Superoxide Dismutase Status in Chronic Periodontitis Patients after Scaling and Root Planing and Vitamin E Supplementation

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Abstract

Context:
Aims: This study investigates the superoxide dismutase (SOD) activity levels in serum of patients with chronic periodontitis (CP). Moreover, the outcome of scaling and root planing (SRP) with and without vitamin E supplementation is evaluated in terms of improvement in periodontal parameters and SOD activity in patients with CP.

Settings and Design:
Methods and Material: Serum SOD activity in 33 patients with CP were compared with those of 9 systemically and periodontally healthy individuals (control group). At periodontal examination, serum samples were obtained. Patients with CP were randomly divided into treatment groups 1 (TG-1) and 2 (TG-2). SRP was performed for both groups, and TG-2 also received 200 mg (300 IU) vitamin E every other day. Periodontal parameters and SOD activity were evaluated after 3 months. SOD activity was determined using Marklund and Marklund technique (1974).

Results:
SOD activity in serum (P <0.05) was lower in patients with CP compared with controls. After 3 months of follow-up, SOD activity improved in both treatment groups; however, the improvement in TG-2 was higher than in TG-1 and controls, along with more improvement in periodontal parameters.

Conclusions:
In CP, systemic SOD levels are lowered. Periodontal healing as well as antioxidant defense are improved by adjunctive vitamin E supplementation.

Keywords: Antioxidants; oxidative stress; periodontitis; reactive oxygen species; scaling and root planing

INTRODUCTION

Periodontitis is an inflammatory disorder of the periodontium affecting the supporting tissues of teeth. Interaction between the pathogenic microorganism and the host defensive mechanisms is the primary etiology of the disease. One important mechanism of host defense is phagocytosis of invading
microorganisms, which involves oxidative and non-oxidative killing of the ingested microorganism. Oxidative killing leads to the formation of toxic, highly reactive metabolites such as superoxide anion (O$_2^-$), collectively known as reactive oxygen species (ROS).$^1$

The role of ROS in the tissue breakdown characteristic of periodontal disease has been widely discussed.$^{2-4}$ Excessive ROS can initiate pathologic reactions in the tissues, including: 1) lysis of the cell membrane; 2) DNA fragmentation (mutagenic response); 3) inactivation of certain proteolytic enzyme inhibitors, e.g., tissue inhibitor of matrix metalloproteinase (TIMP) and a-1 antiproteinase; and 4) activation of proteolytic enzymes such as collagenase, gelatinase, and matrix metalloproteinase (MMP), resulting in collagenolysis and degradation of specific extracellular matrix components such as hyaluronic acid and proteoglycan.$^5$ These events may be partially responsible for the periodontal breakdown in periodontitis.$^{2,4}$

Antioxidants, which are present in all aerobic cells and extracellular fluids, provide protection against ROS.$^{3,6}$ Under pathologic conditions, the balance may be tilted toward the oxidative side, generating oxidative stress. Superoxide dismutase (SOD) is known to be one of the most prominent antioxidant enzymes in the body.$^{6,7}$ SOD concentrations have been estimated in relation to periodontitis in various body fluids, with inconclusive results.$^{7,8}$ Therefore, there is a need for careful assessment of SOD levels and their association with periodontitis.

Vitamin E is a group of _8 naturally occurring tocopherols. This vitamin is essential in humans for normal reproduction, development of muscles, resistance of red blood cells to hemolysis, and a number of other physiologic and biochemical functions. It is a potent antioxidant$^{9-10}$ and has anti-inflammatory properties$^{11-14}$ as well.

Vitamin E administration has been reported to significantly improve the levels of platelet antioxidant enzymes and retard lipid peroxidation.$^{15-17}$ Furthermore, vitamin E-stimulated increase in the activities of antioxidant enzymes such as SOD has been demonstrated in patients with myocardial infarction as well as healthy controls.$^9$ The present study is designed to evaluate the effect of vitamin E administration as an adjunct to SRP on the outcome of periodontal therapy as well as on systemic antioxidant status in terms of SOD activity. This study investigates the serum SOD enzyme activity level in patients with chronic periodontitis (CP) compared with healthy individuals; it also explores the effect of periodontal treatment on systemic SOD activity level of patients with CP. Additionally, an effort is made to explore the effect of adjunctive vitamin E supplementation on SOD levels and periodontal healing in terms of improvement in clinical parameters.

**SUBJECTS AND METHODS:**

The study was conducted in the Department of Periodontics of the institute. An ethical clearance was obtained from Institutional Review Board. Participants for the study were recruited from the patients attending the outpatient department. The study, along with the risks and benefits, was explained in each patient’s language, and written informed consent was given by each patient.

