ORIGINAL ARTICLE

UDC: 616.31-08:615.28
DOI: https://doi.org/10.2298/VSP200921137K

Oxidative/antioxidative effects of colloidal silver ions and chlorhexidine in saliva and gingival fluid of periodontal patients

Oksidativni/antioksidativni efekti jona srebra i rastvora hlorheksidina u salivi i gingivalnoj tečnosti pacijenata sa parodontopatijom

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Abstract

Background/Aim. Chronic periodontitis is an inflammatory disease. Oxidative stress is an important factor in periodontitis progress, hence examining the antioxidative properties of antiseptics, such as chlorhexidine (CHX) and silver ions solution (SSI), is a beneficial biomarker in estimating the recovery of tissue impairment during periodontal disease treatment. **Methods.** This clinical trial was conducted on the control group referred to healthy volunteers and individuals with periodontal disease, divided into two subgroups: before and after applying antiseptic treatments (CHX or SSI). Measurements of oxidative/antioxidative parameters were addressed to determine thiobarbituric acid products (TBARS) concentration and total superoxide dismutase (tSOD) activity in saliva and gingival crevicular fluid (GCF) of periodontal patients. **Results.** TBARS concentration was increased in saliva before the CHX treatment compared to the periodontal group after the CHX treatment, as well as before both CHX and SSI antiseptic treatment in CGF, compared to controls and periodontal groups after the treatment. Patients before SSI treatment had increased tSOD activity in saliva compared to the control group treated with SSI, as well as compared to patients after the SSI treatment. Additionally, tSOD activity was increased in GCF in patients with periodontitis before antiseptic treatment (CHX, SSI) compared to the control or the group of patients after the appropriate treatment. **Conclusion.** Our results revealed elevated lipid peroxidation in CGF, which reflected the promotion of oxidative stress during periodontal inflammation. The study suggests that antiseptics with antioxidant properties may reduce tissue damage initiated by periodontal disease. Moreover, the determination of oxidative/antioxidative parameters can be important for diagnosing, monitoring, and prognosis of the clinical state of periodontal patients.

Key words:
antioxidants; chlorhexidine; oxidative stress; periodontal diseases; silver.

Apstrakt

Uvod/Cilj. Hronična parodontopatija je inflamatorno oboljenje. Oksidativni stres je veoma važan faktor u razvoju parodontopatije, tako da je praćenje mogućnosti antioksidativnih delovanja antiseptika, kao što su hlorheksidin (CHX) i rastvor jona srebra (SSI), koristan biomarker u proceni oporavka oštećenja tkiva tokom lečenja parodontopatije. **Metode.** Kliničko istraživanje obuhvatilo je kontrolnu grupu (grupu zdravih dobrovoljaca), kao i pacijente sa parodontopatijom, koji su bili podeljeni u dve podgrupe: pre terapije antisepticima (CHX ili SSI) i nakon terapije. Merenje parametara oksidativnog stresa i antioksidativne zaštite obuhvatilo je određivanje koncentracije reaktivnih produkata tiobarbiturne kiseline (TBARS) i aktivnosti ukupne superoksid dizmutaze (tSOD) u salivi i gingivalnoj tečnosti (GCF) pacijenata sa parodontopatijom. **Rezultati.** Koncentracija TBARS bila je povećana u salivi pre CHX tretmana u poređenju sa grupom pacijenata sa paradontopatijom posle CHX tretmana, kao i pre CHX ili SSI antiseptičnih tretmana u GCF u poređenju sa kontrolom i grupama pacijenata sa parodontopatijom nakon tretmana. Kod pacijenata pre SSI tretmana aktivnost tSOD u salivi je bila povećana u poređenju sa kontrolnom grupom tretiranom SSI, kao i grupom pacijenata nakon SSI tretmana. Takođe, aktivnost tSOD bila je povećana u GCF kod pacijenata sa parodontopatijom pre tretmana antisepticima (CHX, SSI) u poređenju sa kontrolnom ili grupom pacijenata posle odgovarajućeg tretmana. **Zaključak.** Prikazani rezultati pokazuju indukciju lipidne peroksidacije u GCF, što dovodi do pokretanja oksidativnog stresa

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u toku zapaljenja kod parodontopatije. Studija ukazuje na to da antiseptici sa antioksidativnim delovanjem mogu redukovati pokrenuto oštećenje tkiva u parodontopatiji. Određivanje oksidativnih/antioksidativnih parametara može biti od značaja u dijagnostici, praćenju i prognozi kliničkog stanja pacijenata sa parodontopatijom.

