Original Article

DOI: 10.4274/haseki.galenos.2024.9522
Med Bull Haseki 2024;62:141-148

Evaluation of Serum and Salivary Lipid Peroxidation Levels According to Periodontitis Type

Gizem Omeroglu Demir*, Ahmet Alver**, Esra Baltacioglu*

*Karadeniz Technical University Faculty of Dentistry, Department of Periodontology, Trabzon, Turkey
**Karadeniz Technical University Faculty of Medicine, Department of Biochemistry, Trabzon, Turkey

Abstract

Aim: In this study, we aimed to investigate the role of malondialdehyde (MDA) in the pathophysiology of periodontitis by examining serum and salivary MDA levels in patients with advanced periodontitis and healthy individuals according to the periodontal disease classification revised in the workshop held by the American Association of Chartered Epidemiologists and the European Federation in 2017.

Methods: The study was designed as a cross-sectional study, and a total of 37 patients who applied to Karadeniz Technical University Faculty of Dentistry, Department of Periodontology, in 2022 for periodontal disorders or controls were included. A total of 37 individuals, aged 25-48 years, with stage III grade C periodontitis (Group 1; 13 patients), stage IV grade C periodontitis (Group 2; 12 patients), and a periodontally healthy group (Group 3; 12 individuals) were included in the study. After the demographic characteristics and body mass index (BMI) data were obtained, the clinical periodontal parameters and serum and saliva MDA values of the individuals were measured. All the obtained data were statistically analyzed.

Results: Although BMI was lower and education level was higher in the controls ($p<0.034$), other demographic characteristics did not differ between the groups. When the clinical periodontal parameters were examined, the lowest values were observed in the controls, whereas the highest values were observed in stage IV. The difference between all three groups was statistically significant ($p=0.000$). Although serum MDA levels did not differ between the groups, the highest MDA level was observed in stage III, and the lowest MDA level was observed in the controls. In addition, salivary MDA levels did not differ between the groups, with the highest MDA level observed in stage III and the lowest MDA level observed in stage IV.

Conclusion: The findings of our study showed that systemic and local lipid peroxidation levels increased/decreased in individuals with advanced periodontitis compared with the periodontal healthy group, but this change was not statistically significant. Our findings suggest that different oxidative stress mechanisms may also be involved in advanced periodontitis.

Keywords: Classification, lipid peroxidation, malondialdehyde, periodontitis, saliva, serum

Introduction

Periodontitis is a chronic inflammatory disease that can affect not only oral health but also the general health of individuals and can destroy the periodontal connective tissue and alveoli. Not only does this phenomenon manifest with varying destruction potentials and progression rates in both systemically healthy and sick individuals, but it also affects all age groups in society (1). Periodontal diseases, which are called chronic and aggressive periodontitis according to the periodontal disease classification established by the American Academy of Periodontology (AAP) in 1999, were combined under the name of "periodontitis" with the new classification published in 2017 by the AAP and the European Federation of Periodontology (EFP) (2,3). The 2017 classification of periodontal diseases also developed a staging and grading system, which facilitated the development of individual protocols for diagnosis, treatment planning, and patient response monitoring. The current classification, in addition to having many advantages over previous classifications,

Address for Correspondence: Gizem Omeroglu Demir, Karadeniz Technical University Faculty of Dentistry, Department of Periodontology, Trabzon, Turkey
Phone: +90 506 939 79 56 **E-mail:** gizemomeroglu@gmail.com **ORCID:** orcid.org/0000-0001-7608-4392
Received: 17.09.2023 **Accepted:** 04.07.2024

considers the disease's multifactorial etiology and the risk of disease recurrence or progression, revealing more comprehensive diagnostic and therapeutic approaches.

