



Superoxide Dismutase, Uric Acid, Total Antioxidant Status, and Lipid Peroxidation Assay in Chronic and Aggressive Periodontitis Patients

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ABSTRACT

Aim: It has been suggested that periodontitis may be associated with increased oxidative stress. The objective of this study is to evaluate the possible differences in antioxidant status between chronic periodontitis (CP) and aggressive periodontitis (AP), by assessing the concentrations of antioxidants with total antioxidant status (TAS) and lipid peroxidation status in serum and gingival crevicular fluid (GCF) of these patients.

Materials and methods: Forty-six patients with CP, 32 patients with AP, and 50 healthy controls were included in this study. The level of enzymatic antioxidant, superoxide dismutase (SOD), nonenzymatic antioxidant uric acid, and TAS with lipid peroxidation measured in serum and GCF of patients suffering from CP and AP were compared with the healthy controls.

Results: The TAS is decreased and malondialdehyde (MDA) level is increased in both serum and GCF in CP and AP compared with healthy controls ($p < 0.001$). Superoxide dismutase activities in GCF and serum are found to be low in both the groups of periodontitis ($p < 0.001$). The uric acid levels are found to be inconsistent in GCF and serum in both the groups of periodontitis.

Conclusion: Lipid peroxidation and TAS were affected at systemic level in serum and in GCF of the periodontal pockets, in CP and AP. Similar comments may be made for the decrease in SOD activities and inconsistent uric acid levels.

Clinical significance: Increased oxidative stress may have a role in the pathogenesis of periodontal disease activity.

Keywords: Antioxidants, Blood investigation, Gingival crevicular fluid, Periodontitis, Serum.

How to cite this article: Narendra S, Das UK, Tripathy SK, Sahani NC. Superoxide Dismutase, Uric Acid, Total Antioxidant Status, and Lipid Peroxidation Assay in Chronic and Aggressive Periodontitis Patients. *J Contemp Dent Pract* 2018;19(7):874-880.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Periodontitis is one immune-mediated inflammatory disease characterized by destruction of the periodontal attachment apparatus, caused by specific microorganisms or groups of microorganisms. This disease progresses with recurrent acute episodes of bursts of destructive action on periodontium, with higher frequency during certain periods of an individual's life and can be of chronic or aggressive in nature.¹⁻³ During the progression of periodontal disease, the host response gets triggered by pathogenic biofilm. Reactive oxygen species (ROS) are liberated by inflammatory cells migrated into periodontal tissue and gingival sulcus, during the immune response due to host and bacteria interaction. O^{2-} radicals get converted into different radical and nonradical derivatives like hydroxyl radical (OH \cdot), hypochlorous acid (HOCl \cdot), hydrogen peroxide (H $_2$ O $_2$), and singlet oxygen (1O_2) after being released into phagolysosome and extracellular environment.⁴ Such delivery of antimicrobial ROS by neutrophils involves respiratory burst activity, phagocytosis, and cytolysis.

Antimicrobial components of neutrophils also include nicotinamide adenine dinucleotide phosphate oxidase system, myeloperoxidase, lysozyme, cofactor-binding proteins, defensins, and neutral serine proteases.⁵ It has been hypothesized that hyperactivity to plaque organisms is also shown by peripheral neutrophils to release

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ROS, after Fc-gamma-receptor stimulation, in addition to unstimulated generation of ROS through innate immune response.^{6,7}

Oxidative stress is caused by the shifting of balance toward oxidants during the interaction of oxidants and antioxidants. Pathophysiological conditions elevate the rate of normal productions of oxidants during aerobic metabolism. Antioxidants provide defense through both enzymatic and nonenzymatic pathways.^{8,9} The periodontal treatment includes mechanical debridement procedures with or without antibiotics. The possibility of enhancing the effectiveness of local treatment like mechanical debridement procedures, scaling, and periodontal surgery, by adjunctive antibiotics has been explored by many investigators.

