ABCResearch

Arch Basic Clin Res 2024; 6(3): 228-234 • DOI: 10.5152/ABCR.2024.24295

Original Article

# Oxidant and Antioxidant Parameters in Serum and Saliva Samples of Patients with Periodontal Disease

Yerda Özkan Karasu[1], Fatma Öner[2], Çağrı Onat[1], Lale Duysak[3], Neslihan Yüce[4]

[1]Department of Periodontology, Atatürk University Faculty of Dentistry, Erzurum, Türkiye
[2]Department of Periodontology, Bahçeşehir University Faculty of Dentistry, Istanbul, Türkiye
[3]Department of Biochemistry, Atatürk University Faculty of Pharmacy, Erzurum, Türkiye
[4]Department of Medical Biochemistry, Atatürk University Faculty of Medicine, Erzurum, Türkiye

**Cite this article as:** Özkan Karasu Y, Öner F, Onat Ç, Duysak L, Yüce N. Oxidant and antioxidant parameters in serum and saliva samples of patients with periodontal disease. *Arch Basic Clin Res.* 2024;6(3):228-234.

**ORCID iDs of the authors:** Y.Ö.K. 0000-0002-7333-0524, F.Ö. 0000-0001-6193-2029, Ç.O. 0000-0002-9281-8699, L.D. 0000-0001-7872-3880, N.Y. 0000-0001-6878-0646

## ABSTRACT

**Objective:** Oxidative stress has crucial roles in the pathogenesis of periodontal disease. The balance between oxidant and antioxidant biochemical parameters is detrimental in the progression of periodontal diseases. This study aims to assess the levels of myeloperoxidase (MPO), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) in serum and saliva samples from patients who have periodontal disease.

**Methods:** The study enrolled on 88 individuals divided into 4 groups: (1) periodontally healthy, (2) gingivitis, (3) stage I-II periodontitis, and (4) stage III-IV periodontitis. The participants had their serum and saliva samples taken. Clinical periodontal parameters were measured to determine the periodontal status. Then MPO, MDA, CAT, SOD, and GSH expressions were analyzed with appropriate methods.

**Results:** Salivary-MPO levels improved in parallel with the periodontal disease's severity. The healthy patients had the highest serum-MDA expressions, which decreased as the severity of the disease increased. Glutathione and SOD concentrations were similar in each group. Catalase expressions in serum reached the highest level in stage I-II periodontitis patients. Nonetheless, there were no significant differences between the groups.

**Conclusion:** In conclusion, our results revealed that GSH, SOD, MDA, CAT, and MPO levels are not affected by the progression of periodontal diseases.

**Keywords:** Antioxidant, oxidative stress, periodontal diseases, periodontitis

## INTRODUCTION

Periodontal disease is an inflammatory condition of the gingiva and tooth-supporting tissues, resulting in tooth loss in its severe forms. Microbial dental plaque accumulation on the tooth surface is the primary etiologic cause of periodontal disease; however, progression of the disease depends on the imbalance between oral microbiota and host response in susceptible individuals.[1] Recently it has been well studied that oxidative stress has crucial roles in the pathogenesis of many systemic diseases including periodontal disease. Moreover, oxidative stress has been detected as a link between periodontitis and systemic diseases.[2]

Oxidative stress is primarily caused by the excess production of reactive oxygen species (ROS). Reactive oxygen species is formed with neutrophils via an oxygen-dependent defense mechanism and has crucial roles in cell signaling and gene regulation. Reactive oxygen species also has an antimicrobial effect against pathogenic microorganisms; however, it can also cause pathological tissue destruction in its overactivated conditions.[3] Systemic involvement of hyperactivated ROS in periodontitis and its strong relation with disease progression have been shown previously in different studies.[4-6]

Myeloperoxidase (MPO) is an enzyme mainly secreted by neutrophils and has very effective antibacterial properties

**Corresponding author:** Yerda Özkan Karasu, **E-mail:** yerdaozkan@hotmail.com



**Received:** August 12, 2024
**Revision Requested:** September 16, 2024
**Last Revision Received:** September 17, 2024
**Accepted:** September 23, 2024
**Publication Date:** October 15, 2024

through hypochlorous acid (HClO) formation.[7] However, its excess release outside the cell during oxidative stress causes oxidation of cell components including deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein; tissue damage; and eventually elevated inflammation and impaired biological functions.[8] Oxidative stress leads to the interaction of ROS with polyunsaturated fatty acids, causing lipid peroxidation and exacerbating uncontrollably occurring oxidative stress.[9] Malondialdehyde (MDA) is the end product appearing due to the breakdown of polyunsaturated fatty acids by oxidative stress, and it damages the cell membrane's functions and integrities.[10] Known as strong indicators of oxidative stress, elevated expressions of both MDA and MPO in periodontitis lesions have been well studied.[11-13]