Thirty three individuals were recruited in the study. Nine periodontally and systemically healthy individuals were recruited in the control group (three males and six females, aged 25 to 50 years). Control group participants had no evidence of interproximal attachment loss, no probing depth (PD) ≥ 3 mm at any site on any tooth, and whole-mouth bleeding score (from base of the sulcus) of ≤ 10%.$^4$ Of the 80 individuals assessed for eligibility, 24 patients with CP were included in the test group (eleven males and thirteen females, aged 25 to 55 years) depending on the inclusion and exclusion criteria and willingness to participate. Inclusion criteria for the test group patients were presence of at least 20 teeth and ≥ 2 interproximal sites with attachment loss ≥ 4 mm, or ≥ 2 interproximal sites with PD ≥ 5 mm, not on the same tooth.$^{18}$

Individuals meeting any of the following criteria were excluded from the study: 1) oral prophylaxis within 6 months; 2) use of vitamins, antioxidant supplements, anti-inflammatory agents, or antibiotics within 3 months; 3) regular use of mouthwash; 4) pregnant or lactating; 5) smokers (any form); 6) diagnosis of cystic fibrosis, fat malabsorption, or any form of chronic liver disease; and 7) other medical conditions that could influence the results, e.g., diabetes, rheumatoid arthritis, or other chronic inflammatory disease.

Patients in the test group were randomly assigned to one of the two treatment groups. Patients in treatment...
group 1 (TG-1) received SRP, and those in treatment group 2 (TG-2) received SRP along with oral administration of 200 mg (300 IU) vitamin E in capsule form every other day for 3 months.

Periodontal Examination

Periodontal parameters, which included plaque index (PI), bleeding on probing (BOP), PD, and clinical attachment level (CAL), were recorded. PI and GI were recorded on four sites; BOP, PD, and CAL were recorded at six sites on each tooth. All the examinations were performed at baseline for all the participants and at a 3-month recall for patients in TG-1 and TG-2.

Sampling

All the samples, before and after periodontal therapy (i.e., at baseline and 3-month follow-up), were collected 48 hours after the clinical measurements. Venous blood from an anticubital vein was collected in plain tubes without additive and was centrifuged at 3,500 x g for 5 minutes to separate serum. Serum aliquots were stored at -3°C until analysis.

Treatment

Patients in TG-1 and TG-2 received non-surgical periodontal therapy, 1 week after sample collection, in the form of full-mouth SRP using manual scalers and curettes and an ultrasonic scaler. The patients were given information on periodontal disease, and oral hygiene instructions were given at each appointment. Patients were instructed to use only mechanical methods, e.g., toothbrushes and interdental cleaning aids, during the study period; mouthwashes and antimicrobials were not prescribed. Patients in TG-2 were given vitamin E (200 mg every other day along with a meal for 3 months) adjunctive to SRP. Vitamin E was started immediately after completion of SRP. At the end of each month, patients were asked to return the remaining tablets. Compliance was estimated by counting the number of tablets remaining.

Analyses of SOD Activity

SOD activity in serum was estimated using Marklund and Marklund technique(1974).

Statistical Analyses

Differences among groups were assessed by Mann–Whitney U analysis. Comparisons of periodontal and biochemical parameters between baseline and 3 months were made using Wilcoxon signed rank test. Partial correlation was applied after adjusting for PI to determine correlation among periodontal parameters and SOD activity. All statistical analyses were two-tailed, with significance level at 0.05, and were calculated using statistical software.

RESULTS:

Nine individuals were recruited in the control group, and 24 patients with CP were randomly assigned to TG-1 and TG-2 (12 in each group). All the patients reported to 3-month follow-up. No untoward complication or undesirable response was observed in any of the participants after the treatment regimen provided.

Cross-Sectional Findings

Table 1 and 2 displays the demographic and clinical findings of the control and test groups at baseline. Biochemical parameters in control and test groups at baseline are shown in Table 3. Evaluation of the cross-sectional data revealed that SOD activity in serum (P <0.001) was markedly lower in samples from patients with CP compared with those from the periodontally and systemically healthy individuals.

