ORIGINAL RESEARCH

Clinical and Biochemical Valuation of Enzymatic and Nonenzymatic Stress Markers Following Full-mouth Disinfection in Aggressive Periodontitis

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Abstract

Introduction: Assessment of host response to inflammation will throw light on the critical role of antioxidants (AOs) and free radicle damage in the etiology of periodontal disease. The purpose of the study was to assess the level of plasma oxidative stress in those having aggressive periodontal disease before and after full-mouth disinfection. Objectives were to find the influence of full-mouth disinfection analyzing the level of thiobarbituric acid reactive substances (TBARSs), thereby quantifying the lipid peroxidation (LPO) and also the activities of reduced glutathione (GSH), glutathione peroxidase (GP_X), and catalase (CAT), valuing the AO defense systems in health and disease.

Materials and methods: The valuation composed of 30 subjects with aggressive periodontal disease and 30 healthy controls. Clinical assessment included following periodontal parameters: plaque index (PI), papillary bleeding index (PBI), probing pocket depth (PPD), and clinical attachment level (CAL). Levels of bone loss were assessed by taking full-mouth periapical radiographs. Initial periodontal therapy comprises of full-mouth disinfection which includes subgingival scaling and root planing within 24 hours combined with adjunctive chlorhexidine chemotherapy for aggressive periodontitis subject's at sites indicated. The parameters (clinical) were evaluated at the baseline and 8 weeks after initial periodontal therapy at six sites of teeth indicated. Plasma samples were taken and evaluated by standard procedures as defined in the literature. All the values were weighed and related.

Results: Strong positive associations were detected among periodontal parameters and TBARS, enzymatic/nonenzymatic AO levels ($p < 0.05$), and pre- and postperiodontal management. The plasma levels of patients with aggressive periodontitis had high levels of TBARS and displayed a substantial escalation in the activities of GSH and GP_X levels in the plasma matched to the healthy individuals ($p < 0.05$).

Conclusion: This paper evaluated ROS activity and AO defense before and after treatment to stimulate added periodontal investigation in this part which will give an insight into the therapeutic options with foreseeable results.

Keywords: Aggressive periodontitis, Antioxidants, Biomarkers, Full-mouth disinfection.

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Introduction

Periodontal disease is commenced by settlement of pathogenic bacteria followed by exaggerated inflammatory response resulting in alveolar bone loss.[1] Genetic makeup impact in aggressive periodontitis makes it a unique entity among the various disease patterns which affects periodontium.[2] The rapidly progressive form of destruction which varies with the host response in otherwise healthy individuals is other oddity.[3] As the return to normalcy is unpredictable, it has become clear that there is a dynamically synchronized progression comprising of numerous biochemical pathways, enzymes, and mediators in the pathogenesis of aggressive pattern of disease.[4] Etiopathogensis of periodontal disease relies upon host–bacterial interaction. Polymorphonuclear neutrophils (PMNs) form the first line of cellular host responses counter to bacteria in the gingival sulcus. Numerous studies have pointed out the part played by oxidative stress for the chronic local activation of periodontal inflammation and tissue destruction.[5,6] Ample research has demonstrated that in localized aggressive periodontitis, neutrophils are hyperactive, primed, and seem to discharge enhanced levels of oxygen radicals and inflammatory mediators such as cytokines and matrix-degrading enzymes.[7,8] These hyperresponsive neutrophils are well-thought-out as one of the major causes for the tissue damage noticed in an aggressive form of disease. This hyperactivity and reactivity of neutrophil damages the neighboring host tissues and backs the destructive alterations witnessed in inflammatory periodontal disease.[9] Even though the contribution of ROS to tissue destruction has been identified, the role of AOs in this sequence remains hazy.[10,11]

In research studies, biomarkers can serve as a stand-in measure of a disease process.[12] Biomarkers have a certain level of prognostic strength, but complete authentication of this relation is deficient.[13]