Thanks to current approaches, a different perspective on the etiopathogenesis of periodontal disease has emerged. Pathogenic organisms in plaque flora now establish the disease onset, and the host response plays a vital role in disease progression and severity. Periodontitis results from complex interactions between pathogenic bacteria and the host's immune response (4). The host's inflammatory and immune responses to bacteria colonizing periodontal and related tissues cover the systemic circulation path and ultimately all body systems (5). This process creates a complex, bidirectional array of host-microbe interactions involving networks of cellular and humoral factors, cytokines, chemokines, and growth factors (6). Although pathogenic organisms are the primary etiological agents, most periodontal tissue destruction is due to host responses to microorganisms and their products. It is known that reactive oxygen species (ROS) also play a role in the etiopathogenesis of periodontitis, in addition to activating the immune system (7).

ROS cause tissue damage via various mechanisms, such as deoxyribonucleic acid (DNA) damage, lipid peroxidation (LPO), protein damage (including hyaluronic acid and proteoglycans), and stimulation of pro-inflammatory cytokines, and they play an important role in the pathogenesis of many inflammatory diseases, including periodontitis (7). The destructive effects of ROS are neutralized by various protective antioxidant defense systems developed by cells. More specifically, a lack of balance between proteolytic enzymes, their inhibitors, ROS, and antioxidant defense systems causes oxidative stress. It is believed that oxidative stress causes cellular and molecular damage, which in turn leads to tissue destruction (7-9). Although the exact cause or result of inflammatory diseases remains unknown, determining oxidative stress is believed to be effective in revealing the pathogenic mechanisms of many diseases (7). Measuring LPO products such as malondialdehyde (MDA) was used to determine the level of oxidative stress (10).

Our hypothesis in this study was that MDA (serum and saliva) may play a role in the pathophysiology of different types of periodontitis, according to the 2017 revised classification.

This study aimed to investigate the role of MDA in the pathophysiology of periodontitis by examining serum and salivary MDA levels in patients with advanced periodontitis and healthy individuals, according to the periodontal disease classification revised in a workshop held by the American Association of Pediatricians and the European Federation in 2017 (3).

Methods

Compliance with Ethical Standards

The Scientific Research Ethics Committee of Karadeniz Technical University Faculty of Medicine approved the study (approval no.: 2022/8, date: 17.02.2022). Before starting the study, all participants were informed in detail about the contents of the study and provided consent.

Study Design

The study was designed as a cross-sectional study, and a total of 37 patients who applied to Karadeniz Technical University Faculty of Medicine, Department of Periodontology, in 2022 for periodontal disorders or controls were included in the study. According to the criteria determined by AAP and the EFP as a result of the joint study conducted at the end of 2017, the patients were divided into 3 groups (Figure 1), and the groups were formed as follows:

Group 1: Stage III, grade C periodontitis (n=13 patients).
Group 2: Stage IV, grade C periodontitis (n=12 patients).
Group 3: Control group (periodontally healthy individuals) (n=12 patients).

Stage III, Grade C Periodontitis

Patients were evaluated clinically and radiographically for stage III periodontitis according to the criteria adopted by AAP and EFP in 2017, and the stage III periodontitis group was formed according to the following criteria:

- Individuals who are systemically healthy and have a body mass index (BMI) <25.
- Patients with interdental clinical attachment loss ≥5 mm, radiographic bone loss extended to or exceeding the middle third, ≤4 tooth loss.

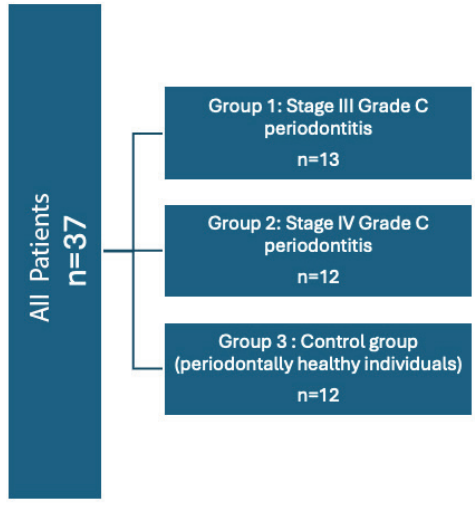

Figure 1. Diagram showing the distribution of groups

- Patients with probing depth ≥6 mm and vertical bone loss ≥3 mm.
- Patients with class 2 and 3 furcation problems and moderate alveolar defects.

