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Research Article

COMPARATIVE ESTIMATION OF SALIVARY TOTAL ANTIOXIDANT CAPACITY IN PERIODONTAL HEALTH AND CHRONIC PERIODONTITIS - A PILOT STUDY

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ABSTRACT

Objective: Gram-negative bacteria provoke polymorphonuclear leukocyte (PMN) to release reactive oxygen species in chronic periodontitis (CP). Inability to maintain a balance between oxidative stress and antioxidant levels makes patients more susceptible to periodontal disease. The present study aims to estimate and compare salivary total antioxidant capacity (TAOC) in subjects with clinically healthy periodontium and patients with CP.

Methods: After fulfilling the selection criteria, a total of 20 subjects (10 with clinically healthy periodontium and 10 with CP) were subjected to unstimulated salivary sample collection for biochemical estimation of TAOC by spectrophotometric assay using Kovacevic method. Analysis of data was done with unpaired student t-test, using SPSS version 22 statistical program.

Results: Salivary TAOC was significantly higher in subjects with clinically healthy periodontium compared to CP patients. It was statistically significant ($p<0.001$).

Conclusion: This study indicated increased levels of salivary TAOC in patients with CP compared to clinically healthy periodontium. Alteration in defensive antioxidant status could be a risk factor in the progression of periodontal disease.

Keywords: Total antioxidant capacity, Chronic periodontitis, Polymorphonuclear leukocyte, Reactive oxygen species, Free radicals, Oxidative stress, Antioxidants.

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INTRODUCTION

Chronic periodontitis (CP) is an inflammatory disease of microbial origin with progressing destruction of supporting structures of the teeth and is the most prevalent cause of tooth loss worldwide [1]. It is initiated by the complex interaction of periodontopathogens and a susceptible host. The tissue destruction mainly occurs in individuals with hyperinflammatory response to the specific colonizing bacteria [2]. Host-microbial response generates oxidative stress leading to the increased production of reactive oxygen species (ROS), which are detrimental to periodontal health. In all the chronic inflammatory diseases production of ROS is inevitable. ROS are released from numerous sources such as the bacteria themselves, or as a consequence of the immune response due to infiltration of polymorphonuclear leukocytes (PMN) [3]. This, in turn, releases cytokines and pro-inflammatory mediators (interleukin [IL]-1α, IL-1β, IL-6, IL-8, and tumor necrosis factor-α) causing exaggeration of an inflammatory process.

Human body unfolds definite defense and repair mechanisms essentially to arrest the accumulation of oxidatively damaging toxic molecules such as H_2O_2, HOCL, and OH^-. This is achieved through antioxidant defense mechanisms (nonenzymatic and enzymatic antioxidants). The antioxidant defense mechanism aids in scavenging the free oxygen radicals and thus prevents their harmful sequela on the host by modulating inflammatory response [4].

Total antioxidant capacity (TAOC) in saliva assays is useful in giving a quantitative measure of the capacity of biological systems to withstand oxidative attack. They also throw light on the various biomolecular interactions among individual antioxidant species. There are various reports suggesting a reduction in the TAOC in serum and gingival crevicular fluid in CP [5,6]. The results in various studies are not able to establish the correlation between salivary TAOC in health and CP. Conflicting results of different studies about the importance of AO and oxidative stress levels in prevention, initiation, progression, and treatment of periodontal diseases exist in the literature [7]. The present study aims to estimate and compare salivary TAOC in health and CP.

METHODS

A total of 20 systemically healthy subjects reporting to the routine outpatient department of periodontics, were recruited in a single centered, case–control study after obtaining informed consent from them. Ethical clearance was obtained from the Institutional Ethical Committee before the study. Confidentiality of the data was maintained throughout the study period. The study group was divided into two groups.

Group I: 10 subjects with healthy periodontium
Group II: 10 subjects with CP.

Inclusion criteria

Male and female subjects within the age group of 30–50 years and with a minimum of 20 scorable teeth were included in the study. Group I included subjects with healthy periodontium, who showed no signs of periodontal disease as determined by the absence of the evidence of interproximal (CAL ≤1 mm), no PD >3 mm at any sites, whole-mouth bleeding scores <10%, and had no clinical signs of gingival inflammation. Group I included subjects with CP according to American Academy of Periodontology -1999 [8] as two or more tooth sites with PD ≥4mm or CAL of 4 mm that bled on probing.