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Apart from cell signaling and metabolic processes, ROS play a significant part in causing a variety of inflammatory disorders. The oxidative reactions are deleterious to health so every organism possesses a range of enzymatic and nonenzymatic systems to safeguard against them.[14] On one hand, logical usage of biomarkers of oxidative stress to clinical trials and observational studies of AOs are a few of the challenges that need to be overcome to understand about the influences of reactive species in the periodontal disease. On the other hand, without gauging the factors important to denote the prominence of AO defense and oxidative stress, it will be difficult to define the selection, dose, and duration of an AO intervention that will achieve its intended biochemical or physiological endpoint or to identify the enrolled subjects that were even present with oxidative stress.[15–17] Among various potential biomarkers, DNA, protein, and lipid oxidation products are of paramount importance but our knowledge pertaining to their role in tissues and body fluids need more explanation.[18]

The neutrophil activation, xanthine oxidase activation, and arachidonic acid metabolism form the main sources of ROS which up to a threshold concentration modulate normal physiologic functions like cell growth, adhesion, differentiation, and protection.[19,20] During pathologic condition, ROS exceeds a threshold level causing adverse effects directly or by influencing the effectors of different signaling trails. The human body is equipped with commanding AO defense mechanism that counteracts oxidative damage in the form of enzymatic and nonenzymatic systems such as superoxide dismutase (SOD), CAT, GP_X, uric acid, ascorbic acid, glutathione, lipoic acid, carotenoids, vitamin E, and ubiquinol.

Upsurge in the making of the oxidants and/or reduction in shielding AOs symbolizes oxidative stress. Unbalanced equilibrium between various forms of ROS can injure periodontium by diverse pathways comprising of lipid membrane peroxidation, stimulation of cytokine production, and protein inactivation.[21,22] LPO begins when the reactive oxygen species (ROS) intermingle with polyunsaturated fatty acids in lipoproteins or the cell membranes; as a result, lipid peroxides are produced. Cellular integrity gets compromised when there is frenzied production of lipid peroxides resulting in oxidative stress. As the LPO is an aftermath of the oxidative stress, monitoring this process is of great diagnostic value. The quantifiable products were the levels of GP_X, CAT, and GSH which depict the AO level and TBARS, a quantification of ROS damage. For cell homeostasis, the enzymatic AOs such as SOD, CAT, GP_X, nonenzymatic AOs, and GSH vitamin E act in tandem with one another to nullify the effects of LPO. So the purpose of the study was to estimate the level of plasma oxidative stress in healthy and in aggressive periodontitis patient's pre- and post phase 1 periodontal management. Objectives were to find the effects of initial periodontal treatment analyzing the prominence of LPO (as the level of TBARS) and the AO defense systems (as the levels and/or activities of GSH, GP_X, and CAT) in health and disease.

Materials and Methods

Experimental Design

Sixty female subjects with an age range of 18–45 years were included in the study, 30 being aggressive periodontitis patients and 30 being periodontally healthy controls. The subjects were selected among individuals who were given a reference to a private dental specialty center from the general oral health awareness camp, due to periodontal complications or for routine controls. The subjects diagnosed with aggressive periodontitis[23] formed the case group, while the control group composed of individuals with healthy periodontal conditions.[24] The inclusion criteria for the study were those received no periodontal treatment, systemic disease, nor taken anti-inflammatory, antibiotics, or other drugs in the last 6 months, nonsmokers, not consumed alcohol, or AO vitamin users and not going through menopause, menstruation, pregnancy, or lactation at the time of the study. Participants belonged to a similar geographic area, with matching socioeconomic status and had comparable nutritional practices. Ethical clearance was acquired from the institutional Ethical Committee. The study was conducted in full agreement with the ethical principles of the World Medical Association Declaration of Helsinki. The subjects were well versed about the study and their agreement was acquired.