Through enzymatic and non-enzymatic processes, antioxidant mechanisms are established to prevent tissue damage induced by oxidative stress. Enzymatic antioxidant defense mechanisms include superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) production; meanwhile, glutathione (GSH), vitamin C, and vitamin E are part of the non-enzymatic antioxidant defense mechanisms of the host.[14] Antioxidants are critical in eliminating the harmful effects of oxidative stress on host cells.[15] Reducing oxidative stress through medications or natural extracts has been shown to improve the periodontal status in animal models.[16,17] Previous studies have shown lower total antioxidant capacity in periodontitis that could be a reason for the progression of periodontal disease.[15,18]

Reactive oxygen species formation is a very fast process and ends up quickly, hence it is not stable biochemically; therefore, it is not easy to compare the ROS levels and the periodontal status of patients. Instead, measuring destructive products of ROS and antioxidant levels is preferred as an easier and accurate way to evaluate the effect of oxidative stress in the pathogenesis of periodontal disease.[19] In this study, we tested the hypothesis that an imbalance between antioxidant defense mechanisms and oxidative parameters results in the progression of periodontal disease. In this context, we aimed to investigate MPO, MDA, SOD, CAT, and GSH expressions in the serum and saliva of healthy, gingivitis, and stage I-II-III-IV periodontitis patients. By investigating both sides of the content, we also aimed to detect whether excess free radical production and/or defects in antioxidant defense mechanism cause the progression of periodontal disease.

### MAIN POINTS

- Periodontal disease development has no impact on GSH, SOD, MDA, CAT, or MPO levels.
- Compared to the stage I-II periodontitis group, the stage III-IV periodontitis group had considerably higher levels of bleeding on probing (BOP) and interdental clinical attachment level (iCAL).
- Positive relationships were discovered between serum MDA and CAT levels and periodontal pocket depth, as well as between bleeding upon probing and serum MPO expression.
- Probing revealed a positive connection between salivary GSH and MPO concentrations and bleeding.

## MATERIAL AND METHODS

### Study Population

This cross-sectional study included 88 patients (36 females and 52 males) who applied to Atatürk University, Türkiye. Participants were chosen from among the systematically healthy patients who were not smoking (never smoked or quit smoking at least 6 months ago), had not received antibiotic/anti-inflammatory therapy or periodontal treatment in the last 6 months, and had no pregnancy or lactation status. Patients were divided into 4 groups: (1) periodontally healthy, (2) gingivitis, (3) stage I-II periodontitis, and (4) stage III-IV periodontitis.

The Atatürk University School of Medicine's Ethics Committee granted approval for the study's use of human samples for research purposes (B.30.2.ATA.0.01.00/463, Date: 12.07.2024), and it was conducted in compliance with the Helsinki Declaration. Each participant received an explanation of the study's approach and gave signed informed consent prior to periodontal therapy.

### Saliva and Serum Sampling

Before the periodontal examination, each patient had unstimulated whole saliva samples taken in the morning at the same time interval. The instructions for the participants were to sit comfortably, collect saliva in their mouths, and spit into 5-mL polypropylene tubes (ISOLAB SantrifugeTube, Eschau, Germany).[20] Venous blood samples were taken from the antecubital vein using the conventional venipuncture technique. The acquired blood was allowed to coagulate at room temperature for 2 hours in order to prevent clotting. Saliva and blood samples were then centrifuged at 1000 × g for 20 minutes and transferred into tubes. All samples were maintained at −80◦C until they were analyzed biochemically.

### Clinical Diagnosis and Periodontal Examination

One calibrated examiner (YOK) completed all periodontal measures. Using a periodontal probe (Williams, Hu-Friedy, Chicago, IL), 6 sites (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, and disto-lingual) of each tooth—aside from third molars—were measured for plaque index (PI), bleeding on probing (BOP), probing depth (PD), and interdental clinical attachment level (iCAL). The clinical

and radiographic evaluations were used to diagnose the patients, and the consensus reports from the "2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions" were used to categorize the patients (Table 1).[21,22]

### Biochemical Analyses of Glutathione, Malondialdehyde, Myeloperoxidase, Superoxide Dismutase, and Catalase

#### Determination of Myeloperoxidase

The basis for this measurement is the kinetic analysis of the absorbance of the complex with a yellowish-orange color that is produced when o-dianisidine is oxidized with MPO in the presence of hydrogen peroxide at a wavelength of 460 nm.[23]

#### Determination of Lipid Peroxidation (Malondialdehyde)

The method relies on measuring the absorbance of the pink complex created by thiobarbituric acid and MDA using spectrophotometry at a wavelength of 532 nm following a 60-minute incubation time at 95°C.[24] About 200 μmol/L of 1,1,3,3-tetraethoxypropane was used to create a stock standard solution. Standard solutions were produced by serial dilution from the stock standard at various concentrations (200 μM-0.78 μM).