Ključne reči:
antioksidansi; hlorheksidin; stres, oksidativni; periodontalne bolesti; srebro.

Introduction

Periodontal disease is one of the most common chronic diseases, with a progressively increasing prevalence spread throughout the world [1]. Periodontitis is reflected as an inflammatory disorder that damages tissue through the complex interactions between periodontopathic bacteria and host defense systems [2]. Oxidative stress is an imbalance between prooxidants and antioxidants and has been linked with both onsets of periodontal tissue damage and systemic inflammation. Reactive oxygen species (ROS) comprise oxygen-derived free radicals, such as superoxide anion, hydroxyl radical, nitric oxide, and hydrogen peroxides, created through the bacteria-host mediated pathway. ROS also excite polymorphonuclear leukocytes (PMNL) to produce radicals by the oxidative burst. ROS cause tissue impairment through several mechanisms, which contain lipid peroxidation (LPO), DNA destruction, oxidation of main enzymes, damage of proteins, and release of proinflammatory cytokines by monocytes and macrophages [3]. ROS are extremely toxic to the affected infectious agent as well as to the extracellular structure and can encourage LPO, causing effects on the periodontal tissue [4]. Terminated production of LPO can result in oxidative stress and, therefore, destroy cellular integrity. The LPO causes oxidative stress, and various markers have been used to follow this process. Malondialdehyde (MDA) is the major and much-studied product of polyunsaturated fatty acid peroxidation that can specify the spread of oxidative stress [5]. The most frequently applied test for the measurement of MDA is the assessment of thiobarbituric acid reactive substances (TBARS).

Many studies are dedicated to the role of ROS, products of oxidative stress, and LPO as well as antioxidant defense systems in the pathology of periodontitis [6, 7]. The capacity of total antioxidant status in a sample reflects the full range of antioxidant activity against numerous reactive oxygen and nitrogen radicals (RNS).

The human body has different nonenzymatic and enzymatic antioxidants, which eliminate detrimental ROS and prevent their deleterious actions [2]. Protective antioxidants like superoxide dismutase (SOD), catalase, and glutathione peroxidase function by enzymatic removal of superoxide ions. The SOD is found in all the tissues and cells of aerobic organisms. An alteration in antioxidant enzyme activity was a consequence of scaling and root planing, and it also revealed the role of oxidative stress in periodontal destruction. Novakovic et al. [8] suggested that salivary antioxidants like SOD credibly reveal periodontal reaction and the tissue response to treatment.

Potential inflammatory markers of host response can be found in saliva, gingival crevicular fluid (GCF), and serum samples and can be used as diagnostic markers [9]. Saliva is of major importance in the maintenance of oral health [10]. It is a complex secretion and could be used as a non-invasive diagnostic fluid to measure biomarkers circulated during the initiation and progression of periodontal disease [11, 12]. Saliva contains biochemical systems involved in periodontal tissue reparation, as well as numerous antibacterial, antiviral, and antifungal components, including lysosome, salivary peroxidase, and various antioxidants [13]. Instead, GCF is an exudate originating from serum and can be collected from the gingival sulcus surrounding natural teeth [14]. The flow of this biological fluid is an important element for the status of periodontal tissues, which reflects the cellular response in periodontium by the components of serum and influences from the gingival crevice [15].

A very capable periodontal therapy is the mechanical removal of bacterial biofilm and bacterial toxins from the tooth surfaces, which includes scaling and root planing for patients with chronic periodontitis [16]. Microbes compete for the most crucial part in the progress of periodontitis in which chlorhexidine (CHX) and colloidal silver ions solution (SSI) are strong antiseptics that have been utilized in dentistry for a long time [17, 18]. The CHX is considered a “gold standard” for the adjuvant treatment of periodontal patients. However, it has adverse effects since studies tested SSI as a very strong antiseptic with nontoxic and antibacterial power [19, 20]. Silver in any form is not considered toxic to the immune, cardiovascular, nervous, or reproductive systems. Furthermore, SSI has been used as medical aid and dietary supplement [21].