Clinical Measurements

The periodontal status of all subjects was spotted by measuring PI,[25] PBI,[26] PPD,[27] and CAL,[28] and levels of bone loss were assessed by taking full-mouth periapical radiographs. PPD and CAL were measured on six sites of teeth (mesial, median, and distal points at buccal and palatal aspects) using the Williams periodontal probe (Hu-Friedy, Chicago, IL, USA). The same investigator carried out all calibrations to reduce bias. Initial periodontal therapy included oral hygiene education and motivation followed by full-mouth disinfection which included scaling and root planing within 24 hours combined with adjunctive chlorhexidine chemotherapy for aggressive periodontitis subject's at sites indicated.[29] The above-mentioned clinical parameters were assessed at the baseline and 8 weeks after initial periodontal therapy at six sites of all teeth.

Collection of Samples

Blood samples were collected before the periodontal examination at the baseline and 4 weeks after initial periodontal therapy. A fasting blood sample (5 mL of venous blood) was collected from the antecubital fossa by venipuncture using a 20-guage needle with a 5 mL syringe into which an anticoagulant is added and transported within 1 hour in an ice bath container to the research laboratory. Additionally, 0.5 mL of the whole blood sample was added to tubes containing 0.5 mL of 10% TCA, for GSH estimation.

Plasma Separation

The blood samples were then subjected to centrifugation at a speed of 3,500 rpm for 10 minutes to separate plasma. The plasma separated is transferred to a vial and stored in ice till the completion of analysis.

Biochemical Assays

Lipoperoxidation Assay

The level of TBARS consequential from endogenous lipoperoxides, reflecting the status of the LPO process, was assayed colorimetrically at 530–535 nm in the plasma as described by Ohkawa et al.[30]

Assessment of Nonenzymatic AO

The level of GSH was measured by the method of Moron et al., and the color developed was measured at 412 nm.[31]

Assessment of AOs Enzymes

The method described by Sinha was employed to measure the CAT activity[32] and assayed colorimetrically at 570 nm in the plasma.

The activity of GP_X was assayed by the method of Rotruck et al.[33] and was read at 420 nm.

Statistical Analysis

The results were conveyed as mean ± SEM. Statistical differences between groups were determined by an independent Student *t* test analysis. For all analyses, $p < 0.05$ was used to assess overall differences.

Results

Table 1 depicts a comparison of periodontal parameters between case (group I) and control (group II). There is a significant difference in the parameters being checked. The level of plaque accumulation and gingival status was deteriorated in group I. The level of destruction as clinically noted through PPD and CAL was 7.03 ± 0.45 mm and 6.94 ± 0.23 mm in group I, respectively, whereas, in group II, it appeared within normal limits.

Table 2 depicts that the plaque score was 2.89 ± 0.12 before treatment and 1.23 ± 0.34 after treatment in group I. The PPD was 7.03 ± 0.45 mm before periodontal therapy and was 5.45 ± 1.56 mm after treatment. Similarly, the CAL was 6.94 ± 0.23 mm before treatment and was 4.93 ± 1.56 mm after treatment. Reduction in probing depth and gain in attachment were noticed after the periodontal therapy.

Table 3 shows the comparative analysis of levels of oxidative stress and enzymatic and nonenzymatic AOs between groups I and II. In group I, the baseline level of TBARS was 10.22 ± 1.32 nmol/mg and, in group II, it was 5.55 ± 0.90 nmol/mg suggesting a high level of oxidative stress in the aggressive periodontitis state. Both enzymatic and nonenzymatic levels are mentioned in Table 3 for groups I and II.

In Table 4, the score for TBARS after treatment in group II was 6.23 ± 1.56 nmol/mg suggesting a reduction in the oxidative stress level. Similarly, analysis of all biochemical assays pre- and posttreatment is shown in Table 4. All the level except for GSH showed a reduction in the value following treatment.