#### Determination of Superoxide Dismutase

Superoxide dismutase enzyme activity measurement was evaluated according to Sun et al.[25] This method works on the premise that when nitro blue tetrazolium (NBT) is present in the sample, the SOP enzyme's free radicals are inhibited during the reduction of the free oxygen radicals created by the enzymatic process. Spectrophotometric analysis measures the color shift that results from the reaction at 560 nm.

#### Determination of Total Glutathione

Measurement of total GSH was done using the Sedlak and Lindsay method.[26] Incubation was carried out for 30 minutes at 37°C, and absorbance at 412 nm was recorded. The standard was 2-0.0312 mM reduced glutathione. By using the findings of standard measurements and their matching values in the graph from absorbances, the total amount of GSH was calculated.

**Table 1.** Guidelines for Diagnosing Periodontal Disease

| **Periodontally Healthy** | **<10% BOP, ≤3 mm PD** |
|---|---|
| Gingivitis | >10% BOP, ≤3 mm PD |
| Stage I periodontitis | 1-2 mm greatest iCAL,<br>Bone loss in the coronal third of the root |
| Stage II periodontitis | 3-4 mm greatest iCAL,<br>Bone loss in the coronal third of the root |
| Stage III periodontitis | ≤5 mm iCAL<br>Bone loss extending to mid-third and beyond<br>Having 4 or less tooth loss due to periodontitis |
| Stage IV periodontitis | ≤5 mm iCAL<br>Bone loss extending to mid-third and beyond<br>Having 5 or more tooth loss due to periodontitis |

#### Determination of Catalase Activity

Using the Campo et al.[27] approach, the catalytic activity in the serum samples was ascertained. In summary, 100 μL of substrate (65 mmol/L hydrogen peroxide in 60 mmol/L phosphate buffer solution, pH = 7.4) was incubated with 20 μL of supernatant for 1 minute at 37°C. Next, 100 μL of ammonium molybdate (32.4 mmol/L) was added to halt the enzymatic process. Using a spectrophotometer, measurements were made at 405 nm to determine the reaction. Pure CAT enzyme was used to create standards, ranging from 940 U/mL to 14.7 U/mL. The CAT activity in the samples was determined using the equation of this curve, which was represented by a standard graph.

### Statistical Analyses

A statistical analysis application, International Business Machines Statistical Package for the Social Sciences (IBM SPSS) 20 (IBM SPSS Corp.; Armonk, NY, USA, was used. The mean, SD, and the number of the data were displayed. The Kruskal–Wallis test was employed when the normal distribution condition was not met, and the Analysis of Variance (ANOVA) test was used when comparing continuous variables with more than 2 independent groups. If there was a normal distribution, 2 quantitative variables were compared using the Pearson correlation; if not, the Spearman correlation test was performed. Covariance analysis was used to assess the biomarker levels and periodontal features after age correction. A *P*-value of less than .05 indicated that a difference was significant.

## RESULTS

### Demographic and Clinical Periodontal Parameters

There was no difference in the individuals' genders; however, the mean age of the stage I-II (42 ± 10 years) and stage III-IV (42 ± 11 years) periodontitis groups was significantly higher than the periodontally healthy (32 ± 7 years) and gingivitis (31 ± 10 years) groups ($P < .05$). Plaque index, BOP, PD, and iCAL levels increased gradually in line with the severity of the disease. The only significant difference between the gingivitis and periodontally healthy group was observed in the BOP score ($P < .05$). Bleeding on probing and iCAL were significantly higher in stage III-IV periodontitis group than in stage I-II periodontitis group ($P < .05$ for BOP, $P < .05$ for iCAL). Patients with stage I-II periodontitis had significantly higher PD than healthy and gingivitis ($P < 0.001$) and also higher PI and BOP scores than patients with gingivitis ($P = .001$).