Stage IV, Grade C Periodontitis

The patients were evaluated clinically and radiographically for stage IV periodontitis according to the criteria accepted by AAP and EFP in 2017, and the stage IV periodontitis group was formed according to the following criteria:

- Individuals who are systemically healthy and have a BMI <25.
- Patients with interdental clinical attachment loss ≥5 mm, radiographic bone loss extended to or exceeding the middle third, ≤5 tooth loss.
- Patients with probing depth ≥6 mm and vertical bone loss ≥3 mm.
- Patients with class 2 or 3 furcation problems, muscle dysfunction, secondary occlusal trauma, and severe alveolar defects.

Control Group

- Individuals who are systemically healthy and have a BMI <25.
- Individuals who do not have inflammatory features in their gums, with clinical parameters within the normal range, and who receive good oral care.

Exclusion Criteria

Patients with systemic disease, using medications that could affect periodontal health (calcium antagonists, anti-convulsant drugs, immunosuppressive agents, antioxidant drugs), having a BMI >25, smoking habits, and periodontal treatment in the last 6 months were excluded from the study.

Demographic and Clinical Periodontal Parameters

Separate patient follow-up forms were created for all participants, and demographic data such as age, gender, educational status, medical history, BMI, drug use, and smoking were recorded in these forms. The Periodontal Diseases Classification developed by AAP and EFP in 2017 was used for the clinical periodontal evaluation of the participants in the study. Probing pocket depth (PPD), clinical attachment level (CAL), gingival index (GI), probing bleeding index (PBI), and plaque index (PI) were measured to determine the clinical periodontal status of all individuals included in the study. Measurements were made by a single clinician using William's Periodontal Probe (Hu-Friedy, Chicago, IL). Orthopantomography and periapical radiographs of all individuals included in the study were evaluated and used as an aid in grouping patients with stage III and IV periodontal health.

Sampling Processes

Saliva Samples

Saliva samples from the participants were collected in the early morning hours. Participants were instructed not to consume liquids other than water, not to eat, and to come to their morning appointments without daily oral care at least 12 hours before giving the sample. It was confirmed that they followed this before the sample was taken. After the patients were kept with their mouths open, saliva samples were collected without stimulation using a dropper and then transferred to Eppendorf tubes. The collected samples were stored at 80 °C until examination.

Serum Samples

Blood samples from the participants were collected in the same session as saliva samples. To ensure standardization of the samples, all participants were placed in a sitting position. After blood sampling, serum was obtained via centrifugation at 3000 rpm for 10 min. Serum samples were transferred to Eppendorf tubes after centrifugation and stored at 80 °C until examination.

Laboratory Studies

Saliva and serum MDA levels were measured at the Karadeniz Technical University Faculty of Medicine, Department of Medical Biochemistry. The determination of salivary MDA levels is based on measuring the absorbance at 532 nm of the color of the complex formed by MDA and thiobarbituric acid (TBA) in an acidic medium (11). The amount of MDA in serum samples was analyzed using the TBA-reactive substance method developed by Yagi (12). The red formed as a result of the reaction between MDA and TBA was measured using spectrophotometry. Serum lipids and proteins were precipitated using a phosphotungstic acid/sulfuric acid system to remove water-soluble substances that react with TBA and give the same color (12-15).

Statistical Analysis

After collecting data for the study, the Statistical Program for Social Sciences version 20.0 was used for the statistical evaluation of the data. The results of the biochemical analysis of periodontal and laboratory parameters were statistically evaluated. The conformity of the data to a normal distribution was examined using the Shapiro-Wilk test. The comparative measurement of clinical parameters between stage III and stage IV periodontitis and the control group was performed using the Kruskal-Wallis test between all groups and the Mann-Whitney U test between the paired groups. In all these evaluations, $p<0.05$ was considered statistically significant.

Results

Demographic Findings

The participants in our study were aged between 25 and 48. The mean age was 38.1±6.4 years. The patients' ages were 39.7±4.4 in stage III periodontitis, 38.9±7.7 in stage IV periodontitis, and 35.6±6.6 in periodontal health. Of the 37 patients included in our study, 27 (73%) were female and 10 (27%) were male. When the educational status of the individuals included in the study was examined, 24.3% (n=9) were primary school graduates and 43.2% (n=16) were university graduates. The BMI of all 37 individuals (100%) included in our study was <25. The patients' mean BMI was 23.5±1.3. Table 1 presents the demographic characteristics of the patients.

