ORIGINAL RESEARCH

Collocation of Malondialdehyde and Superoxide Dismutase Levels in Chronic Periodontitis and Pruning them Post-scaling and Root Planing Coupled with Vitamin E Supplementation

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Abstract

Aim: The purpose of this study is to determine the effect of vitamin E supplementation on malondialdehyde (MDA) and superoxide dismutase (SOD) status post-scaling and root planing (SRP) in patients with chronic periodontitis.

Materials and methods: A total of 80 randomly selected chronic periodontitis patients were classified into two groups (40 each), group I and group II. Those in group I underwent SRP, whereas those in group II underwent SRP along with orally administered 400 mg of vitamin E every other day for 3 months. Gingival index (GI), probing pocket depth (PPD), and clinical attachment level (CAL) were recorded. A baseline examination and a 3-month recall were conducted for all the patients. Serum, saliva, and gingival crevicular fluid (GCF) were collected at baseline and 3 months posttreatment.

Results: The results of the study showed that oxidative stress is more in chronic periodontitis patients. A reduction in CAL and PPD between group I and group II was observed after 3 months. However, it was not statistically significant. Post-periodontal therapy, along with vitamin E supplementation, showed that salivary and GCF MDA levels showed significant reduction at 3 months, whereas serum MDA levels did not. Salivary and serum SOD values showed significant *p* value after, whereas there was an increase in SOD levels in GCF, which was not statistically significant.

Conclusion: Significant changes in MDA and SOD levels were noted after orally administered vitamin E supplementation. Larger sample sizes along with longitudinal studies were required in different reveries of chronic periodontitis.

Clinical significance: The MDA levels were increased in chronic periodontitis, whereas SOD levels were lowered in chronic periodontitis. Conjugation of vitamin E supplementation improves periodontal healing as well as antioxidant defense.

Keywords: Lipid peroxidation, Malondialdehyde, Oxidative stress, Superoxide dismutase, Vitamin E.

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Introduction

One of the widespread human microbial diseases is considered to be periodontitis. The primary etiology is considered to be the interaction between the pathogenic microorganism and host defensive mechanisms.[1,2] The mechanism of host defense is phagocytosis of invading microorganisms by the oxidative or non-oxidative killing of the ingested microorganism. Oxidative killing forms highly reactive toxic metabolites such as superoxide anion (O_2-), hydroxyl radical, and hydrogen peroxide, collectively known as reactive oxygen species (ROS).[3] Reactive oxygen species include not only oxygen-free radicals but also non-radical oxygen derivatives involved in oxygen radical production.

Tissue damage as a result of an alarming rise of ROS levels causes cell membrane lysis and fluctuation of proteolytic enzyme inhibitors either by hyperactivation or hypoactivation. "Oxidative stress" is an expression used to describe various deleterious processes resulting from an imbalance between the excessive formation of ROS and/or reactive nitrogen species and limited antioxidant defenses. While small fluctuations in the steady-state concentration of these oxidants lead to free radical-mediated chain reactions which indiscriminately target proteins, lipids, polysaccharides, and DNA.

The idea that ROS are associated with the pathogenesis of a variety of inflammatory diseases and have a role in tissue damage has become a major area of research. Supporting evidence for their role in tissue damage is often inadvertent and circumstantial. Halliwell's postulates, those being the criteria required to be fulfilled before ROS can be concluded to be key mediators of tissue injury.[4]

Lipid peroxidation (LPO) is one of the major outcomes of ROS-induced tissue damage. When ROS interacts with lipoproteins, it causes disruption of the cell structure, thereby altering the function of the cells leading to oxidative stress. Lipid peroxidation is more commonly and often studied in terms of MDA. Malondialdehyde

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which is the end product of LPO, can be used to assess oxidative stress levels in various body fluids such as saliva, serum, and GCF.

Antioxidants provide protection against ROS. Interactions between antioxidants and oxidants are necessary for homeostasis. When the oxidants level increases, oxidative stress is generated.[5] Superoxide, oxygen radical that is released in inflammatory pathways and connective tissue breakdown are suppressed by SOD, a prominent antioxidant enzyme. It acts as a homeostatic mechanism in intracellular and extracellular compartments.[6] Superoxide dismutase was estimated in various body fluids such as saliva, serum, and GCF for they are associated with periodontitis.