**Table 2.** Age, Gender, and Clinical Periodontal Parameters

| | Periodontally Healthy | Gingivitis | Stage I-II Periodontitis | Stage III-IV Periodontitis |
|---|---|---|---|---|
| Gender (m/f) | 13M/7F | 12M/10F | 12M/10F | 12M/10F |
| Age (years) | 32 ± 7 | 31 ± 10 | 42 ± 10* | 42 ± 11* |
| PI | 1.3 ± 0.2 | 1.4 ± 0.2 | 1.8 ± 0.3** | 2 ± 0.5* |
| BOP (%) | 5.5 | 18** | 59.6** | 65.8*, ## |
| PD (mm) | 1.4 ± 0.3 | 1.8 ± 0.4 | 2.6 ± 0.5* | 3 ± 0.9* |
| iCAL (mm) | 1.5 ± 0.2 | 1.8 ± 0.5 | 3.8 ± 1.0* | 7.9 ± 1.7*, ## |

Mean ± SD. BOP, bleeding on probing; CAL, clinical attachment level; PD, probing depth; PI, plaque index. *Significantly more than groups with gingivitis and periodontal healthy individuals.**Significantly more than groups with periodontal healthy individuals. #Significantly more than gingivitis groups. ##Significantly more than stage I-II periodontitis groups.

Interdental clinical attachment level was substantially higher in the stage III-IV periodontitis group compared to the stage I-II periodontitis group ($P < .05$) (Table 2).

### Biochemical Parameters

Stage I-II periodontitis had the highest salivary-MPO levels, which were slightly more than gingivitis and stage III-IV patients; however, MPO expressions in serum were similar in each group. Overall, a significant difference was observed in salivary and serum MPO concentrations among groups (Figure 1A). Serum-MDA levels were highest in those in periodontal health, and although they tended to decline with the severity of periodontal disease, this difference was not statistically significant. Gingivitis patients had higher salivary-MDA levels than the other groups, but a significant difference was observed among groups (Figure 1B).

The levels of serum and salivary GSH and SOD in patients with varying degrees of periodontal disease were comparable; these levels were not significantly different from periodontally healthy subjects. Glutathione concentration was higher in serum than in saliva; however, saliva samples had higher SOD concentration than serum samples (Figure 2A-B). Serum-CAT levels reached the highest expressions in stage I-II periodontitis, which were higher than these in gingivitis and stage III-IV groups, but the differences were not significant. There was no difference in salivary-CAT levels among the groups, and its concentration decreased gradually in line with periodontal disease severity (Figure 2C).

### Correlations Between Periodontal and Biochemical Parameters

Serum SOD level was negatively correlated with age, PI, and GI ($P < .008$, $P < .003$, and $P < .013$, respectively). Salivary MPO, salivary catalase, and serum catalase levels were found to positively correlate with PD ($P < .012$, $P < .028$, and $P < .014$, respectively). Serum SOD level and BOP were found to be negatively correlated, while salivary GSH and MPO levels were found to be positively correlated ($P < .013$, $P < .013$, and $P < .027$, respectively).

## DISCUSSION

In this study, we investigated the levels of MPO, MDA, GSH, CAT, and SOD in the saliva and serum of patients with various degrees of periodontal disease severity. Oxidant and antioxidant levels in periodontal disease have

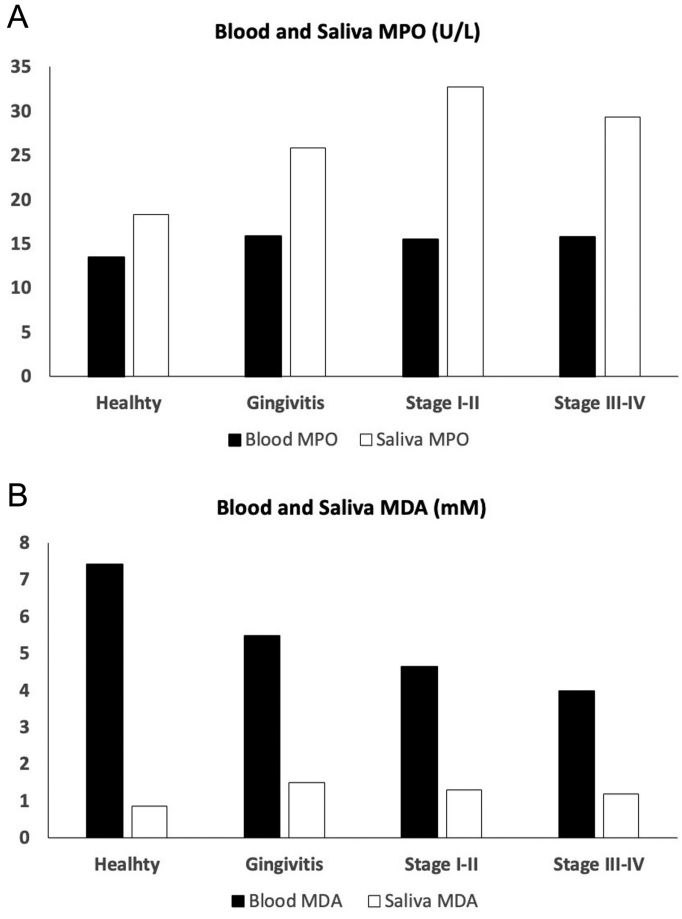


**Figure 1.** (A) Salivary and serum MPO concentrations did not differ significantly across the groups overall; $P > .05$. (B) Serum-MDA levels were highest in those in periodontal health, and although they tended to decline with the severity of periodontal disease, the difference was not statistically significant $P > .05$.