Use of systemically administered antibiotics has not been supported during routine therapy for adult chronic variety of periodontitis, by many clinical studies. However, the use of systemic antibiotics in the treatment of specific periodontal diseases is very much supported.¹⁰ Periodontal disease like localized juvenile periodontitis (LJP) needs optimal treatment with systemic antibiotic therapy along with mechanical debridement procedures, as the role of the causative microbe *Aggregatibacter actinomycetemcomitans* invading the periodontal tissue cannot be disputed.¹¹ Production of superoxide by polymorphonuclear (PMN) leukocytes at a higher level may damage not only periodontopathic bacteria but also PMNs themselves, which could be correlated as one of the causes of the periodontal tissue destruction during host–bacteria interaction. Intracellular and extracellular oxygen radical production by PMNs phagocytosing *Porphyromonas gingivalis* and *A. actinomycetemcomitans* is less than PMNs phagocytosing *Fusobacterium nucleatum*.¹² The less the total antioxidant capacity, the more is the degree of the loss of clinical attachment due to tissue destruction, found in periodontitis.

The total oxidant status (TOS) in saliva of periodontitis patients was found to be the same when compared with healthy controls.¹³ Rapid tissue destruction that is associated with severe form of periodontitis like AP may be due to the reduced total antioxidant capacity of these patients.¹⁴

With this background, the present study was carried out to find out any possible difference at systemic and at local level, by assessment of one enzymatic antioxidant SOD, another nonenzymatic antioxidant uric acid, TAS, and lipid peroxidation status in serum and GCF from periodontal pockets of CP and AP patients compared with healthy controls.

MATERIALS AND METHODS

The study was undertaken on 128 patients, dividing them into three groups, group I—healthy control, group II—

CP, and group III—AP. The diagnosis was made based on the clinical and radiographic criteria proposed by the International World Workshop of American Academy of Periodontology, for “classification of periodontal diseases and conditions” in 1999.³ The AP was differentiated from CP based on the criteria of case definition of world workshop, i.e., rapid attachment loss due to bone destruction associated with inconsistent microbial deposit in younger healthy individuals.^{3,15} Out of 128 subjects, 50 subjects in group I included 33 males, 17 females, aged between 20 and 52 years, with an average age 36.56 ± 6.26 ; 46 patients in group II included 29 males, 17 females, aged between 30 and 62 years, with an average age 47.13 ± 7.00 ; and 32 patients in group III included 21 females, 11 males, aged between 18 and 28 years, with an average age 25.71 ± 3.51 .

For CP, it was ensured that the patients had pocket depth ≥ 4 mm in more than 1/3rd of the total number of teeth, with association of bleeding on probing. Periodontal breakdown was observed either from the clinical or from radiographic follow-up examinations. Aggressive generalized/localized loss of attachments was determined from the records of rapid destruction during last 6 months. The plaque index (PI) was evaluated for all these groups to differentiate the aggressive from the chronic one, ensuring that the amount of accumulated plaque was well correlated with the amount of clinical attachment loss in group II, whereas it was not commensurate for group III. The cases of periodontitis were diagnosed as CP and AP based on the case definitions and finally selected following the exclusion and inclusion criteria. All such selected cases were subjected to biochemical evaluation.

Exclusion Criteria

- Patients with any other systemic disorders
- Patients receiving periodontal treatment
- Patients taking antibiotics or anti-inflammatory or any other drugs for any other disease
- Patients having the habits of smoking or alcohol
- Patients during the time of pregnancy/lactation/menopause

Inclusion Criteria

All the subjects living under the same geographic and climatic condition with more or less similar pattern of living style, food habits, and oral hygiene habits were included in this study. Informed consents were obtained from the participants. The study was approved by the institutional ethical committee.

Clinical Measurements

Periodontal bone support was determined by taking full mouth periapical radiographs. Screening for the

periodontal status of all the patients was done by measuring the clinical attachment level (CAL), gingival index (GI),¹⁶ sulcus bleeding index (SBI),¹⁷ and PI.¹⁸ Clinical attachment levels were recorded at six sites of the teeth. All clinical measurements were done by a single investigator.

Collection of Samples

Collection of GCF

The GCF samples were collected from the periodontal pockets with pocket depth ≥ 4 mm in both the groups of periodontitis and from the gingival sulcus with sulcus depth 1 mm in healthy controls. The GCF samples were collected from each patient after isolation and air drying without getting contaminated by saliva. Mostly upper teeth in anterior region were preferably included for avoiding the contamination of saliva in healthy controls. Standardized paper strips (Oraflow Inc., Amityville, New York, USA), using the orifice method from six sites of each patient, were collected in a similar way by the same investigator from groups II and III. Samples were pooled into Eppendorf tubes separately and frozen at -80°C in liquid nitrogen until the assay. Six GCF samples were collected from each patient. The GCF samples were collected in the same way from the subjects in the control group.

