

EVALUATION OF OXIDANT AND ANTIOXIDANT STATUS AMONG CHRONIC PERIODONTITIS PATIENTS AND HEALTHY SUBJECTS**Dr. Reshama Y. Sawant^{1*} and Dr. Chitra Y. Dhume²**¹Assistant Lecturer, Department of Biochemistry, Goa Medical College, Bambolim-Goa.²Professor and Head, Department of Biochemistry, Goa Medical College, Bambolim-Goa.Article Received on
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Oxidative stress has been implicated in the pathogenesis of inflammatory condition like periodontitis. This study was carried out in order to assess the levels of oxidants and antioxidants in the saliva sample of periodontitis patients and compare it with healthy subjects. This study comprised of 70 subjects, out of which 35 were chronic periodontitis patients and 35 were healthy subjects. Saliva sample was obtained from all the subjects and analysed for levels of MDA which is a marker of oxidative stress. Also total antioxidant capacity (TAC) was determined, along with individual antioxidants like vitamin C, uric acid and reduced glutathione. It was found that the levels of MDA were significantly increased in chronic periodontitis

patients than healthy subjects ($p < 0.05$). Salivary total antioxidant capacity and other antioxidants such as vitamin C and uric acid were significantly lowered in patients with chronic periodontitis as compared to healthy subjects ($p < 0.01$). Glutathione level was also reduced in chronic periodontitis patients than healthy subjects but it was not significant. It was concluded that oxidative stress plays a significant role in the pathogenesis and progression of periodontitis. These parameters would help in evaluating the severity of the disease so that the progression of the disease can be prevented or treated with proper antioxidant therapy.

KEYWORDS: Chronic periodontitis, MDA, Total antioxidant capacity, Uric acid.**INTRODUCTION**

Periodontitis is an inflammatory disease characterised by destruction of the supporting tissue of the teeth. It is initiated by the complex micro biota found as dental plaque, a complex

microbial biofilm^[1]. Activation of host immune response leads to progressive destruction of the periodontal tissue and bone loss. The abnormal response is characterised by exaggerated inflammation involving release of excess proteolytic enzymes and reactive oxygen species (ROS) by the hyper reactive neutrophils during phagocytosis of the bacteria^[2,3]. Released ROS by oxidative mechanism distorts the cell membrane and kill the bacteria. However during defense mechanism overproduction of radicals can lead to oxidative modification of various host biomolecules and damages the host cells^[4]. Reactive oxygen species are highly toxic to the body. These include superoxide radical, hydroxyl radical, nitric oxide, hydrogen peroxide, hypochlorous acid etc. when produced in large amount they can result in DNA lipid peroxidation, protein damage, enzyme oxidation and stimulation of monocytes and macrophages to release proinflammatory cytokines which in turn can cause oxidative stress and damages the host tissue^[5,6]. These can also damage connective tissue and cause bone lysis around tooth root resulting in loosening of the tooth^[7]. Periodontal tissue destruction leads to overproduction of lipid peroxides, inflammatory mediator, and oxidized proteins. These products further activate macrophages, neutrophils, and fibroblasts to generate more ROS. In order to combat the deleterious effects of oxidants, cells are endowed with an antioxidant defence system, consisting of variety of enzymatic and non – enzymatic antioxidants thereby protecting cellular macromolecules such as proteins, lipids, DNA from oxidative damage. Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals or oppose their actions^[8]. Saliva consists of enzymatic antioxidants like superoxide dismutase, glutathione peroxidase, catalase and non enzymatic antioxidants like uric acid, albumin, glutathione, vitamin A and C. In normal cells there is a steady balance between oxidative damage and anti-oxidative protection. Oxidative stress arises when there is imbalance between oxygen free radical generation and scavenging action by antioxidants. Tissue injury due to free radical production has been suggested to be enhanced in individuals with periodontal disease due to lack of adequate antioxidant defense.^[9,10] Salivary markers of oxidative stress are extensively discussed as a possible tool for periodontal diagnostic (Tóthová et al., 2015)^[11]. Since most oxidants have a very short half-life time, the measuring of oxidation products is widely used as an indicator of oxidative stress (Palmieri and Sblendorio, 2007)^[12]. Malondialdehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation that can indicate the increase of oxidative stress.^[13,14] Previous studies show that periodontitis patients have the increased salivary levels of lipid peroxidation products, protein oxidation markers, and DNA damage marker (reviewed in Tóthová et al., 2015). Since these oxidants produced during inflammatory