Discussion

This research considered parameters of oxidative stress and AO mechanism in the plasma on a cohort of female patients with aggressive periodontal disease equated to corresponding control groups. A few studies have been reported regarding the predisposition of periodontitis patients to the deleterious effect of ROS in the periodontium with regard to an imbalance between oxidants and AOs.[10] The attention was on LPO and AO mechanisms (both enzymatic and nonenzymatic) during diseased state and the effect of full-mouth disinfection on these factors. Several approaches are possible to establish the association of oxidative stress in the pathophysiologic mechanisms of periodontitis. Boosted ROS generation by peripheral neutrophils in both chronic and aggressive forms of periodontal disease has been shown to be roused with opsonized bacteria associated with disease. The outcome of the study proposes that the hyperactive phenotype of peripheral neutrophils can have local tissue-damaging concerns.[34] Substantial ROS generation by neutrophils demands a minimum oxygen tension of about 1% and a pH of 7.0–7.5. Both these environments are found within periodontal pockets, specifying that chronic or excess ROS production is possible at the site of tissue damage. Oxidation products produced locally by neutrophil ROS (e.g., oxidized low-density lipoprotein LPO) could further escalate neutrophil ROS generation directly as well as up-regulating adhesion molecules. Factors existing at high levels at diseased sites may boost ROS production by neutrophils locally.

One consequence of ROS damage could be increased LPO, as lipids are among the most readily oxidizable substrates.[35] Uneven local generation of ROS can cause increased LPO, contributing to periodontal disease. An increased level of LPO has been previously reported in the inflamed periodontal tissue.[36] Quantifying the LPO end products such as TBARS is helpful in assessing the tissue destruction induced by oxidative stress in periodontal disease.[37]

Table 1: Comparison of periodontal parameters between the case and control group

Groups	*PI (mean ± SEM)*	*PBI (mean ± SEM)*	*PPD (mean ± SEM)*	*CAL (mean ± SEM)*
Case [Grp I]	2.89 ± 0.12*	58.78 ± 0.89*	7.03 ± 0.45*	6.94 ± 0.23*
Control [Grp II]	0.23 ± 0.02	7.39 ± 0.45	1.34 ± 0.45	1.34 ± 0.45

*$p < 0.05$ significant

Table 2: Comparison of periodontal parameters before and after treatment within the case group

Case	*PI (mean ± SEM)*	*PBI (mean ± SEM)*	*PPD (mean ± SEM)*	*CAL (mean ± SEM)*
Before treatment	2.89 ± 0.12*	58.78 ± 0.89*	7.03 ± 0.45*	6.94 ± 0.23*
After treatment	1.23 ± 0.34	32.45 ± 0.34	5.45 ± 1.56	4.93 ± 1.56

*$p < 0.05$ significant

Table 3: Comparison of anti-oxidant levels between the case and control group

Groups	*TBARS (mean ± SEM)*	*GSH (mean ± SEM)*	*GSHP (mean ± SEM)*	*CAT (mean ± SEM)*
Case	10.22 ± 1.32*	4.43 ± 0.54*	167.54 ± 20.75*	155.64 ± 23.54*
Control	5.55 ± 0.90	7.61 ± 0.69	121.64 ± 12.49	93.75 ± 13.67

*$p < 0.05$ significant

Table 4: Comparison of anti-oxidant levels before and after treatment within the case group

Case	*TBARS (mean ± SEM)*	*GSH (mean ± SEM)*	*GSHP (mean ± SEM)*	*CAT (mean ± SEM)*
Before treatment	10.22 ± 1.32*	4.43 ± 0.54*	167.54 ± 20.75*	155.64 ± 23.54*
After treatment	6.23 ± 1.56	6.19 ± 1.59	130.23 ± 18.23	112.56 ± 12.45

*$p < 0.05$ significant

TBARS causes marked variation in the structural integrity and function of cell membranes.[38] Both enzymatic and nonenzymatic AOs clear off the LPO by-products formed under physiological and pathological conditions.[39] A disproportion between AO defense mechanism and LPO processes results in cell and tissue damage.[40,41]