Serum sampling: Blood drawn from vein and collected in tubes without anticoagulant was centrifuged at 3000 rpm for 10 minutes for separating the serum. Samples were aliquoted into cryogenic vials and stored in liquid nitrogen in -80°C until the assay.

Biochemical Laboratory Studies

Assay of MDA

The lipid peroxidation (MDA) was measured by the method described by Satoh;¹⁹ 0.5 mL of serum or GCF sample pooled was taken in a centrifuge tube to which 2.5 mL of 20 mg/dL trichloroacetic acid (TCA) was added. The tube was left in room temperature for 10 minutes. After centrifugation at 3500 rpm for 10 minutes, the supernatant was decanted. The precipitate was washed with 0.05 M sulfuric acid, 2.5 mL 0.05 M sulfuric acid, and 3 mL of 0.2 mg/dL Thiobarbituric acid (TBA) in 2 M sodium sulfate were added to this precipitate and the coupling of lipid peroxide with TBA was carried out in the boiling water bath for 30 minutes. After cooling in cold water, the resulting chromogen was extracted with 4 mL of butyl alcohol by vortex mixture. The separation of organic phase was facilitated by centrifugation at 3000 rpm for 10 minutes and its absorbance was determined at the wavelength of 530 nm. Working standard was made by dissolving 1, 1, 3, 3-tetraethoxypropane in 0.5 M

sulfuric acid to prepare a 10 nmol/mL solution. Standard was processed the same way as test and for blank instead of serum distilled water used.

Assay of TAS

The TAS was estimated by using kit (Randox total antioxidant status manual kit), 2, 2'-Azino-di-3-ethylbenzthiazoline sulfonate was incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation ABTS^{•+} which had a relatively stable blue-green color and was measured at 600 nm. Antioxidants in the added sample caused suppression of this color production to a degree which was proportional to their concentration. All the observations of the assay results were expressed as mmol/L.

Assay of SOD

Clinical assay of SOD was done by the method described by Sun et al;²⁰ 2.45 mL of assay reagent [0.3 mM of xanthine, 0.6 mM Na_2EDTA , 0.15 mM nitroblue tetrazolium (NBT), 0.4 M Na_2CO_3 , and 1 gm/L bovine serum albumin] was taken; 0.5 mL sample (serum or GCF sample pooled) was added to the assay reagent and mixed. Xanthine oxidase (50 μL , 167 U/L) was added to initiate the reaction; the reduction of NBT by superoxide anion radicals, which were produced by xanthine, xanthine oxidase system, was determined by spectrophotometer at 560 nm absorbance. The activity of SOD was expressed in U/mL.

Assay of Uric Acid

Assay of uric acid of serum and GCF was done by using standard commercial kit, Uricase-TOPS method. Uric acid was acted by uricase to produce allantoin, hydrogen peroxide, and carbon dioxide. Hydrogen peroxide was cleaved by peroxidase enzyme in the presence of 4-aminoantipyrine and TOPS {N-ethyl-N(sulfopropyl)-3-methylaniline} formed a violet color complex which was measured by BIOLIS 24 I at the absorbance of 546 nm. All values were expressed in mg/dL. All these biochemical assays were conducted in the Department of Biochemistry, S.C.B. Medical College.

Statistical Analysis

All the values were expressed as mean \pm standard deviation. The comparison of different parameters and observations of assays between different groups was done by one-way analysis of variance using *post hoc* test to find out the test of significance ($p < 0.05$). The analysis was done by using Statistical Package for the Social Sciences version 24.

RESULTS

Demographic Characters and Clinical Parameters

All the demographic characters and clinical parameters for all the groups are listed in Tables 1 and 2. Significant difference in mean age was found between groups II and III (Table 1).

The clinical parameters CAL, GI, and SBI were found to be significantly on higher side in the CP and AP groups when compared with the control group ($p < 0.001$). The PI is also found to be less in group III when compared with group II ($p < 0.001$) (Table 2).

Biochemical Laboratory Findings

Serum Analysis

The values of serum MDA were found to be at a higher level in both CP and AP, when compared with the healthy control group ($p < 0.001$). The values of MDA levels in AP were found to be on the higher side when compared with CP ($p < 0.001$). The values of activities of SOD and TAS in CP and AP were found to be less compared with the control group ($p < 0.001$). The decreased activities of SOD and TAS in serum are also found to be significant in AP compared with CP. The uric acid level is found to be inconsistent in all groups. Serum analysis of MDA, TAS, SOD, and uric acid is displayed in Table 3 and Graph 1.