Interventional Study Findings

Both TG-1 and TG-2 demonstrated significant improvement in periodontal and biochemical parameters at 3 months of follow-up compared with baseline (Table 4). However, improvement in periodontal parameters and SOD activity in serum (P <0.05) was significantly higher in TG-2 compared with TG-1 (Table 5). An intergroup comparison between TG-1 and TG-2 shows that improvement in all periodontal parameters was significantly higher in TG-2 compared with TG-1 (P <0.05 for PI, GI, PD, and CAL; P <0.001 BOP) (Table 5). The level of SOD activity in TG-2 increased to a level higher than that of the control group (P<0.05) at follow-up (Table 6).

DISCUSSION:

Oxidative stress results in the body when an oxidant–antioxidant balance is disrupted and shifts toward the oxidant side. Many systemic conditions and diseases such as diabetes, rheumatoid arthritis, ulcerative colitis, and oral lichen planus have been found to be related to oxidative stress. An indicator for measuring oxidative stress may be by estimation of ROS-related tissue breakdown products such as malondialdehyde,
total oxidant status, or antioxidant levels such as SOD or glutathione. Several authors have proven the significance of extracellular SOD in plasma and other body fluids, although intracellular SOD is the most prominent antioxidant in mammalian tissue. Earlier, SOD activity in periodontal health and disease in serum and saliva has been evaluated but with inconclusive results. Thus, the present study evaluated, first, the significance of SOD as antioxidant in serum by estimating levels in periodontally healthy individuals and patients with CP, and second, serum SOD levels along with periodontal parameters after SRP. Additionally, this study evaluated the effect of systemic supplementation of antioxidants in the form of vitamin E as adjunct to SRP on antioxidant status in patients with periodontitis.

As the level of antioxidants is affected by aging, in the present study, even distribution of age among the groups, along with a narrow range, allows us to minimize bias in the estimation of SOD. Smoking, one of the factors negatively influencing antioxidant levels, was ruled out in the study population. Sampling, storage of samples, and processing were performed according to recent recommendations. There were no specific limiting conditions, other than CP, for inclusion in the test group; thus the findings of the study are applicable to a large proportion of the population.

Evaluation of the data at baseline revealed that SOD levels in serum (P < 0.05) were significantly lower in patients suffering from CP compared with age-matched periodontally and systemically healthy individuals (Table 3), indicating a compromised oxidant–antioxidant balance in patients with CP. The results are in accordance with previous studies that demonstrated lower antioxidant activity in saliva of patients with CP compared with periodontally healthy individuals. Various authors recorded depressed levels of serum antioxidant levels in patients with CP in comparison to periodontally healthy individuals. Chronic periodontal disease is associated with peripheral neutrophils that are hyperreactive and responsible for the production of ROS in response to Fcy receptor stimulation, which explains the alteration in serum antioxidant capacity.

The test group was randomly divided into treatment groups 1 and 2. Non-surgical periodontal treatment in the form of SRP in TG-1 resulted in significant improvement in clinical as well as biochemical parameters (Table 4). The improvement in SOD levels can be attributed to reduction in the levels of inflammatory burden as a result of SRP. These results are in accordance with those of Kim et al., who also observed significant improvement in SOD levels in saliva. However, in their study the SOD levels at follow-up in the test group remained inferior to those of the control group. In the present study, the SOD levels in serum were improved and were statistically comparable to the baseline values of the healthy group.

Vitamin E is a naturally occurring antioxidant of significance. The literature provides evidence on the negative association of vitamin E levels with oxidative stress and its efficacy in the treatment of various oxidative stress–induced diseases owing to its ability to increase antioxidant levels, especially SOD. Vitamin E was assessed as an adjunct to non-surgical periodontal therapy.

The recommended dose of vitamin E for an adult is 200 to 400 IU once daily as a routine nutritional supplement. Patients in TG-2 received a moderate dose of 200 mg (300 IU) vitamin E on alternate days for the 3-month observation period. There are studies with alternate-day use of vitamin E without any untoward response.

The intragroup comparison for TG-2 at baseline and follow-up showed that all the periodontal parameters (P < 0.001) as well as SOD levels (P < 0.001 for serum) were significantly improved. Intergroup comparison between TG-1 and TG-2 demonstrated that improvement in all the periodontal parameters after SRP was significantly higher in TG-2 compared with TG-1 (Table 5). Although the contribution of better plaque control in TG-2 cannot be ignored, adjunctive vitamin E administration may have played an additional role in resolution of the periodontal inflammatory burden, which manifests in the form of better healing response in TG-2. Inhibition of biosynthesis of prostaglandins, suppression of proinflammatory cytokine production, decrease of reactive protein levels, and prevention of activation of nuclear factor kB by free radicals may be some of the properties of vitamin E that contribute toward inhibition of inflammation. Inhibitory effects of vitamin E on healing of fresh wounds in animal studies has been reported, which may be due to the
anti-inflammatory nature of the compound. Resolution of inflammation is one of the important factors influencing the outcome of non-surgical periodontal therapy. As such, the anti-inflammatory properties of vitamin E are found to be beneficial in the management of chronic inflammatory diseases.\textsuperscript{12,13}