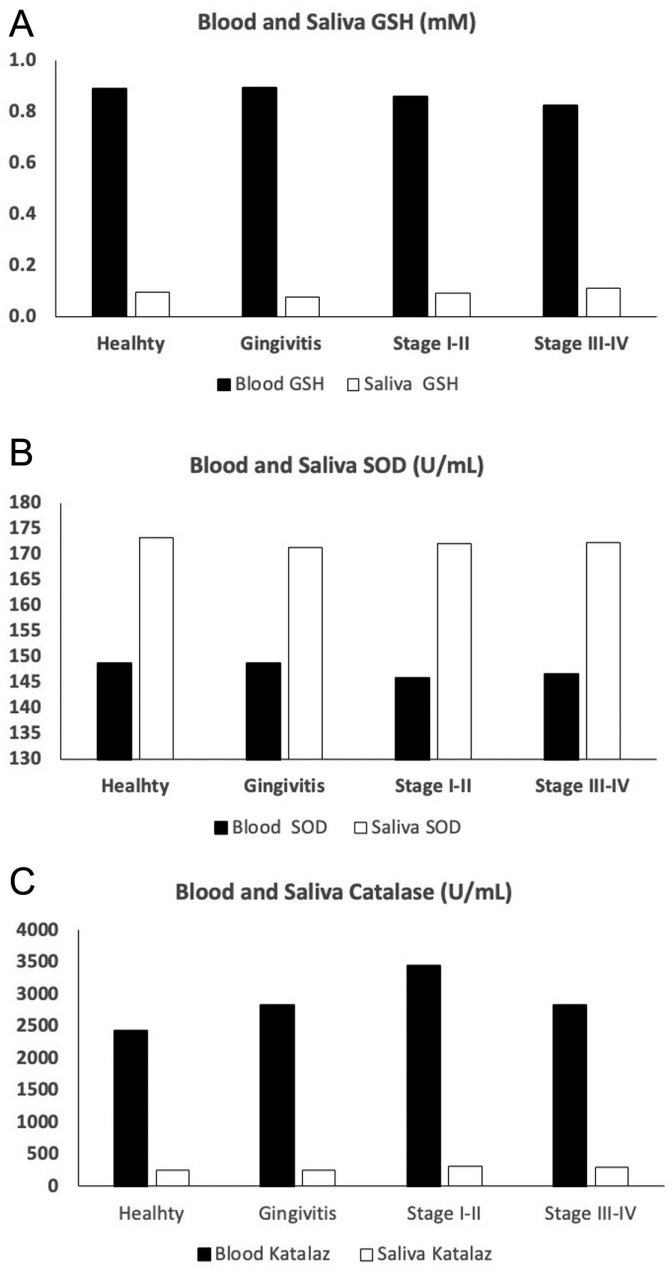


**Figure 2.** (A) Glutathione concentration was higher in serum than in saliva, $P > .05$. (B) Saliva samples had higher superoxide dismutase concentration than serum samples $P > .05$. (C) There was no difference in salivary catalase levels among the groups, $P > .05$.

been evaluated before, but in most studies, saliva and GCF were used for analyses, and systemic impacts of these markers were underestimated. Additionally, periodontal diseases were not classified according to their severities. In our study, we had healthy, gingivitis, and stage I-II and stage III-IV periodontitis groups; therefore, in addition to analyzing the impact of oxidative stress on periodontal disease, we also evaluated the effect of periodontal disease on oxidative stress. Generally, antioxidants are taken in through nutrients, and therefore their systemic impacts are crucial in preventing chronic inflammation. We analyzed the systemic impacts of antioxidants on periodontal disease severity by including serum samples and comparing their local and systemic effects in disease progression.