GCF Analysis

The values of MDA in GCF were found to be on the higher side in both CP and AP when compared with the control group ($p < 0.001$). The MDA levels were also on the higher side in AP compared with CP ($p < 0.001$). There

Table 1: Demographic characteristics of different study groups

	Control (n = 50) (group I)	CP (n = 46) (group II)	AP (n = 32) (group III)
Age (in years)	36.56 ± 6.26	47.13 ± 7.00	25.71 ± 3.51*
Sex			
Male	33	29	11
Female	17	17	21

*Significant difference in mean age between groups II and III ($p < 0.001$)

Table 2: Clinical parameters of different groups at baseline (cross-sectional analysis at baseline)

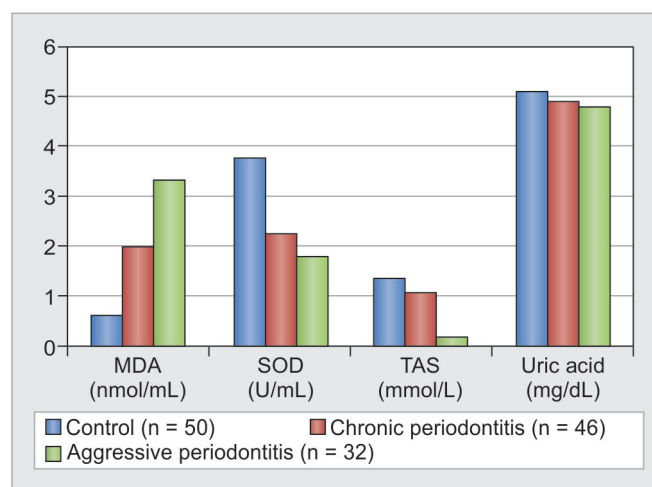
Parameters	Groups		
	Control (n = 50)	CP (n = 46)	AP (n = 32)
CAL	1.49 ± 0.25	4.51 ± 0.5*	4.73 ± 0.51*
GI	0.52 ± 0.15	2.44 ± 0.17*	2.53 ± 0.22*
PI	0.65 ± 0.13	2.47 ± 0.1**	0.94 ± 0.24**
SBI	0.62 ± 0.21	2.48 ± 0.19*	2.61 ± 0.12*

*Significant increase in clinical parameters in the CP and AP groups when compared with the control group; **Significant decrease in PI of group III when compared with group II ($p < 0.001$)

Table 3: Comparison of biochemical parameter in control, chronic, and aggressive periodontitis in serum

Biochemical parameter	Control (n = 50)	CP (n = 46)	AP (n = 32)
MDA (nmol/mL)	0.59 ± 0.14	2.02 ± 0.32*	3.61 ± 0.39*
SOD (U/mL)	3.95 ± 0.26	2.73 ± 0.29**	1.55 ± 0.15**
TAS (mmol/L)	1.23 ± 0.22	0.82 ± 0.12**	0.16 ± 0.03**
Uric acid (mg/dL)	5.11 ± 0.54	5.12 ± 0.32#	5.07 ± 0.30#

*The value of serum MDA shows a significant rise in both CP and AP when compared with the control group ($p < 0.001$); **There is a significant decrease in values of serum SOD and TAS in CP and AP in respect to control group ($p < 0.001$); #There is no significant variation in the uric acid value in all groups



Graph 1: Comparison of MDA, SOD, TAS, and uric acid in chronic and aggressive periodontitis in serum