Clinical Findings

Table 2 presents comparisons of the clinical periodontal parameters among all patients and the paired groups. A statistically significant difference was found in the comparison of the clinical periodontal parameters of the patients in all groups and in the comparison between the paired groups (p=0.000).

Laboratory Findings

Table 3 presents the salivary and serum MDA levels of all groups included in our study. No statistically significant difference was observed in the salivary and serum MDA levels between the groups.

Table 1. Comparative analysis of demographic characteristics of individuals participating in the study

	All patients	Stage III (G1)	Stage IV (G2)	Control (G3)	p-value (G1-G2-G3)
Age	38.1±6.4	39.7±4.4	38.9±7.7	35.6±6.6	>0.05
Gender F. M.	 73% 27%	 84.6% 15.4%	 66.7% 33.3%	 66.7% 33.3%	>0.05
Educational status Primary Middle High Associate degree University	 9 (24.3%) 1 (2.7%) 9 (24.3%) 2 (5.4%) 16 (43.2%)	 4 (30.8%) - 5 (38.5%) 1 (7.7%) 3 (23.1%)	 5 (41.7%) 1 (8.3%) 3 (25%) - 3 (25%)	 - - 1 (8.3%) 1 (8.3%) 10 (83.3%)	**0.034**
BMI (kg/m^2)	23.5±1.3	24.1±0.8	23.9±0.9	22.5±1.4^	**0.004**

^Statistical significance was found in the comparative analysis of G3 with G1 and G2 (p<0.05), F.: Female, M.: Male, BMI: Body mass index

Table 2. Intergroup comparison of clinical periodontal parameters

Clinical parameters	Groups	N	X ± SD	Median	Chi-square	p-value[&]
PPD	Stage III	13	3.3±0.53	3.1	32.009	**0.000**
	Stage IV	12	4.5±0.25	4.6		
	Control	12	1.8±0.19	1.8		
CAL	Stage III	13	3.4±0.48	3.3	32.025	**0.000**
	Stage IV	12	4.6±0.22	4.7		
	Control	12	1.9±0.23	1.9		
PBI	Stage III	13	0.92±0.02	0.9	26.636	**0.000**
	Stage IV	12	0.95±0.02	0.9		
	Control	12	0.06±0.03	0.5		
GI	Stage III	13	1.9±0.06	1.9	32.059	**0.000**
	Stage IV	12	2.6±0.21	2.7		
	Control	12	0.06±0.03	0.05		
PI	Stage III	13	1.9±0.15	1.9	32.074	**0.000**
	Stage IV	12	2.6±0.18	2.5		
	Control	12	0.05±0.03	0.05		

[&]Kruskal-Wallis test
PPD: Probing pocket depth, CAL: Clinical attachment level, PBI: Probing bleeding index, GI: Gingival index, PI: Plaque index, SD: Standard deviation

Table 3. Intergroup comparison of saliva and serum malondialdehyde concentrations

Clinical parameters	Groups	N	X ± SD	Median	Chi-square	p-value[&]
Saliva MDA level (nM)	Stage III	13	8.3±1.7	7.4	0.396	0.820
	Stage IV	12	7.8±0.9	7.5		
	Control	12	7.9±0.8	8.1		
Serum MDA level (nM)	Stage III	13	8.9±5.2	6.7	0.753	0.686
	Stage IV	12	7.9±2.0	7.3		
	Control	12	7.1±0.9	7.0		

[&]Kruskal-Wallis test
MDA: Malondialdehyde, SD: Standard deviation

Discussion

The 1999 classification of periodontal diseases has remained valid for approximately 20 years and has been extremely effective in determining diagnosis and treatment planning, supportive periodontal treatment, and, most importantly, establishing a nonsurgical periodontal treatment protocol (16). However, the 1999 classification's inability to distinguish between chronic and aggressive periodontitis in diagnosis and the inapplicability of certain treatment protocols necessitated its revision over time. Also, progress in molecular biology has shown how important host susceptibility is in diagnosis and prognosis, as shown by many immunological studies. Personalized medicine has also been helped by many biomarkers, such as oxidative stress parameters. To sum up, periodontal disease is a multifactorial disease where different immune-inflammatory mechanisms are at work, host sensitivity is a big part of how the disease will progress, and systemic and environmental factors like smoking, stress, and diabetes are big parts of how the disease starts.