In our study, stage I-II periodontitis patients had higher yet insignificant salivary MPO levels compared to those in periodontally healthy, gingivitis, and stage III-IV periodontitis groups, but MPO concentrations in serum were similar in each group. In a similar manner, previous studies found that salivary MPO concentrations in chronic periodontitis patients were higher than those in periodontally healthy patients, but serum MPO levels were similar to each other.[28,29] The oral microenvironment could influence the induction of MPO activity. Also, a local increase in MPO levels against periodontal pathogens may be more effective than their systemic impact. Furthermore, our investigation showed that MPO levels in saliva tend to decline as the disease advances to stage III-IV periodontitis. Myeloperoxidase is released with neutrophils upon stimulation with pathogens and is strongly related to promoting inflammation.[8] Since neutrophils take part in the early response against inflammation in the pathogenesis of periodontal disease, it is excepted that MPO levels increase in line with the severity of the disease. In advanced lesions where lymphocytes dominate, the cell profile neutrophil response may decrease, and this could explain the tendency to decrease MPO levels in saliva seen in this study.[30]

Although MDA levels in serum were the same, an increase in saliva-MDA concentration was observed in chronic periodontitis patients,[9] on the other hand, increased salivary and serum MDA concentrations were seen in other studies.[15,18] In our study, there was no significant difference in salivary and serum MDA levels among groups. However, there was a positive correlation between periodontal pocket depth and serum-MDA concentration, but this was not the case for salivary MDA levels. Our results confirm previous studies showing a positive correlation between bleeding on probing and serum MDA levels.[31] This shows us that despite other studies,[9,32] lipid oxidation end products are systematically regulating the progressive tissue destruction in periodontitis.

There are few studies evaluating antioxidant levels in periodontal disease with different severities. It was discovered that the degree of the disease correlates with an increase in superoxide scavenging activity.[33] However, other research indicates that periodontal disease is associated with lower SOD levels.[34,35] Also, in a previous study, SOD activation in gingiva was found to be higher in chronic periodontitis patients, while no difference was observed in SOD levels in GCF samples.[36] Our results revealed that SOD levels in periodontal disease with different severities

did not show any difference in both saliva and serum samples. Superoxide dismutase activity mainly occurs in cells and tissues; therefore, it may be undetectable in body fluid;[37] there is a possibility that changes in SOD levels could not be reflected in our study. Additionally, SOD activity might be suppressed by the excess superoxide radical anion productions in inflammation.[38]

SOD activity influences the GSH concentration through peroxidase production;[39] in our study, GSH levels among groups did not show significant difference, which may be due to the unaltered SOD activity. In accordance with SOD activity, GSH concentrations in chronic periodontitis were elevated compared with those in periodontally healthy individuals.[39] In contrast, higher SOD and GSH expressions in periodontally healthy individuals than in patients with periodontal disease have been shown before.[40] However, similar to our results, it was shown that periodontitis does not influence GSH levels in animal models,[41] and total GSH levels were not affected in chronic periodontitis patients.[42] Similar to other antioxidants, conflicting results are seen in CAT levels; there are studies showing higher CAT activity in periodontitis, but there are also other studies showing vice versa.[43] In our study, stage I-II periodontitis patients have the highest serum-CAT expressions insignificantly more than stage III-IV periodontitis groups. Superoxide anion expressed in inflammation suppresses the activity of CAT enzyme,[44] which could be the reason of unaltered change seen in our study and other studies.[42]

In our study, significant differences in oxidative stress and antioxidant levels were not observed among groups; serum and saliva may not be the ideal medium to analyze these markers as their expressions are higher in GCF than in serum and saliva.[34] Also, periodontal inflammation may suppress the activity of the antioxidant mechanism rather than being under the influence of it.[45] In conclusion, our results revealed that GSH, SOD, MDA, CAT, and MPO levels are not affected by the progression of disease; therefore, they are not appropriate prognostic salivary biomarkers for determining the severity of periodontal disease...

**Data Availability Statement:** The data that support the findings of this study are available on request from the corresponding author.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ethics Committee of Atatürk University (Date: 12.07.2024, Number: B.30.2.ATA.0.01.00/463).

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept – Y.Ö.K., F.Ö.; Design – Y.Ö.K., F.Ö.; Supervision – Y.Ö.K.; Resources – Ç.O.; Materials – Ç.O.; Data Collection and/or Processing – Y.Ö.K., Ç.O.; Analysis and/or Interpretation – L.D., N.Y.; Literature Search – F.Ö.; Writing Manuscript – F.Ö.; Critical Review – Y.Ö.K., F.Ö.; Other – Y.O.K., F.Ö., Ç.O.