Table 4: Comparison of MDA, SOD, TAS, and uric acid in CP and AP in GCF

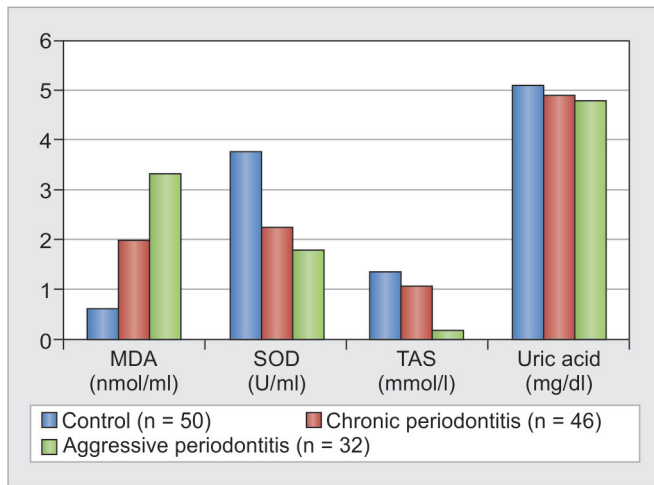
Biochemical parameter	Control (n = 50)	CP (n = 46)	AP (n = 32)
MDA (nmol/mL)	0.63 ± 0.12	1.98 ± 0.32*	3.33 ± 0.38*
SOD (U/mL)	3.78 ± 0.32	2.26 ± 0.31**	1.8 ± 0.45**
TAS (mmol/L)	1.36 ± 0.11	1.10 ± 0.26**	0.21 ± 0.06**
Uric acid (mg/dL)	5.11 ± 0.53	4.91 ± 0.40#	4.82 ± 0.29#

*The value of MDA in GCF shows a significant rise in both CP and AP when compared with the control group ($p < 0.001$); **there is a significant decrease in values of SOD and TAS in GCF in CP and AP in respect to control group ($p < 0.001$); #There is no significant variation in the uric acid value in all groups

is a significant decrease in values of activities of SOD and TAS in GCF in CP and AP compared with the control group ($p < 0.001$). The decrease in levels of activities of SOD and TAS in GCF is also found to be significant in AP compared with CP. There is no significant variation in uric acid levels in all groups. Analysis of MDA, TAS, SOD, and uric acid in GCF is displayed in Table 4 and Graph 2.

DISCUSSION

Interests among the investigators have been observed for assessing the possible differences in local and systemic



Graph 2: Comparison of MDA, SOD, TAS, and uric acid in chronic and aggressive periodontitis in GCF

antioxidant status between CP and AP. Reactive oxygen species are liberated by inflammatory cells during the periodontal destruction due to alteration in host response to pathogenic biofilm, inside the periodontal tissue and gingival sulcus. Different radical and nonradical derivatives like hydroxyl radical (OH^-), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1\text{O}_2^-$) are released into phagolysosome and extracellular environment. Delivery of such antimicrobial molecular agents by neutrophils involves respiratory burst activity, phagocytosis, and cytolysis/apoptosis. Antioxidants provide defense through both enzymatic and non-enzymatic pathways to balance the activity of oxidants to prevent oxidative stress. The decision for use of systemic antibiotics or mechanical debridement and local drug delivery systems depends upon the type and pattern of periodontal disease. Periodontal disease like AP needs optimal treatment with systemic antibiotic therapy with mechanical debridement, whereas CP needs mechanical debridement and local drug delivery systems. It has been hypothesized that the less the total antioxidant capacity at both local and systemic levels, the more is the degree of loss of clinical attachment due to periodontal tissue destruction. The TOS in saliva of periodontitis patients was found to be the same when compared with healthy controls. Severe tissue destruction associated with AP may be due to the reduced total antioxidant capacity of these patients.¹⁻¹⁴

The present study was carried out for the assessment of one enzymatic antioxidant, SOD, another nonenzymatic antioxidant uric acid, TAS, and lipid peroxidation status in serum and GCF of CP and AP patients compared with healthy controls. The objective of the assessment was for observing the possible differences in local and systemic antioxidant status between CP and AP. As per the case definition of 1999 workshop of the American

Academy of Periodontology, the AP differs from CP based on the criteria of rapid attachment loss due to bone destruction associated with inconsistent microbial deposit in younger healthy individuals. Clinical parameters were assessed by using GI, SBI, and PI, and biochemical assays were done in the Department of Biochemistry.^{3,15-20}

It was stated that most common inflammatory cells believed to be the predominant source of ROS in periodontitis are PMN leukocytes. It has been found that there was no significant difference in the hydrogen peroxide-dependent oxidative product formation by PMN leukocytes in the blood from the patients with various types of periodontal diseases, like LJP, generalized juvenile periodontitis, and adult periodontitis.²¹

The hyperreactivity of peripheral neutrophils in periodontitis was compared with that with normal individuals. It was found that hyperreactivity to opsonized bacteria seems to be related to the stimulation of factors like tumor necrosis factor- α to the outer cell membrane lipopolysaccharides, or to the liberation of ROS. No significant difference was found in hydrogen peroxide production between the periodontal patients and the controls during liberation of ROS.²²

