Comparative evaluation of serum antioxidant levels in periodontally diseased patients: An interventional study

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Abstract

Background: Periodontal disease is an immune-inflammatory disease characterized by connective tissue breakdown, loss of attachment and alveolar bone resorption. In normal physiology, there is a dynamic equilibrium between reactive oxygen species activity and antioxidant defense capacity and when that equilibrium shifts in favor of reactive oxygen species, oxidative stress results. Oxidative stress is thought to play a causative role in the pathogenesis of periodontal diseases. Catalase (CAT) protects cells from hydrogen peroxide generated within them. Even though, CAT is not essential for some cell types under normal conditions, it plays an important role countering the effects of oxidative stress on the cell. Aim: This study was designed to estimate and compare the CAT and total antioxidant capacity (TAOC) levels in the serum of periodontitis, gingivitis, and healthy individuals before and after nonsurgical periodontal therapy. Materials and Methods: This study was conducted in the Department of Periodontics, A. B. Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore. The study was designed as a single blinded interventional study comprising of 75 subjects, inclusive of both sexes and divided into three groups of 25 patients each. Patients were categorized into chronic periodontitis, gingivitis and healthy. The severity of inflammation was assessed by using gingival index and pocket probing depth. Biochemical analysis was done to estimate the TAOC and CAT levels before and after nonsurgical periodontal therapy. Results obtained were then statistically analyzed using ANOVA test and paired t-test. Results: The results showed a higher level of serum TAOC and CAT in the healthy group compared with the other groups. The difference was found to be statistically significant (P < 0.0001). The posttreatment levels of TAOC were statistically higher than the pretreatment levels in periodontitis group.

Keywords: Antioxidant defense, catalase, interventional study, periodontitis, total antioxidants

Introduction

Periodontitis is a term used to describe an inflammatory process, initiated by the formation of plaque biofilm, that leads to loss of periodontal attachment to the root surface and adjacent alveolar bone and which ultimately results in tooth loss. Plaque is the main contributor to periodontal disease and after stimulation by bacterial pathogens neutrophils produce free radicals. Periodontal tissue destruction is also contributed by an inappropriate host response to these microorganisms and their products thus causing more damage to the periodontium.[1] Several reactive oxygen species (ROS) and lipid peroxidation products are produced in physiological quantities in the human body, but it has been well established that over-production of ROS occurs at sites of chronic inflammation. The human body does contain an array of antioxidant defense mechanisms (non-enzymatic and enzymatic antioxidants) to remove harmful ROS as soon as they are formed and to prevent their deleterious effects. The enzymatic antioxidants include superoxide dismutase, catalase (CAT), and glutathione peroxidase, while the nonenzymatic antioxidants include vitamins E and C, and reduced glutathione.[1] Thus, the aim of this study was to determine the serum levels of total antioxidant capacity (TAOC) and CAT in gingivitis and periodontitis cases, before and after nonsurgical periodontal therapy and to compare the obtained result with healthy controls.

Materials and Methods

The subjects for this study were selected from the Department of Periodontics A. B. Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore. Ethical clearance was taken from the Institutional Ethical Committee before the start of the study. Written consent was taken from each patient prior to sample collection. A total of 75 patients who reported to the outpatient department of periodontics were
divided into three: Group A consisting of 25 subjects with chronic periodontitis, group B consisting of 25 subjects with chronic gingivitis, and group C (control group) with a total of 25 periodontally healthy subjects.

**Clinical examination**

The screening examination consisted of patient’s medical history, dental history, gingival index (as given by Loe and Silness, 1967) probing depth and loss of attachment recorded with Williams periodontal probe. The subjects were categorized according to the following criteria: All subjects were systemically healthy, with no medical condition that would affect their participation in the study with a minimum complement of 20 teeth. Controls were periodontally healthy. Gingivitis was defined as patients with bleeding on probing in >30% of sites and with a mean Loe and Silness gingival index score ≥1. Periodontitis was defined as the presence of at least 30% of sites with clinical attachment loss ≥5 mm measured with a Williams periodontal probe. The subjects with a history of any antibiotic or anti-inflammatory therapy 2 months prior to study, pregnant, pre-eclamptic or lactating women, subjects who had received periodontal therapy in the past 3 months, smokers and subjects taking vitamin supplements were excluded from the study. All measurements and readings were done before the collection of the blood samples. Group A and group B patients underwent nonsurgical periodontal therapy and were followed-up after 3 weeks for respective blood parameters.

**Results**

Table 1 compares the serum TAOC and CAT levels among the three groups and shows a highly significant increase in the healthy group than the periodontitis and gingivitis group.

Table 2 compares the mean and standard deviation of serum TAOC levels in the pretreatment and posttreatment groups by the paired $t$-test. It shows a highly significant increase in the posttreatment levels in periodontitis group and no significant difference in the gingivitis group.