Vitamin E is a group of naturally occurring tocopherols. It is essential in human beings for reproduction, development of muscles, resistance of red blood cells to hemolysis, etc. Vitamin E administration has been significant in improving the levels of antioxidant enzymes. This balances the prooxidant and antioxidant levels and could lower the oxidative stress; when used along with SRP, vitamin E could play a role in reducing free radicals, oxidative stress, and inflammation.[7]

Currently, literature shows a lot of emphasis on the role of ROS, LPO products, antioxidant systems, and oxidative stress in the pathology of periodontal disease. There is a variation in the levels of these products in various diseases, and has been used as a marker. The present study aims to explore the effect of SRP with vitamin E supplementation as an adjunct in salivary, GCF and serum SOD, and MDA levels in patients with generalized chronic periodontitis.

Materials and Methods

A total of 80 individuals belonging to both the sexes and aged between 30 and 45 years with generalized chronic periodontitis were recruited from the outpatient pool of the Department of Periodontology, Thai Moogambigai Dental College and Hospital, Chennai. An ethical committee involving the institutional review board assessed the study protocol and cleared it. Patients who participated in the study also gave written consent before taking part in the study.

Patients with at least 20 teeth, having CAL ≥4 mm, 5 mm of PPD, or two sites interproximally with 5 mm PPD and GI ≥2 were included in this study. Patients who (1) Had oral prophylaxis 6 months prior to the trial, (2) Were using any vitamins supplements or any other medication, (3) Were using mouthwash regularly, (4) Were pregnant or lactating, (5) Had any form of habits, and (6) Had any medical conditions were excluded from the study as it would have a negative impact on the results.

The study population was divided into two groups (40 each), group I and group II. Patients in group I received SRP, and those in treatment group II received SRP along with orally administered vitamin E, 400 mg (300 IU) in capsule form every other day for 3 months.

Clinical attachment level, GI, and PPD were documented at baseline and at 3 months recall. Gingival index was recorded on four sites, while PPD and CAL were recorded at six sites of each tooth.

At baseline and after 3 months post nonsurgical therapy, saliva, serum, and GCF samples were collected. Disposable, sterile, and clean tubes were used for collecting unstimulated whole saliva (2 mL) and were immediately centrifuged to remove cell debris. Venous blood (3 mL) from an antecubital vein was collected in plain tubes without additive and was centrifuged at 3500 rpm for 5 minutes to separate serum. Gingival crevicular fluid was collected using micropipettes.

Malondialdehyde levels in the saliva, GCF, and serum were estimated as per the method of Biochemist Jayasekharan.[8] Around 2.5 mL of trichloroacetic acid was taken and mixed with 0.5 mL of sample (saliva, serum, and GCF). The contents were mixed well and incubated for 15 minutes at 90°C. The sample tubes were cooled with cold water. The contents were centrifuged at 3000 rpm for 10 minutes. A total of 2 mL of supernatant was transferred to a new tube. To this, 1 mL 0.675% thiobarbituric acid was added. The tubes were sealed and incubated at 90°C for 15 minutes. And the content was measured at 586 nm using microplate reader (Thermo Fisher Scientific Oy, FI-01,621 Vantaa, Finland). The concentration of MDA levels in the samples were expressed as µg/dL.

Marklund and Marklund method was used to assay SOD activity to which 0.25 mL ethanol and 1.25 mL chloroform were added to 1 mL of the sample (saliva, serum, and GCF) and was placed in a mechanical shaker for 15 minutes, followed by centrifugation.[9] To 0.5 mL of the supernatant solution, 2.0 mL of 0.1 M Tris-HCl buffer pH 8.2; 1.5 mL of distilled water, and 0.5 mL of pyrogallol were added. Optical density was read at 420 nm in a spectrophotometer at 0, 1, and 3 minutes (Shimadzu, UV-1700 PharmaSpec). Control tubes containing 0.5 mL distilled water were also treated in a similar manner against a buffer blank. A total of 1 mg protein was the unit used to express the enzyme activity. The amount of enzyme needed to bring about 50% inhibition of pyrogallol autoxidation corresponded to one enzyme unit.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) (IBM Corporation, Chicago, Illinois, USA) software program version 16 was used to perform statistical analysis. Pearson's correlation was used to analyze the clinical parameters with MDA levels in GCP patients after SRP with vitamin E supplementation. Intergroup comparison was done using paired *t*-test.