**Declaration of Interests:** The authors have no conflict of interest to declare.

**Funding:** The authors declared that this study has received no financial support.

## REFERENCES

1. Slots J. Periodontitis: facts, fallacies and the future. *Periodontol 2000*. 2017;75(1):7-23. [CrossRef]
2. Monea A, Mezei T, Popsor S, Monea M. Oxidative stress: a link between diabetes mellitus and periodontal disease. *Int J Endocrinol*. 2014;2014:917631. [CrossRef]
3. Sczepanik FSC, Grossi ML, Casati M, et al. Periodontitis is an inflammatory disease of oxidative stress: we should treat it that way. *Periodontol 2000*. 2020;84(1):45-68. [CrossRef]
4. Chapple ILC, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontol 2000*. 2007;43:160-232. [CrossRef]
5. D'Aiuto F, Nibali L, Parkar M, Patel K, Suvan J, Donos N. Oxidative stress, systemic inflammation, and severe periodontitis. *J Dent Res*. 2010;89(11):1241-1246. [CrossRef]
6. Tamaki N, Tomofuji T, Maruyama T, et al. Relationship between periodontal condition and plasma reactive oxygen metabolites in patients in the maintenance phase of periodontal treatment. *J Periodontol*. 2008;79(11):2136-2142. [CrossRef]
7. Strzepa A, Pritchard KA, Dittel BN. Myeloperoxidase: a new player in autoimmunity. *Cell Immunol*. 2017;317:1-8. [CrossRef]
8. Lin W, Chen H, Chen X, Guo C. The roles of neutrophil-derived myeloperoxidase (MPO) in diseases: the new progress. *Antioxidants (Basel)*. 2024;13(1). [CrossRef]
9. Akalin FA, Baltacioğlu E, Alver A, Karabulut E. Lipid peroxidation levels and total oxidant status in serum, saliva and gingival crevicular fluid in patients with chronic periodontitis. *J Clin Periodontol*. 2007;34(7):558-565. [CrossRef]
10. Guentsch A, Preshaw PM, Bremer-Streck S, Klinger G, Glockmann E, Sigusch BW. Lipid peroxidation and antioxidant activity in saliva of periodontitis patients: effect of smoking and periodontal treatment. *Clin Oral Investig*. 2008;12(4):345-352. [CrossRef]
11. Lutfioğlu M, Aydoğdu A, Atabay VE, Sakallioğlu EE, Avci B. Gingival crevicular fluid oxidative stress level in patients with periodontal disease and hyperlipidemia. *Braz Oral Res*. 2017;31:e110. [CrossRef]
12. Wei PF, Ho KY, Ho YP, Wu YM, Yang YH, Tsai CC. The investigation of glutathione peroxidase, lactoferrin, myeloperoxidase and interleukin-1beta in gingival crevicular fluid: implications for oxidative stress in human periodontal diseases. *J Periodont Res*. 2004;39(5):287-293. [CrossRef]
13. Altıngöz SM, Kurgan Ş, Önder C, et al. Salivary and serum oxidative stress biomarkers and advanced glycation end products in periodontitis patients with or without diabetes: a cross-sectional study. *J Periodontol*. 2021;92(9):1274-1285. [CrossRef]

14. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39(1):44-84. [CrossRef]
15. Ahmadi-Motamayel F, Goodarzi MT, Jamshidi Z, Kebriaei R. Evaluation of salivary and serum antioxidant and oxidative stress statuses in patients with chronic periodontitis: a case-control study. *Front Physiol*. 2017;8:189. [CrossRef]
16. Araújo AA, Morais HB, Medeiros CACX, et al. Gliclazide reduced oxidative stress, inflammation, and bone loss in an experimental periodontal disease model. *J Appl Oral Sci*. 2019;27:e20180211. [CrossRef]
17. Fan Q, Zhou XH, Wang TF, et al. Effects of epigallocatechin-3-gallate on oxidative stress, inflammation, and bone loss in a rat periodontitis model. *J Dent Sci*. 2023;18(4):1567-1575. [CrossRef]
18. Miricescu D, Totan A, Calenic B, et al. Salivary biomarkers: relationship between oxidative stress and alveolar bone loss in chronic periodontitis. *Acta Odontol Scand*. 2014;72(1):42-47. [CrossRef]
19. Chen M, Cai W, Zhao S, et al. Oxidative stress-related biomarkers in saliva and gingival crevicular fluid associated with chronic periodontitis: a systematic review and meta-analysis. *J Clin Periodontol*. 2019;46(6):608-622. [CrossRef]
20. Navazesh M, Kumar SKS, University of Southern California School of Dentistry. Measuring salivary flow: challenges and opportunities. *J Am Dent Assoc*. 2008;139(suppl):35S-40S. [CrossRef]
21. Chapple ILC, Mealey BL, Van Dyke TE, et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Clin Periodontol*. 2018;45(suppl 20):S68-S77. [CrossRef]
22. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol*. 2018;89(suppl 1):S173-S182. [CrossRef]
23. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol*. 1982;78(3):206-209. [CrossRef]
24. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95(2):351-358. [CrossRef]
25. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem*. 1988;34(3):497-500. [CrossRef]
26. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem*. 1968;25(1):192-205. [CrossRef]
27. Campo GM, Avenoso A, Campo S, et al. Hyaluronic acid and chondroitin-4-sulphate treatment reduces damage in carbon tetrachloride-induced acute rat liver injury. *Life Sci*. 2004;74(10):1289-1305. [CrossRef]
28. Lahdentausta LSJ, Paju S, Mäntylä P, et al. Saliva and serum biomarkers in periodontitis and coronary artery disease. *J Clin Periodontol*. 2018;45(9):1045-1055. [CrossRef]
29. Yilmaz D, Niskanen K, Gonullu E, Tervahartiala T, Gürsoy UK, Sorsa T. Salivary and serum levels of neutrophil proteases in periodontitis and rheumatoid arthritis. *Oral Dis*. 2024;30(3):1660-1668. [CrossRef]
30. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. *Periodontol 2000*. 2014;64(1):57-80. [CrossRef]
31. Andreu R, Santos-Del-Riego S, Payri F. Serum inflammatory and prooxidant marker levels in different periodontal disease stages. *Healthcare (Basel)*. 2021;9(8). [CrossRef]
32. Nguyen TT, Ngo LQ, Promsudthi A, Surarit R. Salivary lipid peroxidation in patients with generalized chronic periodontitis and acute coronary syndrome. *J Periodontol*. 2016;87(2):134-141. [CrossRef]
33. Tamaki N, Yoshino F, Fukui M, et al. Relationship among salivary antioxidant activity, cytokines, and periodontitis: the Nagasaki Island study. *J Clin Periodontol*. 2015;42(8):711-718. [CrossRef]
34. Ghallab NA, Hamdy E, Shaker OG. Malondialdehyde, superoxide dismutase and melatonin levels in gingival crevicular fluid of aggressive and chronic periodontitis patients. *Aust Dent J*. 2016;61(1):53-61. [CrossRef]
35. Baltacioğlu E, Akalin FA, Alver A, Balaban F, Unsal M, Karabulut E. Total antioxidant capacity and superoxide dismutase activity levels in serum and gingival crevicular fluid in postmenopausal women with chronic periodontitis. *J Clin Periodontol*. 2006;33(6):385-392. [CrossRef]
36. Akalin FA, Toklu E, Renda N. Analysis of superoxide dismutase activity levels in gingiva and gingival crevicular fluid in patients with chronic periodontitis and periodontally healthy controls. *J Clin Periodontol*. 2005;32(3):238-243. [CrossRef]
37. Bauer V, Bauer F. Reactive oxygen species as mediators of tissue protection and injury. *Gen Physiol Biophys*. 1999;18(Spec No):7-14.
38. Toczewska J, Zalewska A, Konopka T, Maciejczyk M. Enzymatic antioxidants activity in gingival crevicular fluid and saliva in advanced periodontitis. *Oral Dis*. 2023;29(8):3559-3570. [CrossRef]
39. Kluknavská J, Krajčíková K, Bolerázska B, et al. Possible prognostic biomarkers of periodontitis in saliva. *Eur Rev Med Pharmacol Sci*. 2021;25(8):3154-3161. [CrossRef]
40. Biju T, Shabeer MM, Amitha R, Rajendra BP, Suchetha K. Comparative evaluation of serum superoxide dismutase and glutathione levels in periodontally diseased patients: an interventional study. *Indian J Dent Res*. 2014;25(5):613-616. [CrossRef]
41. Kose O, Altin A, Kurt Bayrakdar S, et al. Influences of periodontitis on hippocampal inflammation, oxidative stress, and apoptosis in rats. *J Periodont Res*. 2021;56(6):1154-1162. [CrossRef]
42. Borges I, Moreira EAM, Filho DW, de Oliveira TB, da Silva MBS, Fröde TS. Proinflammatory and oxidative stress markers in patients with periodontal disease. *Mediators Inflammm*. 2007;2007:45794. [CrossRef]
43. Toczewska J, Konopka T. Activity of enzymatic antioxidants in periodontitis: a systematic overview of the literature. *Dent Med Probl*. 2019;56(4):419-426. [CrossRef]
44. Petelin M, Pavlica Z, Ivanusa T, Sentjurc M, Skaleric U. Local delivery of liposome-encapsulated superoxide dismutase and catalase suppress periodontal inflammation in beagles. *J Clin Periodontol*. 2000;27(12):918-925. [CrossRef]
45. Chapple ILC, Brock GR, Milward MR, Ling N, Matthews JB. Compromised GCF total antioxidant capacity in periodontitis: cause or effect? *J Clin Periodontol*. 2007;34(2):103-110. [CrossRef]