The various details mentioned above have revealed the necessity of a more comprehensive classification of periodontal disease. The 2017 classification is a comprehensive classification that distinguishes periodontal health, gingivitis, and suspected periodontitis, establishes the differential diagnosis between periodontitis and decreased periodontitis, improves the staging system to assess the severity and complexity of periodontitis, and improves the grading system to assess the risk profile. According to the current classification, performing different immunological or molecular studies will shed light on the formation of new information in the literature that can help determine diagnosis and treatment planning. Based on this concept, our study aimed to contribute to the existing literature on rapidly progressive forms of periodontitis by measuring LPO, a measure of oxidative stress, in individuals with advanced stage and grade C periodontal disease. This study is the first to examine the systemic and local mechanisms of LPO in individuals with advanced periodontitis, according to the 2017 classification.

In our study, serum samples collected from patients were examined to assess systemic responses, and total unstimulated saliva samples were analyzed to assess local responses. Stimulation of saliva increases the flow of gingival crevicular fluid (GCF) from the periodontal pocket during chewing. As a result, it can increase the number of antioxidant and plasma-related molecules in saliva (10). It has been reported that changes in the salivary flow rate of individuals with periodontitis significantly affect the concentrations of markers in saliva (17-19).

The relationship between oxidative stress and aging has been demonstrated by various studies for many years (20,21). On the other hand, over-reactive aldehyde production and protein oxidation caused by oxidative stress induced by LPO and glycoxidation reactions have been shown to play a role in aging and various age-related chronic diseases (22). Considering the findings of the abovementioned studies and the fact that oxidative stress and LPO increase with age, patients in similar age groups were included in the study to avoid age differences between the groups. In addition, there were no gender differences between the three groups in this study. Since it is known that vascular oxidative stress affects sex and hormones, attention was paid to the fact that the sex ratios between the groups were similar in the study (23). As a result, there was no difference between the three groups in terms of age and sex, and it was appropriate to ensure standardization between the groups and not to be affected by age and sex in the oxidative stress picture. It has been known for many years that obesity causes oxidative stress and plays a role in numerous chronic inflammatory diseases, including cancer, hypertension, diabetes... and periodontitis (24-26). Therefore, the BMIs of individuals were also measured in this study, and it was observed that the values obtained were between 20 and 25 kg/m^2 in all groups.

Many studies have shown an increase in oral health-related quality of life with increasing education levels (27,28). In the literature, these data have been associated

with an increase in the level of education of individuals, the attention shown to personal oral care and awareness of oral health, as well as the regular visits of individuals to their routine dental examinations (29). Owing to the increase in income and education level, there is an increase in the number of dental visits made not only for the complaints of individuals but also for routine checkups (30-32). When the results of our study are evaluated together; stage III and stage IV periodontitis patients, whose socio-economic and socio-cultural levels are lower than the controls, seem to have progressed in the stage of periodontal disease because the importance they attach to oral-dental health is less than that of the healthy group, their oral hygiene practices are inadequate, and they do not go to the dentist regularly. Our findings are consistent with those of the literature.

In our study, clinical periodontal parameters were also measured along with a radiographic examination to diagnose periodontitis in healthy individuals. Probing pocket depth, CAL, GI, PPI, and PI values measured within the scope of clinical periodontal parameters form the basis of periodontal clinical examination. According to the "2017 Current Periodontal Disease Classification", which follows the measurements of clinical periodontal patients, dividing patients diagnosed according to periodontal examination into 3 separate groups as stage III and stage IV periodontitis, periodontal health improves the clinical periodontal effect. Despite observing the lowest values in the control group and the highest values in stage IV, there was a statistically significant difference across all three groups.