Results

In comparison of the demographic variables, the mean age of the participants in group I was 43.35 and group II was 45.75, which showed no statistically significant difference. Group I included 19 males (47.5%), 21 females (52.5 %) and group II included 22 males (55%), 18 females (45%).

In intragroup comparison of salivary and GCF MDA levels in group II between baseline and 3 months, a statistically significant difference was found. However, serum MDA levels did not show any significant difference ($p < 0.28$) between baseline and 3 months (Table 1).

When GI values were compared between baseline and 3 months after SRP in group II, a statistically significant difference was seen ($p < 0.001$). However, PPD and CAL values did not show statistical significant differences between baseline and 3 months, with a *p*-value of 0.681 and 0.712, respectively (Table 2).

When GI and PPD values were correlated with GCF, saliva, and serum MDA levels in group II, there was a statistically significant correlation. When CAL values were correlated with saliva and serum MDA values in group II patients, there was no correlation (0.280 and 0.445, respectively). However, there was a significant correlation with GCF MDA value ($p = 0.037$) (Table 3).

In intergroup comparison of salivary SOD levels between baseline and 3 months in group I and group II, a statistically significant difference was seen ($p < 0.010$). However, presence of

SOD in GCF did not show any significant results when compared between the groups ($p < 0.250$). On comparison of serum SOD levels between baseline and 3 months, the difference was statistically significant ($p < 0.010$) (Table 4).

On comparison of GI values between saliva, serum, and GCF SOD levels in group I and group II patients, the values were statistically significant. However, PPD and CAL values between GCF, saliva, and serum SOD levels were not statistically significant ($p = 0.640$ and $p = 0.726$), respectively (Table 5).

When GI and PPD values were correlated with GCF, saliva, and serum SOD values in group II, it was statistically significant. However, CAL values did not show statistical significance in correlation with GCF, saliva, and serum SOD levels ($p = 0.620$, 0.311, and 0.169) (Table 6).

Table 1: Malondialdehyde levels in patients with generalized chronic periodontitis after SRP with vitamin E supplementation (group II) at baseline and 3 months (group II)

	Baseline				*3 months*				
Group II	*Mean*	*SD*	*Median*	*IQR*	*Mean*	*SD*	*Median*	*IQR*	*p-value*
Saliva (µg/dL)	2.265	0.28702	2.3538	0.5077	1.5107	0.29519	1.6153	0.5538	0.001
GCF (µg/dL)	1.158	0.12123	1.1692	0.2462	0.8830	0.14359	0.8615	0.2000	0.002
Serum (µg/dL)	0.526	0.11537	0.5076	0.2385	0.4697	0.17238	0.4923	0.1846	0.28

Table 2: Comparison of clinical parameters and malondialdehyde levels in generalized chronic periodontitis after SRP with vitamin E supplementation (group II)

	Group II	*N*	*Mean*	*p-value*
GI	SRP + vitamin E at baseline	40	1.63 ± 0.8	<0.001
	At 3 months	40	0. 62 ± 0.54	
PPD	SRP + vitamin E at baseline	40	3.66 ± 0.19	0.681
	At 3 months	40	3.43 ± 0.55	
CAL	SRP + vitamin E at baseline	40	2.76 ± 0.11	0.712
	At 3 months	40	2.53 ± 0.61	

±, standard deviation; N, number; *p*-value, probability value; Significant at the level of 0.01

Table 3: Correlation of clinical parameters with malondialdehyde levels in generalized chronic periodontitis patients after SRP with vitamin E supplementation (group II)

