied with CP diagnose, GAgP; non-surgical periodontal therapy applied with AgP diagnose, Control; periodontally healthy individuals. Then, patient groups were divided into 2 subgroups: Baseline (B), 90th days after the end of the treatment (AT).

The patients in the study groups were otherwise healthy, with no history of systemic disease and consumption of anti-inflammatory or other drugs and antioxidants for at least six months. Subjects having past illness and undergoing any periodontal treatment, pregnant, lactating mothers, alcoholics and smokers were not included the study. Control subjects also had the same criteria and also did not have any history of periodontal disease.

According to research methods, periodontal treatment which includes scaling and root planing (SRP) was applied to the CMG, CP and GAgP patients. Whole saliva samples were taken at baseline and 90th days after the end of the treatment. All patients were checked once in a month in a 3 months follow up period and if necessary, oral hygiene instructions were repeated and professional dental cleaning wasdone to the patients.

Clinical Measurements and Periodontal Therapy

The periodontal status of all participants was evaluated by measurement of plaque index (PI) as developed by Löe H and Silness P (11), gingival index (GI) as developed by Silness P and Löe H (12) and pocket depth (PD) and clinical attachment loss (CAL). The periodontal examination of the study was carried out at 4 sites per tooth. PD and CAL were measured on sites of each tooth such as mesial, distal, median points at vestibular and lingual surfaces. All clinical measurements were saved at baseline and 90 days after the end of the initial periodontal therapy in CMG, CP, GAgP groups and one time point in periodontally healthy group after the collection of saliva samples.

Patients with periodontal diseases received the initial periodontal therapy, including scaling and root planing within 14 days and oral hygiene was taught to each one. All patients were checked once in a month in a 3 months follow up period and if necessary, oral hygiene instructions were repeated and professional dental cleaning was done to the patients.

Collection of Samples

Unstimulated whole saliva samples of the groups were collected in the morning following an overnight fast and the collection of the saliva was performed at the same time of the day, as much as possible. The participants were told not to drink or eat that morning before collection of the saliva. The saliva samples of the individuals were gotten in the morning while the patients were seated with the instructions to allow saliva to pool in the bottom of the mouth and drain into a tube for collection. The saliva was aliquoted into storage vials and kept in -20°C until analysis.

Determination of Glutathione

Glutathione (GSH) concentrations of samples were determined by the method of Beutler (13) and the results are expressed in % mg GSH.

Determination of Lipid Peroxidation

Yagi's method was used for determination as thiobarbituric acid reactive substances (14). Results are expressed as nmol malondialdehyde (MDA)/ml.

Determination of Glutathione-S-transferase

Glutathione-S-Transferase (GST) activities of samples are monitored at 340 nm by a spectrophotometer and results were expressed as U GST/ml.min (15).

Determination of Catalase Activity

Catalase (CAT) activities of samples were measured with the Aebi's method (16) and results were expressed as U CAT/ml.min.

Determination of Superoxide Dismutase Activity

Superoxide dismutase (SOD) activities of samples were assayed by the previously described method and results were expressed in U SOD/ml.min (17).

Determination of Tissue Factor Activity

Tissue factor (TF) activities of samples were evaluated according to Quick's method (18). The clotting time is inversely proportional to the TF activity and the lengthed clotting time is a manifestation of decreased TF activity.

Statistical Analysis

All data were presented as mean ±SD. Differences in biochemical parameters between groups were analyzed using the Mann–Whitney U-test and an unpaired two-tailed Student t test was used for comparing two independent groups. Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, California, USA).

RESULTS

Enzymatic Biochemical Parameters

The results of salivary GST, SOD and CAT activities are shown in Figure 1.

In salivary GST activity, a significant decrease was observed in the CP-AT group compared with the control group ($p<0.05$) and also in the CMG-AT group compared with the CMG-B and CP-AT group compared with the CP-B group ($p<0.05$).

A significant decrease in salivary SOD activity was detected in the CMG-AT and GAgP-AT groups compared with the control group ($p<0.05$), additionally in the CMG-AT group compared with the CMG-B and CP-AT group compared with the CP-B ($p<0.01$, $p<0.05$, respectively).

In salivary CAT activity, a significant decrease was detected in the CMG-AT, CP-AT and GAgP-AT groups compared with the control group ($p<0.05$), also in the CMG-AT group compared with the CMG-B, CP-AT group compared with the CP-B and GAgP-AT group compared with the GAgP-B group ($p<0.01$, $p<0.001$, $p<0.05$, respectively).

Nonenzymatic Biochemical Parameters

The results of saliva GSH, lipid peroxidation (LPO) levels and TF activities are shown in Figure 2.