Exclusion criteria

Subject with a history of nonsteroidal anti-inflammatory drugs or antimicrobial drugs, mouthwashes, or vitamin/antioxidant supplements within a 3 month period before the study commences. Subjects with special dietary requirements, history of medication which influences antioxidant status, history of periodontal therapy in the previous 6 months, pregnant and lactating women, and subject with habits such as smoking, tobacco chewing, and alcoholics were excluded from the study.

Before study, ethical clearance was obtained from the Institution's Ethics Committee (Ref. No. YUEC/2015/045), and informed consent was obtained from all the study subjects.

After enrolment, a total of 20 subjects (10 with CP and 10 with clinically healthy periodontium) who fulfill the selection criteria were subjected to unstimulated salivary sample collection for biochemical estimation of TAOC.

Collection of saliva

Subjects were asked not to eat or drink 1 h before the sample collection. 2 mL of unstimulated whole saliva was collected by expectorating into disposable tubes before clinical measurements into a sterile container. Collected sample was centrifuged at 3000 rpm for 15 min, and the supernatant was stored in small aliquots at −20°C until the biochemical analysis [9]. The biochemical analysis of TAOC was done using Koracevic's method [10].

The principle behind Kovacevic method

Standardized solution of Fe–EDTA complex interacts with hydrogen peroxide by a Fenton-type reaction, leading to the formation of hydroxyl radicals (•OH). These ROS dissolute benzoate, leading to liberation of thiobarbituric acid reactive substances (TBARS). Antioxidants from the added sample of saliva cause suppression of the production of TBARS [10]. Analysis of this reaction is done spectrophotometrically [11].

Statistical analysis

Data were compiled in an Excel sheet and analyzed using software IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Mean and the standard deviation was calculated and presented in Table 1. TAOC was compared among the Group I (healthy periodontium) and Group II (CP) using unpaired students t-test. $p<0.05$ was considered statistically significant.

RESULTS

A study was conducted among 10 subjects with healthy periodontium and 10 patients diagnosed with CP to assess salivary TAOC.

Biochemical assessment of salivary TAOC

Table 1 and Fig. 1 show the intergroup comparison among Group I and Group II. The mean salivary TAOC was higher in Group I (clinically healthy periodontium) (796.10±46.49) compared to Group II (CP) (512.05±50.28), and it differs significantly ($p<0.001$) (Fig. 1).

DISCUSSION

CP is caused by various hyper-responsive and destructive products of immune response stimulated by microbial plaque around the gingival margin. Inability to maintain the balance between oxidative stress and antioxidant levels makes patients more susceptible to periodontal disease [1]. The major reason for depletion of antioxidant levels in the periodontal disease is due to excessive production of ROS such as H_2O_2 and OH− ion by infiltration of hyperactive PMN leading to the destruction of the connective tissue of periodontal ligament and bone [12,13]. Presence of periodontal inflammation is said to be linked with systemic oxidative stress, which, in turn, causes systemic inflammation.

The present study attempts to estimate levels of TAOC in saliva in patients suffering from CP and compares it with subjects with healthy periodontium. A sample size of 10 subjects with clinically healthy periodontium and 10 subjects with CP were subjected to salivary estimation of TAOC using Kovacevic method [10].

Baltacıoğlu *et al.* [14] found that both lipid peroxidation levels and TAOC in saliva and serum is reduced in patients with CP while Ahmadi-Motamayel *et al.* [15] reported slightly lower TAOC in the periodontitis group compared to healthy control but was not statistically significant. A study was done by Miricescu *et al*. [16] reported that the salivary antioxidant activity was reduced significantly in CP patients ($p<0.05$), which is in an accordance with our study. Results of our study showed higher levels of salivary TAOC in Group I (subjects with clinically healthy periodontium) compare to Group II (subjects with CP). It shows that the oxidative stress in the body is effectively counteracted by the antioxidants. Lower levels of antioxidants in CP direct toward the fact that the disease process is a result of an imbalance between free radical production and antioxidant levels, with the failure of antioxidants to balance the oxidant levels.