responses are neutralized by different antioxidants system. Therefore, measuring salivary total antioxidant capacity (TAC) can be considered as an important tool for periodontal diagnostic. Total antioxidant capacity has therefore been developed to reduce the costly and time-consuming task of measuring individual antioxidant species. In present study we have also evaluated levels of individual antioxidants like vitamin C, reduced glutathione and uric acid. The study of individual antioxidant species greatly improves our understanding of their role in pathogenesis of the disease. Vitamin C is a chain breaking antioxidant. It protects critical macromolecules from oxidative damage. Vitamin C also plays a role in recycling of tocopherol and indirectly involved in neutralisation of reactive oxygen species^[15]. Thus vitamin E and vitamin C acts synergistically to reduce lipid peroxidation and protects cell membrane from oxidative damage. Reduced glutathione is the major endogenous antioxidant produced by cells and participates directly in neutralisation of free radicals as well as maintaining vitamin C and E in reduced form. Uric acid is the most common antioxidant of saliva and it represents 70% of the antioxidant capacity of saliva.

Thus the aim of this study is to evaluate and compare salivary oxidant/ antioxidant status in patients with periodontitis and healthy subjects and their possible role in the pathophysiology of periodontitis.

MATERIALS AND METHODS

Selection of Subjects

A total of 70 subjects of both genders with age range of 25 to 60 years were included in this study. Patients were selected from the OPD of Department of Periodontics, Goa Dental College, Bambolim Goa and biochemical research was done at the Biochemistry Laboratory of Goa Medical College, Bambolim. The informed consent of the subjects was taken after obtaining ethical clearance from institution's ethical committee. Detailed medical and dental history was taken prior to inclusion into the study and control groups. The selected subjects were divided into two groups: group I included 35 subjects with chronic periodontitis (radiographic evidence of generalized alveolar bone loss >30%, presence of at least one pocket with PPD >5 mm per quadrant with positive bleeding on probing – BOP with clinical attachment loss of >5mm) and group II included 35 healthy subjects. Exclusion criteria were pregnant or lactating women, use of food supplements, such as vitamin C and E supplements over the previous three months or some restrictive diet, history of smoking, use of antibiotics for last 6 months and presence of any systemic disease.

Periodontal examination

The periodontal status of all individuals was detected by measurements of probing depth (PD), clinical attachment level (CAL), gingival index (GI), gingival bleeding index (GBI) and plaque index (Pi) in CP and control groups. Full mouth periapical radiographs were taken to determine the level of periodontal bone loss. PD and CAL were measured on six sites of teeth (mesial, median and distal points at buccal and palatal aspects). All clinical measurements and radiographic examinations were performed by a single investigator. It was ensured that the total number of teeth in the mouth was >20.

Saliva Collection

Unstimulated whole saliva samples were used in this study. Salivary collection was carried out between 8:00 and 11:00 a.m. Participants were asked to refrain from eating, drinking, smoking or brushing their teeth after midnight on the day of sampling. Unstimulated saliva was collected for 5min according to the protocol of Navazeshetal.(2008), aliquoted and stored at -80°C until analysis.

Biochemical analyses Saliva sample was used for measurement of MDA, vitamin E, vitamin C, reduced glutathione and total antioxidant capacity. All the estimations were done within 24 to 48 hours after specimen collection.

Determination of salivary ascorbate by 2, 6 dichlorophenolindophenol titration method^[16]

Titration with 2, 6 Dichlorophenolindophenol in acid solution. This blue coloured compound is red in acidic solution and on titration with ascorbic acid is oxidised to D- L ascorbic acid.

Determination of salivary MDA using satoh kei's method^[17]

Specimen was treated with Thiobarbituric acid in sodium sulphate to form chromogen. This chromogen is allowed to form a pink coloured complex in boiling water bath and extracted with butanol which is then measured at 530 nm filter.

Estimation of salivary reduced glutathione (beutler's method, 1963)^[18]

Glutathione (GSH) in the saliva is maintained in reduced state through reduced nicotinamide adenine dinucleotide phosphate and glutathione reductase. The function of reduced glutathione is to be to keep sulfhydryl groups in their active reduced state and through glutathione peroxidase to remove peroxide. Photometric method adapted by Beutler using 5

5' Dithiobis 2'Nitro benzoic acid (DTNB) was used for the assay of salivary glutathione levels. The method is based upon the development of a relatively stable yellow colour when DTNB is added to sulfhydryl compound.

Measurement of total antioxidant capacity^[19]

The total antioxidant capacity of saliva was evaluated using the spectrophotometric assay. The method is based on the principle that, when a standardized solution of Fe-EDTA [Iron-Ethylenediaminetetraacetic acid] complex reacts with hydrogen peroxide by a Fenton-type reaction, it leads to the formation of hydroxyl radicals (OH). These ROS degrade benzoate, resulting in the release of thiobarbituric acid reactive substances (TBARS). Antioxidants from the added sample of saliva cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of color is development defined as the antioxidant capacity.