When improvement in SOD levels was compared between TG-1 and TG-2, SOD serum demonstrated a significant difference which may be partially due to the resolution of periodontal inflammation and partially because of the additional antioxidant effect of vitamin E. Known to be a lysosomal stabilizer, Vitamin E is a major lipophilic antioxidant. It prevents lipids peroxidation and results in improved stability and integrity of biologic membranes. Stable biologic membranes inhibit release of ROS,\textsuperscript{14} thus lowers the oxidant status and also prevents the damage caused by ROS. This includes prevention of DNA degradation and cell lysis, inactivation of tissue degradation enzymes, and activation of inhibitors of tissue degradation enzymes.\textsuperscript{29} Results of this study in terms of increase in the levels of serum SOD agree with those of Dwivedi et al.,\textsuperscript{9} who demonstrated a significant increase in the activities of antioxidant enzymes, including SOD, in patients of myocardial infarction as well as healthy individuals after vitamin E administration compared with no vitamin E.

Assaying other components of lipid peroxidation and the antioxidant network would have improved the quality of the study as components of the antioxidant system work in synergy and not in isolation.\textsuperscript{16} However, there are studies that demonstrate a concomitant rise in the levels of glutathione peroxidase and catalase activity along with SOD activity following vitamin E administration.\textsuperscript{15,17} Thus, rather than the SOD component alone, the basis of effectiveness of adjunctive vitamin E supplementation is the total antioxidant system working in harmony. Moreover, the literature also provides evidence of reduction in the levels of lipid peroxidation after vitamin E administration\textsuperscript{15,16} and negative correlation between peroxidation of lipid and SOD activity.\textsuperscript{30} Therefore these results must be comprehended as one of the pioneer findings that open a gateway for future research involving assays of other markers of lipid peroxidation and antioxidants.

CONCLUSIONS

Vitamin E may be effective as an adjunct to SRP in the management of periodontitis and in improving systemic antioxidant status. Possible recommendation of vitamin E supplementation with routine SRP can be considered only after the validation of these preliminary findings by multiple studies with larger sample sizes.

ACKNOWLEDGEMENT:

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REFERENCES:


7. Akalin FA, Toklu E, Renda N. Analysis of superoxide dismutase activity levels in gingiva and gingival crevicular fluid in patients with chronic...


TABLES:

Table 1

DEMOGRAPHIC OF THE STUDY POPULATION

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TOTAL NO. OF PATIENTS</th>
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<td>TEST</td>
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<td>25-55</td>
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Table 2

CLINICAL PARAMETERS FOR CONTROL AND TEST GROUP AT BASELINE

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<th>TEST GROUP (n=24)</th>
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<td>GI</td>
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<td>BOP</td>
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<td>PD (mm)</td>
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<tr>
<td>CAL (mm)</td>
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Table 3

BIOCHEMICAL PARAMETER FOR CONTROL AND TEST GROUP AT BASELINE

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<tr>
<td>SOD (U/ml)</td>
<td>3.15</td>
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Table 4
PERIODONTAL AND BIOCHEMICAL PARAMETERS AT BASELINE AND AFTER 3-MONTH FOLLOW-UP

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<th>P VALUE</th>
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<th>P VALUE</th>
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<td>BASELINE</td>
<td>FOLLOW-UP</td>
<td>P VALUE</td>
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<td>FOLLOW-UP</td>
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<td>CAL (mm)</td>
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<td>SOD (U/ml)</td>
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<td>2.33</td>
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Table 5
IMPROVEMENT IN PERIODONTAL AND BIOCHEMICAL PARAMETERS

<table>
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<th>TG-2</th>
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<td>CHANGE IN CAL (mm)</td>
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<td>&lt;0.05</td>
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<tr>
<td>CHANGE IN SOD (U/ml)</td>
<td>0.7</td>
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Table 6
COMPARISON OF BIOCHEMICAL PARAMETER OF TG-1 AND TG-2 WITH CONTROL GROUP AT FOLLOW-UP

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<th>PARAMETER</th>
<th>CONTROL GROUP(n=9)</th>
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<th>TG-2 (n=12)</th>
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<tr>
<td>SOD (U/ml)</td>
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<td>2.95</td>
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