Periodontal disease consists of active periods, in which destruction is evident and severe, and silent inactive periods, in which the inflammatory response of the host decreases and destruction slows or even almost stops. The burst hypothesis refers to this process, which persists in the form of active and inactive periods (33). The active phase of periodontal disease begins with the formation of gram-negative dental plaque. In this process, active periodontal destruction occurs with attachment loss, resulting in a periodontal pocket or deepening of the existing periodontal pocket. In this period, gingival bleeding may occur due to probing or spontaneous bleeding, and an increase in inflammatory fluid may be observed in the gingiva. Further biochemical, immunological, genetic, and microbiological studies on samples such as saliva, serum, GCF, gingival tissue, and plaque are the most appropriate diagnostic criteria for determining periodontal disease activity (34). However, in addition to clinically measuring PPD and CAL levels, GI and PBI parameters are also important in determining periodontal disease activity. Furthermore, our findings confirmed the idea that inflammation was present in both groups, but more severe inflammation was observed in parallel with the severity of destruction in the Stage IV group.

LPO is a very harmful mechanism that destroys the structure of cells through chain reactions that cannot be intervened with (7,35,36). Measuring LPO products readily determines tissue destruction due to ROS. MDA is the most studied end-product of polychasia unsaturated fatty acid peroxidation (37). In our serum MDA levels, although there was no statistical difference between the groups with periodontitis and healthy controls, the highest value was in Stage III, and the lowest value was in healthy controls. When our salivary MDA findings were examined, it was observed that the highest value was in Stage III and the lowest value was in Stage IV, although there was no significant difference between the periodontitis and healthy groups. Although the results of the studies examining the serum, salivary, GCF, and gingival tissues MDA levels of patients with periodontitis differ from each other, it has been shown that GCF and salivary MDA levels are significantly higher than those without periodontitis in general, whereas systemic MDA levels do not change or increase compared with controls (10,14,18,38-42). When we evaluate the findings of the abovementioned studies together, even though it is revealed that systemic and local LPO increases or does not change in periodontitis, it is widely believed that LPO, as an oxidative stress marker, may be associated with periodontal inflammation and bone loss and may play an important role in the pathogenesis of periodontal disease.

Study Limitations

When we evaluated our LPO findings under the guidance of the above studies, we observed that systemic and local LPO did not change according to periodontal health in periodontitis and in Stage III periodontitis compared with Stage IV periodontitis. However, the highest LPO value was detected in patients with stage III periodontitis. We recognized that the small number of patients was a major limitation. However, despite this limitation, our results suggest that various oxidative stress markers may play a part in the harmful effects of advanced periodontitis, taking into account the current system for grading and staging the disease. To learn more about how oxidative stress changes in different stages of periodontitis, it will be helpful to measure markers like total antioxidant capacity and oxidative stress index, as well as protein oxidation and DNA damage. Instead, studying enzymatic antioxidants like glutathione peroxidase, superoxide dismutase, and catalase, as well as non-enzymatic antioxidants, may help figure out how oxidative stress affects the development of periodontitis at different stages.

Conclusion

Simultaneously, the observation of the lowest salivary MDA values in stage IV of our study implies that an increase in inflammation may trigger the activation of adaptive mechanisms like antioxidants, which could potentially mitigate oxidative damage resulting from LPO. Further studies examining more individuals and more comprehensive parameters to shed light on this issue will be beneficial in terms of understanding the place of oxidative stress in the pathogenic mechanisms of periodontitis at different stages and developing new treatment approaches.

Ethics

Ethics Committee Approval: The Scientific Research Ethics Committee of Karadeniz Technical University Faculty of Medicine approved the study (approval no.: 2022/8, date: 17.02.2022).

Informed Consent: Before starting the study, all participants were informed in detail about the contents of the study and provided consent.

Authorship Contributions

Surgical and Medical Practices: G.O.D., E.B., Concept: G.O.D., A.A., E.B., Design: G.O.D., E.B., Data Collection or Processing: G.O.D., A.A., Analysis or Interpretation: G.O.D., A.A., E.B., Literature Search: G.O.D., E.B., Writing: G.O.D., E.B.