Determination of salivary uric acid by uricase method^[20]

Uric acid is oxidized to allantoin by uricase with the production of hydrogen peroxide. Hydrogen peroxide reacts with 4 aminoantipyrine and 2,4,6 tribromo 3 hydroxybenzoic acid in presence of peroxidase to yield quinoneimine dye. The resulting change in absorbance at 548 nm is directly proportional to uric acid concentration in the sample.

Statistical analysis

The statistical comparison of the mean values of systemic biochemical parameters including total salivary antioxidants capacity between the study group and control group was evaluated using Student t test with SPSS data processing software.

Table 1: Distribution of Total Subjects

Group	Number of cases
Chronic periodontitis	35
Controls	35
Total	70

Table 2: Table showing mean levels of salivary antioxidants and MDA between chronic periodontitis patients and control group

Group	No. of cases	VITC (mg/l)	Uric acid(μ mol/l)	GSH (mg %)	Total antioxidant capacity(μ mol/dl)	MDA (nmol/ml)
Chronic periodontitis	35	0.36 \pm 0.30	153.9 \pm 41.87	2.02 \pm 0.54	1.09 \pm 0.19	1.03 \pm 0.22
Controls	35	1.39 \pm 0.55	198.43 \pm 87.41	3.00 \pm 0.66	4.68 \pm 0.31	0.51 \pm 0.19

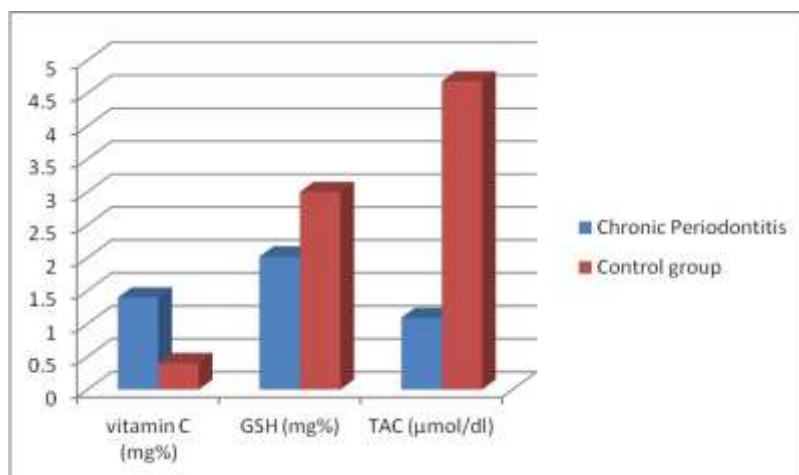


Figure 1: Mean levels of salivary vitamin C, reduced glutathione and total antioxidant capacity among chronic periodontitis patients and control group

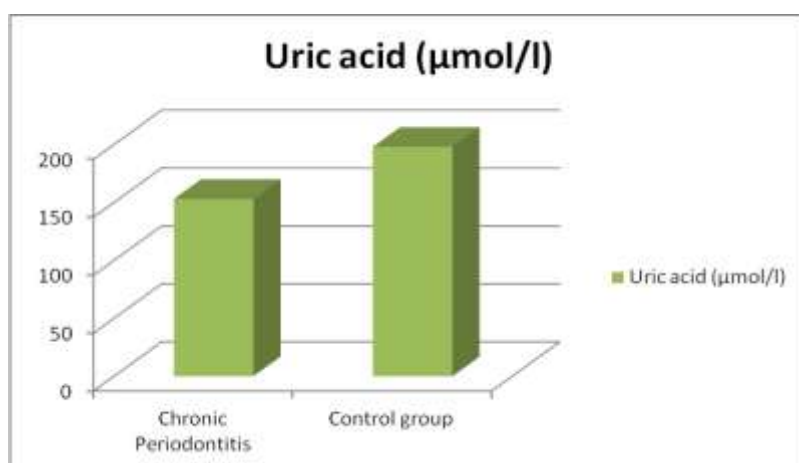


Figure 2: Mean levels of salivary uric acid among chronic periodontitis patients and control group