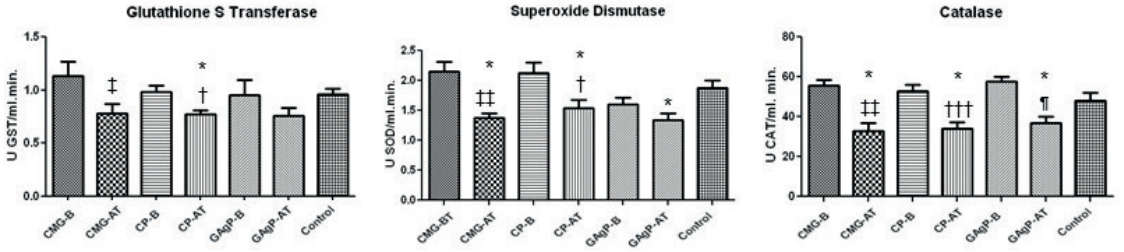

Figure 1. Enzymatic biochemical parameters in saliva. GST: Glutathione-S-Transferase, SOD: Superoxide Dismutase, CAT: Catalase, CMG-AT: Chronic Marginal Gingivitis-After Treatment, CMG-B: Chronic Marginal Gingivitis-Baseline, CP-AT: Chronic Periodontitis-After Treatment, CP-B: Chronic Periodontitis-Baseline, GAgP-AT: Generalized Aggressive Periodontitis-After Treatment, GAgP-B: Generalized Aggressive Periodontitis-Baseline. The bars represent mean ± SD for each group. *p <0.05 vr Control; ‡p<0.05, ‡‡p<0.01 vr CMG-B; †p<0.05, †††p<0.001 vr CP-B; ¶p<0.05 vr GAgP-B.

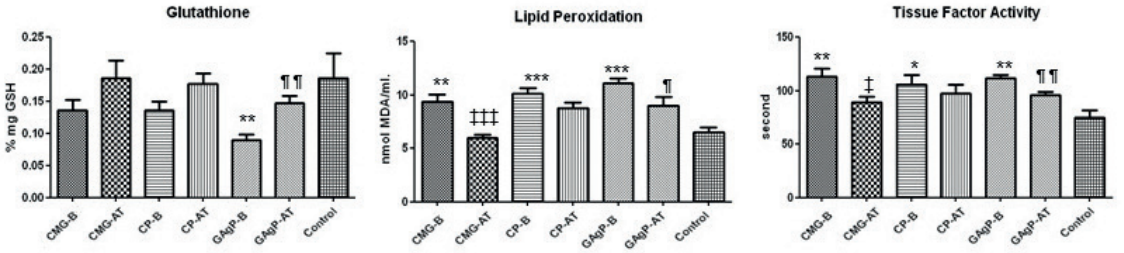

Figure 2. Nonenzymatic biochemical parameters in saliva. GSH: Glutathione, LPO: Lipid Peroxidation, TF: Tissue Factor, CMG-AT: Chronic Marginal Gingivitis-After Treatment, CMG-B: Chronic Marginal Gingivitis-Baseline, CP-AT: Chronic Periodontitis-After Treatment, CP-B: Chronic Periodontitis-Baseline, GAgP-AT: Generalized Aggressive Periodontitis-After Treatment, GAgP-B: Generalized Aggressive Periodontitis-Baseline. Since the clotting time is inversely proportional to the TF activity, the lengthening of the clotting time is a manifestation of decreased TF activity. The bars represent mean ± SD for each group. *p <0.05, **p<0.01, ***p<0.001 vr Control; ‡p<0.05, ‡‡‡p<0.001 vr CMG-B; ¶p<0.05, ¶¶p<0.01 vr GAgP-B. In salivary GSH levels, a significant decrease was detected in GAgP-B group compared with the control group ($p<0.01$), on the other hand a significant increase was detected in GAgP-AT group compared with GAgP-B group ($p<0.01$).

Significant increases in salivary LPO levels were detected in the CMG-B, CP-B and GAgP-B groups compared with the control group ($p<0.01$, $p<0.001$, $p<0.001$, respectively) while significant decreases were observed in the CMG-AT group compared with the CMG-B and GAgP-AT group compared with the GAgP-B ($p<0.001$, $p<0.05$, respectively).

Significant decreases in salivary TF activity were detected in the CMG-B, CP-B and GAgP-B groups compared with the control group ($p<0.01$, $p<0.05$, $p<0.01$ respectively). On the other hand significant increases were observed in the CMG-AT group compared with the CMG-B group and in the GAgP-AT group compared with the GAgP-B group. ($p<0.05$, $p<0.01$, respectively).

DISCUSSION

In the present study the results show that the presence of inflammation and excessive production of ROS accelerate tissue damage and actuate coagulation cascade in periodontium. Supporting periodontitis treatment by antioxidants may facilitate these damage and provides to tissue renewing itself faster.