Consistent results have shown that peripheral blood neutrophils of people with CP or AP generate significantly more ROS upon simulation with purified immunoglobulin opsonized *Staphylococcus aureus* compared with peripheral blood neutrophils of healthy controls, suggesting that sites with periodontal destruction have a hyperreactive phenotype of neutrophils and those can be stimulated by the Fc-gamma receptor pathway.²³

Investigations on TOS revealed that patients with CP were having increased lipid peroxidation levels and oxidative stress both at systemic level and at local sites.²⁴

Total oxidative status, SOD, and MDA levels were significantly higher in the patients suffering from CP when compared with the healthy control group. Local and systemic control and restoration of the subject's antioxidant capacity by nonsurgical therapy can be achieved in patients suffering from CP.²⁵ Oxidative stress can be reduced in patients suffering from CP through periodontal treatment in smokers and nonsmokers.²⁶

Antioxidant status is found to be increased, reducing the oxidative stress after periodontal treatment in patients suffering from CP with familial Mediterranean fever and also in patients with CP alone.²⁷ The possible role of oxidative stress for tissue destruction during progression of periodontitis cannot be ruled out. The total antioxidant capacity of saliva was found to be lower in patients suffering from periodontitis than the healthy controls. The more the loss of clinical attachment due to tissue destruction, the less is the total antioxidant capacity found in the patients suffering from periodontitis. The difference in the

salivary TOS between periodontitis patients and healthy controls was not significant.¹³ Severe tissue destruction associated with aggressive form of periodontitis may be due to the reduced total antioxidant capacity of the patients.¹⁴

Action of antioxidant enzyme SOD triggered against superoxide, ROS, in inflammatory pathways is for protection against connective tissue breakdown. There may be an increase in SOD activity in gingival connective tissue for protection against destruction without any change in its level in GCF.²⁸

One of the approaches to demonstrate the involvement of oxidative stress in the pathophysiologic mechanisms of periodontal tissue destruction is the assay of end products of lipid peroxidation. Lipids are among the most readily oxidizable substrates. Increased lipid peroxidation is one of the consequences of ROS-induced tissue damage. Significant changes in MDA levels with little or no change in antioxidant status were found in saliva and serum of patients with CP, indicating increased oxidative stress.^{24,25,29,30}

The review on studies regarding the activities of enzymatic antioxidants revealed that the results of the studies are contradicting to each other. Some studies showed that increase in SOD is associated with periodontitis, whereas others showed increased activities of enzymatic antioxidants as a protective reaction found among the people with periodontitis. The results of studies on total antioxidant capacity are consistent and indicate compromised antioxidant capacity in periodontitis.³⁰ Saliva of periodontitis patients revealed reduced activity of uric acid. The activity of uric acid is also negatively correlated with C-terminal telopeptide of type I collagen and matrix metalloproteinases.³¹ The values of uric acid were found to be within the normal limits in the patients suffering from periodontitis. However, there is statistically significant difference between the values of uric acid in periodontitis patients when compared with normal individuals even if values in both the groups were within normal limits.³² Ellis et al³³ found reduced activities of SOD and catalase when these activities were measured in human gingival tissue with the increase in periodontal pocket depth. Canakci et al³⁴ found the activities of SOD and glutathione peroxidase in saliva to be decreased in periodontitis patients. In contrast to these studies, the elevated activities of enzymatic antioxidants like SOD and catalase measured in gingival tissues and plasma, in periodontitis patients, were found by Panjamurthy et al.³⁵ Similarly, higher activities of enzymatic antioxidants including SOD was shown in the saliva of periodontitis patients by Novakovic et al.³⁶

In this study, GCF and serum MDA levels were found to be on a higher side in both CP and AP when compared

with the healthy control group ($p < 0.001$). The MDA levels were also found to be more in AP compared with CP ($p < 0.001$). The values of activities of serum SOD and TAS in CP and AP were found to be less when compared with healthy controls ($p < 0.001$). There was also decrease in serum levels of SOD and TAS in AP compared with CP. Levels of uric acid in GCF and serum of patients in both the groups of periodontitis were found to be inconsistent.

CONCLUSION

These results are in agreement with earlier studies revealing the facts that reduced antioxidant status and increase of oxidative stress may have a role in playing as a risk factor in the pathogenesis of CP and AP.

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