Table 3 compares the mean and standard deviation of serum CAT levels in the pretreatment and posttreatment groups by the paired $t$-test. It shows no significant difference in posttreatment levels in periodontitis group and gingivitis groups.

Graph 1 represents a bar graph comparing serum levels of TAOC (mM/L) among the three groups.

Graph 2 represents a bar graph comparing the serum levels of Catalase (U/mgHb) among the three groups.

Graph 3 represents a bar graph comparing the serum TAOC levels before and after non surgical periodontal therapy in Group A and B.

Graph 4 represents a bar graph comparing the serum Catalase levels before and after non surgical periodontal therapy in periodontitis group.

**Discussion**

Inflammatory and immune reactions to microbial plaque are the predominant features of gingivitis and periodontitis. There are reports demonstrating the ability of perio-pathogens and their products to induce the generation of ROS by polymorphonuclear leukocytes (PMNs). One of the host defense mechanism involves the production of powerful oxidizing agents, which is characterized by a rapid increase in the uptake of oxygen. This phenomenon is known as the respiratory burst and results in the production of reactive oxidants such as superoxide, hydrogen peroxide, and hydroxyl radicals. Chronic exposure to ROS can initiate a broad spectrum of pathologic reactions in the adjacent tissues. Thus, normal tissue destruction is part of the typical inflammatory response. The prevention of lipid peroxidation is an essential process in all the aerobic organisms, as lipid peroxidation products can cause DNA damage. Increased lipid peroxidation and decreased antioxidant protection frequently occurs.

**Table 1:** Comparison of serum TAOC (in mM/L) and catalase (U/mgHb) levels among the three groups: One-way ANOVA with Tukey post-hoc test

| Groups                        | n  | Mean   | Standard deviation | Statistics/mean squares | df 2 (Welch)/F (ANOVA) | P value |
|-------------------------------|----|--------|--------------------|-------------------------|-------------------------|---------|
| **TAOC in conc. in Mm/L**     |    |        |                    |                         |                         |         |
| Periodontitis (A)             | 25 | 0.49724| 0.225029           | 43.041                  | 44.832                  | <0.001  |
| Gingivitis (B)                | 25 | 0.86372| 0.172003           |                         |                         |         |
| Healthy individuals (C)       | 25 | 1.25852| 0.368255           |                         |                         |         |
| Total                         | 75 | 0.87316| 0.409808           |                         |                         |         |
| **Catalase in U/mg Hb**       |    |        |                    |                         |                         |         |
| Healthy individuals           | 25 | 6021.608| 1157.685          | 1.09E+08                | 51.882                  | <0.001  |
| Gingivitis                    | 25 | 3930.494| 1615.705          |                         |                         |         |
| Periodontitis                 | 25 | 1840.865| 1538.174          |                         |                         |         |
| Total                         | 75 | 3930.989| 2236.322          |                         |                         |         |

**TAOC:** Total antioxidant capacity; **ANOVA:** Analysis of variance
Table 2: Comparison of the pre- and the post-serum TAOC (in mM/L) values in gingivitis and periodontitis using paired t-test

| Disease group | Treatment group | Mean    | n   | Standard deviation | Paired difference mean | Standard deviation | t    | df  | P value |
|---------------|----------------|---------|-----|--------------------|-----------------------|--------------------|------|-----|---------|
| Group A       | Pre-Rx         | 0.4972  | 25  | 0.225029           | -0.4595               | 0.19840            | -11.58| 24  | <0.001  |
|               | Post-Rx        | 0.9568  | 25  | 0.187936           |                       |                    |      |     |         |
| Group B       | Pre-Rx         | 0.8637  | 25  | 0.172003           | -0.0494               | 0.22717            | -1.08| 24  | 0.287   |
|               | Post-Rx        | 0.9131  | 25  | 0.267394           |                       |                    |      |     |         |

TAOC: Total antioxidant capacity

Table 3: Comparison of the pre- and the post-serum catalase (U/mgHb) values in gingivitis and periodontitis using paired t-test

| Disease group | Treatment group | Mean    | n   | Standard deviation | Paired difference mean | Standard deviation | T   | df  | P value |
|---------------|----------------|---------|-----|--------------------|-----------------------|--------------------|-----|-----|---------|
| Group A       | Pre-Rx         | 1840.86 | 25  | 1538.174           | -10.486               | 640.754            | -0.082| 24  | 0.935   |
|               | Post-Rx        | 1851.35 | 25  | 1796.32            |                       |                    |      |     |         |
| Group B       | Pre-Rx         | 3930.49 | 25  | 1615.705           | -76.856               | 391.328            | -0.982| 24  | 0.336   |
|               | Post-Rx        | 4007.35 | 25  | 1675.579           |                       |                    |      |     |         |