Periodontitis is a common, chronic inflammation disease caused by infection of periodontium with especially gram negative bacteria. The response of the host is generally chronic inflammation and this response has both local and systemic inflammatory symptoms (19).

In many studies it was shown that ROS is the cause of LPO and oxidative stress in the pathogenesis of periodontal disease. As it was demonstrated by various studies that ROS trigger and/or progress of periodontal disease (6,20-22). Free radicals are capable of inducing and increasing destruction of periodontal tissue and are associated with bone resorption, also free radical induced tissue injury increased in individuals with periodontitis (23). Additionally, an increase in free radicals causes overproduction of MDA, is one of the final products of LPO in the cell. Enhanced levels of LPO was reported by previous studies with periodontitis patients (24-28). In accordance to this results, we found increased LPO levels in the CMG-B, CP-B, CP-AT, GAgP-B and GAgP-AT groups compared with the controls and significantly decreased levels of LPO in the CMG and GAgP groups after treatment compared with baseline which means treatment provides protection to cell against cellular damage via the inhibition of LPO.

Glutathione transferases are a family of detoxifying enzymes that have been shown to be over-expressed in tumor tissues and suggested as biomarkers for some specific tissues. However there are a few reports about the level and the presence of these enzymes in human saliva (29). Accordingly there are few studies focused on the activity of GST in periodontitis patients and the results are contradictory. Borges et al. found increased GST activities in periodontitis patients compared with control group while Amarnath et al. found decreased activity of this enzyme (23,30). In the present study, increased GST activities were determined in periodontitis groups, additionally a decrease was observed in treatment groups. As these enzymes are devoted to cell protection in organism, catalyzing the conjugation reaction to the center of the toxic compounds increased activities in periodontitis groups may be related with the defence mechanism of the organism.

SOD is the antioxidant enzyme which catalyzes dismutation of superoxide radicals to hydrogen peroxide. It has an important function to remove ROS from the cellular environment and avoid the cell from damaging effect of ROS. Previous studies have found increased SOD activities in periodontitis patients before treatment that support our results (21,28,30-33). CAT is also an antioxidant enzyme of the defence system in organisms and helps to detoxify hydrogen peroxide. Increased levels of CAT activities were found in previous studies in periodontitis patients in accordance with our results (21,25,28,30).

The antioxidant enzymes are protective molecules of organism which prevent ROS and they play an important role in periodontal disease by providing protection against oxidative stress. Excessive production of free radicals trigger immune system and activate antioxidant enzymes. For that reason, increased activities of GST, SOD, CAT may be a defense system of saliva against destructivity of radicals in periodontal tissues. Increased levels of GST may indicate the oxidative damage in cell and increased superoxide radicals via bacterial inflammation may induce an increase in SOD production in cell. Also, increased CAT activity in periodontitis patients may be attributed to elevated oxidative damage via ROS.

Reduced form of GSH, is a nonenzymatic antioxidant, has many functions including the removing hydroperoxides and detoxification of membranes. Similar to GST, there are limited studies about GSH in periodontitis and the results are conflincting. Panjamurthy et al. found decreased levels of GSH levels in plasma but increased levels in gingival tissue in periodontitis patients (21). Also Tsai et al. found decreased levels of GSH levels in their study which supports our result (26). This findings may suggest that immune system needs large amount of GSH for protection the periodontal tissues and GSH is consumed during inflammatory defense.

Due to the inflammation caused by periodontal pathogens, inflammatory and endothelial cells get activated and by the beginning of the inflammatory response, onset and progression of atherosclerosis is induced (34). TF is the key initiator of the coagulation cascade and has a crucial role in thrombosis. TF initiates coagulation and thrombus formation following to injury of vessel wall by a reason. Previous studies have demonstrated that periodontitis patients have tendency to atherosclerotic complications (35-37). In the present study, we found decreased activity of TF in periodontitis patients which means there is a tendency to bleeding and after treatment, an increase was observed in TF activity. Periodontal pathogens may cause defects in coagulation mechanism by progression of inflammation and damage to the veins.

CONCLUSION

In this study, we support the fact that patients with periodontitis have a tendency towards the destruction of periodontal tissues due to excessive free radical production. The severity of periodontitis may effect the immune response and the consumption of antioxidants. Thus, selection of a periodontal treatment which supports antioxidant system may be effective in preventing of other inflammatory diseases. Increased levels of radicals during periodontitis makes the organism unprotective, and therefore, an additional antioxidant treatment may be useful during periodontal treatment. Also based on the results from our study, saliva analysis to determine oxidant-antioxidant parameters in inflammatory oral diseases may be suggested as an alternative to other invasive methods.

Periodontal diseases have been recently re-classified and some descriptions to define the diseases have been changed according to the new classification report (38). But due to the present study started before the publications of these report, the descriptions were not changed.

The limited number of patients in this study may impair the validity of results. Further studies need to be conducted with large study population.

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