Kim *et al*. [17] found that the salivary total antioxidant level was higher in severe CP patients than in the healthy or gingivitis controls before scaling and root planning, which was in contrast to our present study. Brock *et a*l. [7] found that the salivary antioxidant capacity was lower with periodontitis, but this difference was not significant. Moore *et al*. [18] reported in their study that salivary TAOC was not statistically different between cases and controls. Wei et al. [19] also showed higher levels of lipid peroxidation levels, total oxidant status

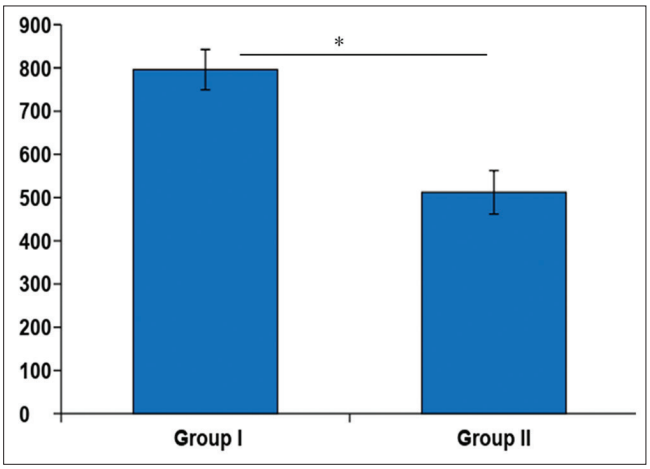

Fig. 1: Comparison of salivary total antioxidant capacity between Groups 1 and 2. Group 1: Subjects with healthy periodontium who showed no signs of periodontal disease. Group 2: Subjects with CP as defined according to American Academy of Periodontology. Bar indicates the standard deviation within the group. *Statistical significance with Wilcoxon signed-rank test is p<0.01

Table 1: Comparison of salivary TAOC of between Group I and Group II

Group	*n*	Mean	SD	Mean difference	p-value	95% Confidence interval of the difference
I	10	796.1	46.491	284.05	<0.001*	(238.5519, 329.5481)
II	10	512.05	50.284			

*Statistically significant, SD: Standard deviation. TAOC: Total antioxidant capacity

and superoxide dismutase in serum, saliva and gingival crevicular fluid in CP patients. The differences in the results of TAOC among different studies could be explained by different analytical methods used by TAOC assessment [11,20].

We evaluated salivary TAOC as they furnish information on the biological interactions among individual antioxidants. It is also more advantageous to estimate TAOC than specific antioxidant level as some of them might be difficult to assay, are expensive, and time-consuming [21].

In the present study, we estimated TAOC in whole unstimulated saliva because it contains major salivary gland composition, saliva may constitute the first line of defense against free radical-mediated oxidative stress, and in addition, it also has elements of gingival crevicular fluid. It is easily available, non-invasive diagnostic medium for detection of TAOC.

The result of the present study confirms our primary hypothesis that reduction in antioxidant capacity accentuates susceptibility to oxidative stress, and the resulting damage is thought to be involved in the pathogenesis of CP [22].

Within the limitation of our study which had the smaller sample size, we were able to find a statistically significant difference in salivary TAOC in healthy controls and CP. Estimation of oxidative stress and salivary TAOC levels in terms of severity of CP might be more helpful in the thorough understanding of ROS versus antioxidant role in the pathogenesis of CP. Long-term studies with a larger sample size are required to explicate the role played by antioxidants in the progression of periodontal disease.

CONCLUSION

The result of the current study shows lower levels of salivary TAOC in CP compared to healthy periodontium. Reduction in TAOC of saliva in the progression of periodontal destruction provides possibilities in future to evolve novel antioxidant treatment strategies to overcome the burden of the free radicals and to play a possible role as a host modulating agent in CP.

AUTHOR'S CONTRIBUTIONS

All authors have made substantial contributions to the work reported in the manuscript. Raghavendra U and Anupama Rao: Concepting of the study, data collection, and data analysis. Anupama Rao, Jyothi D'esoza, Vinita Ramanath Pai, Sindhu Nair, and Vijaya Kumar: Lab experiment, interpretation, and revision of the article, Bhuvanesh Sukhlal Kalal, and Anupama Rao: Drafting the article and revising the article. All of the authors read and approved the final manuscript of the study to be published.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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