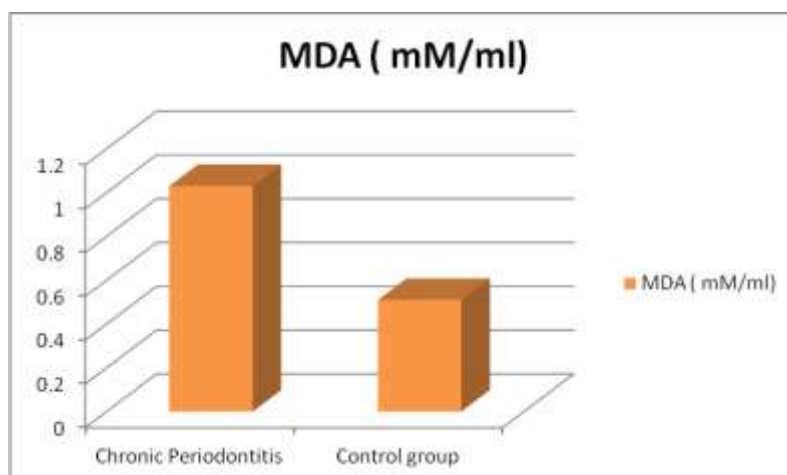


Figure 3: Mean levels of salivary MDA among chronic periodontitis patients and control group

RESULTS AND DISCUSSION

In the present study it was seen that there is increased level of MDA in chronic periodontitis patients as compared to healthy subjects. It was found to be statistically significant ($p < 0.05$). On the contrary, the total antioxidant capacity (TAC) was significantly decreased in chronic periodontitis patients as compared to control group. Upon analyses of individual antioxidant like uric acid, ascorbic acid and reduced glutathione it was found that the levels of uric acid and ascorbic acid were significantly less in chronic periodontitis patients as compared to healthy subjects ($p < 0.05$). Glutathione levels were found to be slightly lower in chronic periodontitis as compared to healthy subjects but there was no statistical significant difference between the two groups. The mean levels of all the parameters are mentioned in table 2. This study indicate that oxidative stress plays a important role in the progression of periodontitis which results due to an up-regulation of pro-inflammatory pathways and progressive tissue damage. Study by Dean V Sculley et al showed that subjects with the worst periodontal health status tended to have greater oxidative injury which is wholly consistent with the hypothesis that there is enhanced reactive oxygen species mediated damage to tissues in the most advanced states of periodontal disease^[21]. Oxidative stress induced progression of periodontitis might be due to antioxidant depletion due to ongoing free radical activity and destruction of scavenging antioxidant species. Study by Sculley and Langley – Evans indicated that chronic periodontal disease is associated with peripheral neutrophils that are hyper-reactive with respect to the production of ROS in response to Fcγ-receptor stimulation.^[22,23] Thus, periodontal disease has been suggested to be associated with reduced salivary antioxidant status and increased oxidative damage within the oral cavity. Malondialdehyde was also found to be raised in gingival crevicular fluid and saliva of chronic periodontitis patients as compared with controls.^[24] Our study showed findings similar to study by Chapple *et al* in which salivary total antioxidant capacity was significantly lowered in chronic periodontitis patients as compared to control group. Several studies also demonstrated significantly lower total antioxidant capacity in serum and plasma samples from periodontitis subjects.^[25-27] Diab-Ladki et al^[28] showed lower saliva total antioxidant capacity in periodontitis subjects than those in healthy subjects. Inorder to avoid host tissue destruction these ROS are neutralized by antioxidants, which might result in decreased TAC. It should be also noted that TAC is a complex parameter which includes the integrated activity of different antioxidants and often depends on their interaction and synergistic effects (Ghiselli et al., 2000)^[29]. Therefore depletion of any of the antioxidant species will result in decreased antioxidant capacity. Panjamurthy et al^[30] reported that disturbance in the

antioxidant defence system due to overproduction of products like MDA at inflammatory sites could be related to a higher level of oxidative stress in periodontitis patients. The results of our study were similar to Miricesue et al^[31], Novakovic et al^[32] which showed reduction of antioxidant status in periodontitis. Urate, ascorbate and albumin appear to contribute most of the antioxidant protection in whole saliva. Moore et al^[33] suggested that urate contributes in excess of 70% of salivary TAA therefore decreased level of uric acid results in lowered antioxidant capacity. Our findings were similar to Tsai et al^[34] who found that salivary glutathione concentrations were significantly reduced in periodontitis subjects relative to controls and that treatment increased glutathione concentrations. These study findings suggest that the equilibrium between the free radicals/ROS and antioxidant is the main prerequisite for healthy periodontal tissue. The disturbance of this equilibrium either due to increased free radicals or decreased (or insufficiently increased) antioxidant might result in oxidative damage to periodontal tissue.

CONCLUSION

It can be concluded from the study that oxidative stress plays an important role in the pathology and progression of periodontitis. Thus appropriate antioxidant therapy along with conventional treatment can be used to prevent and treat chronic periodontitis patients.

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