The aim of this study was to determine oxidative/antioxidative status in saliva and GCF in periodontal patients and to evaluate the diagnostic power of antiseptic treatments with SSI and CHX, especially with regard to the inflammatory process.

Methods

Participants and ethical conditions

This randomized prospective clinical study was conducted at the Periodontology and Implantology Departments of the Dental Clinic, Military Medical Academy in Belgrade, Serbia. Ninety examinees of both sexes participated in the study and they were divided into study and control groups, each of them having two subgroups depending on the treatment time (for the study groups) or the antioxidant used (for the control group): the control group – healthy individuals (with no prior history of periodontal disease), the probing depth in this latter group did not exceed 3 mm [22] (a) treated

by rinsing with a 0.2% solution of chlorhexidine (n = 20), (b) treated by rinsing with a 5 mg/mL colloidal SSI (n = 20); the CHX study group (n = 25) – periodontal patients treated by rinsing with a 0.2% solution of CHX after scaling and root planing of periodontal pockets (a) before the treatment, (b) after the treatment; the colloidal SSI study group (n = 25) – periodontal patients treated with a 5 mg/mL SSI rinsing, in addition to the treatment of periodontal pockets by scaling and root planing (a) before the treatment, (b) after the treatment.

This study was in agreement with the ethical principles of the World Medical Association Declaration of Helsinki (1964). Permission for this study was obtained from the Ethics Committee of the Military Medical Academy, Belgrade, Serbia (project No. 13/03/2014), and the study included only individuals who agreed to participate after reading and signing a free and informed consent form, except those with difficulty in understanding and communicating, with a physical handicap, or both, which could have compromised the sample collection.

Inclusion criteria were: the presence of chronic inflammation (pain, redness, heat, swelling), diagnosed according to bleeding on probing, at least 5 or 6 sites with probing depth ≥ 5 mm, attachment loss ≥ 3 mm, and extensive radiographic bone loss [22].

For all groups, the following items were considered exclusion criteria: infection, cardiovascular and/or neurological illness, renal insufficiency and/or diabetes; pregnancy; smoking; use of antibiotics and/or hormonal or nonhormonal anti-inflammatory drugs 6 months prior to tissue collection.

Clinical examination

Samples of unstimulated saliva and GCF were taken at the beginning of the study. One mL of unstimulated saliva was collected using sterile injectors, centrifuged for 15 min at 3,000 × g to remove cell elements, and then stored at -80 °C. GCF samples were taken using sterile paper points from the area of periodontal pockets depth ≥ 5 mm for 30 sec. Paper points were immediately immersed in 50 μL of buffered physiological solution, vortexed for 10 sec, and centrifuged at 3,000 × g for 5 min to remove plaque and cellular elements. Finally, the paper points were frozen at -80 °C until the time of determining the laboratory parameters [23].

After sampling saliva and GCF in the next few days, mechanical processing was carried out in several sessions of periodontal pockets by quadrants. Periodontal pockets of one group of subjects were washed with aqueous solution SSI (concentrations of 5 mg/mL), while in the other group, they were washed with CHX solution (0.2%). Furthermore, patients could choose one of the two offered antiseptics for rinsing the oral cavity 10 days after starting therapy. On the 30th day after the end of the therapy, the gingival and periodontal parameters were measured again, after which samples of unstimulated saliva and GCF were collected. In the control groups which received one of the antiseptics (CHX, SSI), a sample of unstimulated saliva and GCF was taken from the gingival sulcus.

The nonsurgical periodontal treatment was performed using a method of scaling and root planing per quadrant (in the following four days, one quadrant was processed). After the scaling and root planing, the application of a particular adjuvant antiseptic (CHX or SSI) was carried out by injecting 10 mL into the periodontal pockets. The patient was asked to mouthwash a given amount of antiseptic for 60 sec and not to take food for one hour after the treatment. Depending on the allocated group, each patient was given the same solution for home use for the next 10 days with precise usage instructions. A month after scaling and root planing, including a particular adjuvant antiseptic, all parameters' control measurement was performed to compare the values to the baselines.

Examined parameters

The oxidative/antioxidative status parameters (TBARS, tSOD) were determined in both saliva and GCF of control groups (C_{CHX}, C_{SSI}), as well as periodontal groups before (P_{CHX} before, P_{SSI} before) and after (P_{CHX} after, P_{SSI} after) the treatment. Total protein concentration was estimated with bovine serum albumin as a standard [24].