Conflict of Interest: No conflicts of interest were declared by the authors.

Financial Disclosure: This study received no financial support.

References

1. Bartold PM, Cantley MD, Haynes DR. Mechanisms and control of pathologic bone loss in periodontitis. Periodontol 2000. 2010;53:55-69.
2. Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. J Clin Periodontol. 2018;45(Suppl 20):S1-S8.
3. Fine DH, Patil AG, Loos BG. Classification and diagnosis of aggressive periodontitis. J Clin Periodontol. 2018;45(Suppl 20):S95-S111.
4. Bartold PM, Van Dyke TE. An appraisal of the role of specific bacteria in the initial pathogenesis of periodontitis. J Clin Periodontol. 2019;46:6-11.
5. 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;32:238-43.
6. Braz-Silva PH, Bergamini ML, Mardegan AP, De Rosa CS, Hasseus B, Jonasson P. Inflammatory profile of chronic apical periodontitis: a literature review. Acta Odontol Scand. 2019;77:173-80.
7. Patil RT, Dhadse PV, Salian SS, Punse SD. Role of oxidative stress in periodontal diseases. Cureus. 2024;16:e60779.
8. 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 post-menopausal women with chronic periodontitis. J Clin Periodontol. 2006;33:385-92.
9. Canakci C, Cicek Y, Canakci V. Reactive oxygen species and human inflammatory periodontal diseases. Biochemistry (Mosc). 2005;70:619-28.
10. Aycicek A, Erel O, Kocyigit A. Increased oxidative stress in infants exposed to passive smoking. Eur J Pediatr. 2005;164:775-8.
11. Khalili J, Biloklytska HF. Salivary malondialdehyde levels in clinically healthy and periodontal diseased individuals. Oral Dis. 2008;14:754-60.
12. Yagi K. Assay for blood plasma or serum. Methods Enzymol. 1984;105328-31.
13. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol. 1999;4:1-6.
14. Brock GR, Butterworth CJ, Matthews JB, Chapple IL. Local and systemic total antioxidant capacity in periodontitis and health. J Clin Periodontol. 2004;31:515-21.
15. Veljovic T, Djuric M, Mirnic J, et al. Lipid peroxidation levels in saliva and plasma of patients suffering from periodontitis. J Clin Med. 2022;11:3617.
16. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408:239-47.
17. Mohideen K, Chandrasekar K, Ramsridhar S, Rajkumar C, Ghosh S, Dhungel S. Assessment of oxidative stress by the estimation of lipid peroxidation marker malondialdehyde (MDA) in patients with chronic periodontitis: a systematic review and meta-analysis. Int J Dent. 2023;2023:6014706.
18. Moldogazieva NT, Mokhosoev IM, Mel'nikova TI, Porozov YB, Terentiev AA. Oxidative stress and advanced lipoxidation and glycation end products (ALEs and AGEs) in aging and age-related diseases. Oxid Med Cell Longev. 2019;2019:3085756.
19. Arcas VC, Tig IA, Moga DFC, Vlad AL, Roman-Filip C, Fratila AM. A systematic literature review on inflammatory markers in the saliva of patients with multiple sclerosis: a cause or a consequence of periodontal diseases. Medicina. 2024;60:859.
20. Miller AA, De Silva TM, Jackman KA, Sobey CG. Effect of gender and sex hormones on vascular oxidative stress. Clin Exp Pharmacol Physiol. 2007;34:1037-43.
21. Engin A. Obesity-associated breast cancer: analysis of risk factors. Adv Exp Med Biol. 2017;960:571-606.
22. Iwashita M, Hayashi M, Nishimura Y, Yamashita A. The link between periodontal inflammation and obesity. Curr Oral Health Rep. 2021;8:76-83.

23. Yiengprugsawan V, Somkotra T, Seubsman S-a, Sleigh AC, th TCSTs-assa. Oral health-related quality of life among a large national cohort of 87,134 Thai adults. Health Qual Life Outcomes. 2011;9:42.