Cells sequester essential nutrients from the circulation to meet the demand of body. Thus, the observed decrease in GSH in the plasma of periodontitis patients before and after therapy can be due to consumption of these AOs by the periodontal tissues or to compensate for the excessive oxidative stress in circulation. In the present study, the levels of GSH were inconsistent when the case and control groups were compared. GSH levels in the case group were reduced before treatment and were found to be increasing after phase I therapy levels. One of the justifications for varying responses of the GSH levels can be related to the distinct stages of periodontal disease the patient was going through. Severity and extension of the disease as well as long-term chronic conditions would have affected AO defenses of body which determines the AO responses in the patient.[42] GSH levels revealed a tendency to increase in the study, reason can be directed to a process called phase II reactions where GSH acts directly on ROS scavenging it or as a cofactor of GP_X and GST, either by catalyzing the reduction of hydrogen peroxide and lipid hydroperoxides or by the conjugation/excretion process.[43]

The unevenness between oxidative stress and AO mechanisms could also result from reduced AO capacity in periodontitis.[44] The results observed in the current study have a similarity with related studies done by Panjamurthy which reinforces the study value.[45] The observed increase in CAT levels in the plasma of periodontitis sufferers can be due to the scavenging of excessive generated LPO by-products at the inflammatory sites.

GP_X speeds up the reduction of H_2O_2 and various hydroperoxides using glutathione as a reducing agent. Metabolism of glutathione is one of the most important antioxidative defense mechanisms, whereas GP_X safeguards the body against low levels of oxidative stress.[46] Before the phase I therapy, the levels of enzyme GP_X were increased in the case group. Similar results were observed in other studies done in dogs and humans pertaining to the GP_X level.[45,47] The upsurge in GP_X in periodontal disease happens in reaction to the oxidative stress which occurs in inflamed periodontal tissues. The rise of GP_X in the case group pretreatment may represent possible AO compensation in detoxification reactions of organic peroxides produced during oxidative stress.[48] After the treatment, the recovery observed in the case group, in terms of clinical parameters, was found to be significantly higher. Initially, the extent of AO activity increases to safeguard tissues against oxidative stress; however, in the case of further advancement of disease, the amount of AO decreases because of an increase in pocket depth. We can conclude that betterment in clinical parameters and decrease of AO levels' value with the outcome of nonsurgical periodontal management, oxidative stress was reduced at periodontal tissues.

Studies have reported in various diseases that there exists a close association between oxidative stress processes and alterations in the immune response, thus, both aspects also seem to have a relationship to the development of periodontal disease, which was detected in the plasma of patients with periodontitis supporting the current study.[42,44,45]

The present study analysis elucidates the immense effort that has been attempted to evaluate the evidence for new periodontal treatment concepts. Even though the paper had limitations to provide the scientific exactness of a systematic research methodology but it is adequate to draw the following conclusions about the effectiveness of full-mouth disinfection and its impact on betterment in clinical parameters and level of oxidative stress.

Conclusion

Our understanding of ROS and AO enzymes relation to the periodontal disease is in the stage of evolution. In the years to come, intervention aimed at modulating the host response will have a greater therapeutic value. Based on the results from the current study, we can summarize that among various risk factors, reduced level of AOs should be given equal leverage in the etiology and pathogenesis of periodontal diseases. Release of free radicals occurs due to bacterial clearance and killing; to surpass this oxidative stress, the tissues of the periodontium relay on natural AOs. Ultimately, the ability of tissues to maintain normalcy by overcoming the oxidative stress thereby combating infection get compromised when the AO level is diminished. However, the level to which overproduction of ROS has an impact on initiation and progression of periodontal diseases is still unknown. In spite of the limitation in the sample size, the results suggest that the phase I therapy has a positive impact on the prognosis of periodontal therapy.

The reasons for selecting only female patients for the study are the following: subjects chosen in the study were referred from a general oral health awareness camp. Based on screening, an increased number of subjects diagnosed with aggressive periodontitis were females. Moreover, males diagnosed with aggressive periodontitis were either active smokers or those who have quit the habit. As smoking is considered as a risk factor for periodontal disease, to eliminate bias females who never smoked in their life were solely included as participants for this study.

Clinical significance

Altered LPO and AO level in aggressive periodontal disease that may be associated with their compensatory changes in the AOs defense system will give more insight into the etiopathogensis and therapeutic options.

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