		Saliva	*GCF*	*Serum*
GI	Pearson's correlation	0.638**	0.611**	0.318**
	Sig. (two-tailed)	0.000	0.000	0.004
	N	80	80	80
PPD	Pearson's correlation	0.557**	0.531**	0.226*
	Sig. (two-tailed)	0.000	0.000	0.044
	N	80	80	80
CAL	Pearson's correlation	0.122	0.234*	0.087
	Sig. (two-tailed)	0.280	0.037	0.445
	N	80	80	80

*Correlation is significant at the 0.05 level (two-tailed); **Correlation is significant at the 0.01 level (two-tailed); N, number; Sig., significance

Table 4: Comparison of salivary, serum, and GCF SOD levels between group I and group II after 3 months

	Group	*No. of patients*	*Mean*	*SD*	*p-value*
Salivary SOD	I	40	43.33	6.66	0.010
	II	40	63.0	7.94	
Serum SOD	I	40	72.33	4.04	0.010
	II	40	86.0	5.29	
GCF SOD	I	40	20.33	2.52	0.250
	II	40	24.63	1.52	

p-value, probability value; Significant at the level of 0.01

Table 5: Comparison of clinical parameters in generalized chronic periodontitis patients after SRP with vitamin E supplementation between group I and group II

	Group	*N*	*Mean*	*p-value*
GI	I	40	1.13 ± 0.83	<0.001
	II	40	0.26 ± 0.45	
PPD	I	40	3.26 ± 0.79	0.640
	II	40	3.13 ± 0.75	
CAL	I	40	2.46 ± 0.51	0.726
	II	40	2.43 ± 0.51	

±, standard deviation; N, number; *p*-value, probability value; Significant at the level of 0.01

Table 6: Correlation of clinical parameters with SOD values in generalized chronic periodontitis patients after SRP with vitamin E supplementation (group II)

		GCF	*Saliva*	*Serum*
GI	Correlation	–0.535**	–0.823**	–0.794**
	Sig. (two-tailed)	0.000	0.000	0.000
	N	80	80	80
PPD	Correlation	–0.334**	–0.483**	–0.307
	Sig. (two-tailed)	0.035	0.002	0.054
	N	80	80	80
CAL	Correlation	0.081	0.164	0.222
	Sig. (two-tailed)	0.620	0.311	0.169
	N	80	80	80

** correlation significant in value 0.05; N, number of samples; Sig., significance; Significant at the level of 0.05

The present study results showed that there was a significant change in MDA levels and a significant increase in SOD levels in group II patients after vitamin E supplementation.

Discussion

Diseases of periodontal tissues are among the most widespread inflammatory disorders. Currently, there has been extensive emphasis on the oxidant and antioxidant system in periodontal etiology. One of the reasons could be the lack of proportion between oxidants and antioxidants.[10,11] Reactive oxygen species play a role in tissue breakdown, but in excess initiate various pathologic reactions. They are toxic not only to internalized microbial agent but also to the extracellular structure and induce LPO having an effect on cells. Redundant production of LPO can result in oxidative stress and consequently damage cell integrity. Because LPO results from oxidative stress, numerous markers have been used to monitor this process. Malondialdehyde is the principal and most studied biomarker that results from the decomposition of unstable peroxides derived from polyunsaturated fatty acids and can indicate oxidative stress. They are known to contribute to numerous pathogenic processes like periodontitis. The results from the present study revealed that MDA levels were significantly increased in periodontitis patients, which were similar to the study done by Dhotre et al. and Khalili and Biloklytska.[12,13]