24. Caglayan F, Altun O, Miloglu O, Kaya MD, Yilmaz AB. Correlation between oral health-related quality of life (OHQoL) and oral disorders in a Turkish patient population. Med Oral Patol Oral Cir Bucal. 2009;14:e573-8.

25. Sabbah W, Tsakos G, Sheiham A, Watt RG. The role of health-related behaviors in the socioeconomic disparities in oral health. Soc Sci Med. 2009;68:298-303.

26. Checa-Ros A, Hsueh WC, Merck B, González-Torres H, Bermúdez V, D'Marco L. Obesity and Oral Health: The Link Between Adipokines and Periodontitis. touchREV Endocrinol. 2024;20:25-31.

27. Sakki TK, Knuuttila ML, Anttila SS. Lifestyle, gender and occupational status as determinants of dental health behavior. J Clin Periodontol. 1998;25:566-70.

28. Kumar S, Bhargav P, Patel A, et al. Does dental anxiety influence oral health-related quality of life? Observations from a cross-sectional study among adults in Udaipur district, India. J Oral Sci. 2009;51:245-54.

29. Socransky SS, Haffajee AD, Goodson JM, Lindhe J. New concepts of destructive periodontal disease. J Clin Periodontol. 1984;11:21-32.

30. Takane M, Sugano N, Ezawa T, Uchiyama T, Ito K. A marker of oxidative stress in saliva: association with periodontally-involved teeth of a hopeless prognosis. J Oral Sci. 2005;47:53-7.

31. Akbayram S, Dogan M, Akgün C, et al. The association of oxidant status and antioxidant capacity in children with acute and chronic ITP. J Pediatr Hematol Oncol. 2010;32:277-81.

32. Chisini LA, Vargas-Ferreira F, Demarco GT, et al. Socioeconomic status in life course is associated with dental appearance dissatisfaction. Braz Oral Res. 2024;38:e051.

33. Wei D, Zhang XL, Wang YZ, Yang CX, Chen G. Lipid peroxidation levels, total oxidant status and superoxide dismutase in serum, saliva and gingival crevicular fluid in chronic periodontitis patients before and after periodontal therapy. Aust Dent J. 2010;55:70-8.

34. Su H, Gornitsky M, Velly AM, Yu H, Benarroch M, Schipper HM. Salivary DNA, lipid, and protein oxidation in nonsmokers with periodontal disease. Free Radic Biol Med. 2009;46:914-21.

35. Sobaniec H, Sobaniec-Lotowska M. Morphological examinations of hard tissues of periodontium and evaluation of selected processes of lipid peroxidation in blood serum of rats in the course of experimental periodontitis. Med Sci Monit. 2000;6:875-81.

36. Diab R, Choufani A, Dagher J, Chahine N. The influence of circadian rhythm on the antioxidant capacity of saliva in periodontal diseases. Cureus. 2024;16:e56174.

37. Panjamurthy K, Manoharan S, Ramachandran CR. Lipid peroxidation and antioxidant status in patients with periodontitis. Cell Mol Biol Lett. 2005;10:255-64.

38. Marton IJ, Balla G, Hegedus C, et al. The role of reactive oxygen intermediates in the pathogenesis of chronic apical periodontitis. Oral Microbiol Immunol. 1993;8:254-7.

39. 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;46:608-22.

40. Tonguç MÖ, Öztürk Ö, Sütçü R, et al. The impact of smoking status on antioxidant enzyme activity and malondialdehyde levels in chronic periodontitis. J Periodontol. 2011;82:1320-8.

41. Canakci V, Yildirim A, Canakci CF, Eltas A, Cicek Y, Canakci H. Total antioxidant capacity and antioxidant enzymes in serum, saliva, and gingival crevicular fluid of preeclamptic women with and without periodontal disease. J Periodontol. 2007;78:1602-11.

42. Tüter G, Kurtiş B, Serdar M. Interleukin-1beta and thiobarbituric acid reactive substance (TBARS) levels after phase I periodontal therapy in patients with chronic periodontitis. J Periodontol. 2001;72:883-8.