Graph 1: Comparison between serum levels of total antioxidant capacity (mM/L) among the three groups

Graph 2: Comparison between serum levels of catalase (U/mgHb) among the three groups

Graph 3: Comparison of serum total antioxidant capacity levels before and after nonsurgical periodontal therapy in groups A and B

Graph 4: Comparison of serum catalase levels before and after nonsurgical periodontal therapy in periodontitis group

Thus, this study was aimed to evaluate the significance of TAOC and CAT as an antioxidant in serum, by estimating their levels in patients with chronic periodontitis, gingivitis and periodontally healthy individuals. The interventional part of the study involved the estimation of serum TAOC and CAT levels along with the periodontal parameters 3 weeks after nonsurgical periodontal therapy.
The results of this study for serum TAOC levels are in broad agreement with the observations of Chapple et al. and Brock et al. It demonstrates that the plasma TAOC levels are significantly reduced in chronic periodontitis \((P \leq 0.001)\) subjects and gingivitis subjects \((P \leq 0.001)\) when compared with age and sex matched healthy controls. This can be explained by the destruction caused by and imbalance between the antioxidant and ROS. The basis of increased ROS production is a consequence of inflammatory infiltrate of PMNs as a host response against microbial invasion. The strongest evidence to implicate ROS in the pathological destruction of the connective tissues during periodontal diseases arises in considering PMN infiltration as a key event. Such an infiltration in numbers is likely to lead to an increase in ROS levels causing oxidative stress. Elevated lipid peroxidation and disturbed antioxidant status have also been reported in experimental periodontitis by Tsai and Chen. In this study, it was reported that increased gingival crevicular fluid lipid peroxidation levels reflected the increased ROS damage to the periodontal tissue as well as periodontal inflammation.

This study has also assessed the pretreatment and posttreatment levels and found a significant increase \((P \leq 0.001)\) in posttreatment levels of the periodontitis groups but no significant change in the gingivitis group. Which is in accordance with the recent study done by Novakovic et al. which is ahead of print and Kim et al.

Thus, oxidative stress lies at the heart of the periodontal tissue damage that results from host-microbial interactions, either as a direct result of excess ROS activity/antioxidant deficiency or indirectly as a result of the activation of redox-sensitive transcription factors and the creation of a pro-inflammatory state. A body of literature supports peripheral blood neutrophil hyper-reactivity in chronic and aggressive forms of periodontitis, with respect to total Fcγ-receptor-mediated ROS generation. Furthermore, it seems possible that baseline hyperactivity (i.e., low level extracellular ROS release in the absence of exogenous stimulus) is also a constitutional property of peripheral neutrophils from periodontitis patients. This, together with the evidence for compromised plasma antioxidant capacity, independent of smoking, suggests an underlying environment of oxidative stress, within periodontitis patients.

Catalase protects cells from hydrogen peroxide generated within them. Even though CAT is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress as an adaptive response of cells. \(\text{H}_2\text{O}_2\) is enzymatically catabolized in aerobic organisms by CAT and several peroxidases.

In our study, the CAT levels in subjects of periodontitis group \((P < 0.001)\) and gingivitis group \((P < 0.001)\) was found to be significantly lower than the control group. These results are consistent with the outcomes of recent investigations, which reported low serum levels of CAT in periodontitis subjects. The study found no significant increase in serum CAT levels in the subjects before and after nonsurgical periodontal therapy. This could be due to the short follow-up time after nonsurgical periodontal therapy, which was not sufficient to cause a significant variation in the serum levels of CAT.

Thus, a delicate balance exists between antioxidant defense and repair systems and pro-oxidant mechanisms of tissue destruction, which if tipped in favor of tissue damage could lead to significant attachment loss.

This study may have important therapeutic implications in terms of the use of antioxidants in periodontal therapy to prevent tissue destruction.

However, it is only as data becomes available from other long-term interventional trials done on humans that we will see whether these compounds live up to their potential for the treatment of periodontal diseases.

**Conclusion**

Serum levels of total antioxidants and CAT is decreased in the periodontally compromised groups compared with the control group. The posttreatment serum levels of TAOC showed a significant increase compared to the pretreatment levels in periodontitis group. Whereas the serum levels of CAT did not show any significant variation posttreatment in either of the groups. As periodontitis is a multifactorial disease there are various mechanisms leading to breakdown of periodontal tissues.

To conclude, the reduction in the serum levels of TAOC and CAT in gingivitis and periodontitis patients has led to an imbalance leading to increased ROS and hence periodontal breakdown. Hence, focus should be shifted to the development of antioxidant based approaches to periodontal therapy. Further studies need to be carried out on the efficacy of antioxidant therapies that target the free radicals that lead to periodontal tissue breakdown.

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