The removal of ROS by antioxidant defense systems is a must for a healthy aerobic lifestyle. All mammalian cells contain antioxidants that prevent or limit injury to the cell. They are released locally at the site of inflammation and protect against oxidative stress. The most significant oxidant is SOD which catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen. The current study was done in an effort to explore the effect of adjunctive vitamin E supplementation on SOD levels and periodontal healing. Vitamin E stimulated an increase in the activities of antioxidant enzymes such as SOD. Superoxide dismutase has been demonstrated in patients with myocardial infarction as well as healthy controls. In the present study, group I received SRP and those in group II received SRP along with oral administration of 400 mg of vitamin E in capsule form every other day for 3 months. Saliva, serum, and GCF samples were collected at baseline and 3 months, and were evaluated for SOD level. Between baseline and 3rd month salivary and serum levels showed an increase which was statistically significant in group II ($p < 0.010$). However, the presence of SOD in GCF did not show any significant result when compared between baseline and 3 months in group II ($p < 0.250$). This was in accordance with the study by Ellis et al.[14] Wei et al. reported that SRP controlled and restored the subject's LPO levels and SOD levels in serum, saliva, and GCF.[15] Removal of oxidative stress restores a normal SOD activity. Vitamin E is fat-soluble and thus not secreted much in GCF; the additional antioxidant effect of vitamin E could be appreciated in saliva and serum compared to GCF. This could be one of the reasons for the nonsignificant value in GCF. Vitamin E administration has been reported to significantly improve the levels of antioxidant enzymes and retard LPO.[16]

Vitamin E is a prominent antioxidant enzyme and is known to improve levels of platelet antioxidant enzymes and retard LPO. Vitamin E is a major lipophilic antioxidant that is known to be a lysosomal stabilizer. It prevents the peroxidation of lipids and improves and stabilizes the biological membranes. Stable biological membranes could inhibit the release of ROS, thus lowering the oxidant status and could prevent the damage caused by free radicals. Vitamin E being one of the eight naturally existing tocopherols, is a necessary factor for muscle development, RBC resistance to hemolysis, reproduction, and a number of other physiological and biochemical functions. The antioxidant behavior of vitamin E occurs because of oxidation of a single phenolic OH group by giving rise to vitamin E (tocopheryl) radical. Singh et al.[11] reported on the

use of vitamin E along with SRP in treating periodontal diseases. Micronutrients, vitamins, and antioxidants play an essential role in coping with oxidative stress and also in adequate immune response.

Removal of oxidative stress restores the normal SOD activity. There was a significant increase in SOD levels in group II, which could be due to the additional antioxidant property of vitamin E. Results of this study in terms of increase in levels of serum and salivary SOD were comparable to Dwivedi et al.,[17] who showed a significant improvement in antioxidant enzyme activity in patients having myocardial infarction as well as healthy individuals after vitamin E administration. The contribution of vitamin E could be because of its varying properties, including inhibition of biosynthesis of prostaglandins, suppression of proinflammatory cytokines, and prevention of activation of signaling pathways by free radicals. Ehrlich et al.[18] reported on the inhibitory effect of vitamin E on the healing of wounds. Because moderate inflammation is mandatory for providing necessary chemical mediators for wound healing in fresh wounds, inhibition may lead to poor healing. However, chronic periodontitis being a chronic lesion with established inflammation and proinflammatory mediators, overpower the abundant anti-inflammatory reparative mediators responsible for the clinical presentation of the disease. Results showed that the anti-inflammatory properties of vitamin E proved to be beneficial in healing following SRP.

The additional antioxidant effect of vitamin E could not be appreciated so well in GCF when compared to saliva and serum.[19] In the present study, smokers were excluded as smoking could have an impact on SOD levels.[20–22] Negative correlation was observed between GCF, saliva, and serum SOD levels with GI, PPD, and CAL. This was because SRP decreased oxidative stress and increased antioxidant levels. There was an improvement in the clinical parameters, which could be due to a decrease in bacterial load, which in turn could have decreased the inflammation. Vitamin E could have added the benefits as an adjunct. Thus, the data from the present study suggested that it is reasonable to prescribe vitamin E supplementation as an adjunct to nonsurgical therapy in the management of periodontal disease.

A small study population was taken in the present study to generalize the results. The study should have involved more participants at different levels, and this could be one of the limitations of the study. The long-term follow-up of assessment of vitamin E supplementation might have also improved the quality of the present study.

Conclusion

The current study suggested that vitamin E as an adjunct to SRP improved oxidative stress when compared to SRP alone. It could be used as an adjunct to SRP in improving the systemic antioxidant status. Further longitudinal studies with a larger sample size could be helpful to substantiate the role of vitamin E supplementation in periodontal health and disease.

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