Lipid peroxidation analyses in both saliva and GCF were measured as TBARS production, assayed in the thiobarbituric acid reaction, and described by Girotti et al. [25]. The results are expressed as μmol TBARS/mg proteins.

The total SOD (tSOD; EC 1.15.1.1) activity was measured by a spectrophotometer as the inhibition of spontaneous autoxidation of epinephrine at 480 nm. The kinetics of sample tSOD activity was followed in a 50 mmoL carbonate buffer on pH 10.2, comprising 0.1 mmoL ethylene diamine tetraacetic acid (EDTA), after the addition of 10 mmoL epinephrine [26]. Data were expressed as U tSOD per mg of proteins.

All drug solutions were prepared on the day of the experiment. In addition, all the chemicals used in this study were of analytical grade.

Statistical analysis

The type of study according to which the research was carried out is a prospective cohort study. In one study, the therapeutic efficacy of oral formulations of CHX on a relatively small sample was tested, and statistically significant effects were obtained by registering a difference of 15% between treatments [27]. Application of *t*-test for independent groups (study power 80% and the probability of type 1 error (α) 0.05) showed that the minimum number of subjects in each group was 20.

After confirming a normal distribution in all groups using the Kolmogorov-Smirnov test, the data were presented as mean ± standard deviation (SD). All data were analyzed statistically by one-way ANOVA using Dunnet's C test. The linear regression analysis was performed to determine the relationship between different parameters using the statistical program GraphPad Prism. Statistical significance was described as $p < 0.05$.

Results

The age of the subjects included in the study is shown in Table 1.

In the saliva of periodontal patients before the CHX or SSI treatment, TBARS concentrations were not significantly different compared to controls (Table 2). In the saliva of the periodontal group after the CHX treatment, the TBARS concentration significantly decreased ($p < 0.01$) compared to the group of patients before CHX treatment (Figure 1A). Contrary to this, TBARS concentration in GCF was significantly higher in all periodontal patients before the treatment (CHX, SSI) compared to that in the controls (Table 3). However, these values in both study groups (CHX and SSI) significantly decreased after the treatment with antiseptics (Figure 2B).

Table 1

Demographic variables of the study population

Group	Number of patients	Age (years) mean values ± SD
P_{CHX}	25	49.47 ± 8.58
P_{SSI}	25	51.64 ± 9.41
C_{CHX}	20	42.05 ± 7.19
C_{SSI}	20	40.95 ± 8.19
Total	90	45.29 ± 9.27

SD – standard deviation; P – periodontal patients; C – healthy individuals (control group); CHX – chlorhexidine treatment; SSI – silver ions solution treatment.

Table 2

Thiobarbituric acid reactive substances (TBARS) concentration (μmoL/mg proteins) and total superoxide dismutase (tSOD) activity (U/mg proteins) in the saliva of healthy individuals (C) with the treatment (T) of chlorhexidine – CHX (C_{CHX}) or silver ions solution – SSI (C_{SSI}), periodontal patients (P) before the T with CHX (P_{CHX} before T) or SSI (P_{SSI} before T), as well as P after the T with CHX (P_{CHX} after T) or SSI (P_{SSI} after T)

Parameters	$P_{CHX\ before\ T}$	$P_{CHX\ after\ T}$	C_{CHX}	$P_{SSI\ before\ T}$	$P_{SSI\ after\ T}$	C_{SSI}
TBARS (μM/mg prot.)	21.3 ± 4.7	16.5 ± 2.3$^{\blacklozenge\blacklozenge}$	17.6 ± 5.2	20.6 ± 4.3	16.2 ± 5.4	17.1 ± 7.9
tSOD (U/mg prot.)	422.4 ± 99.5	339.4 ± 119.2	353.0 ± 113.7	464.1 ± 87.3***	320.1 ± 41.7$^{\blacklozenge\blacklozenge\blacklozenge}$	312.7 ± 93.5

All results are represented as mean ± standard deviation (SD). Labels of statistical significance: * – compared to the control group (C); ♦ – compared to P_{CHX} before T or P_{SSI} before T. Statistical significance was considered at: ♦♦$p < 0.01$, *,♦♦♦$p < 0.001$ (one-way ANOVA, Dunnett's C test).**

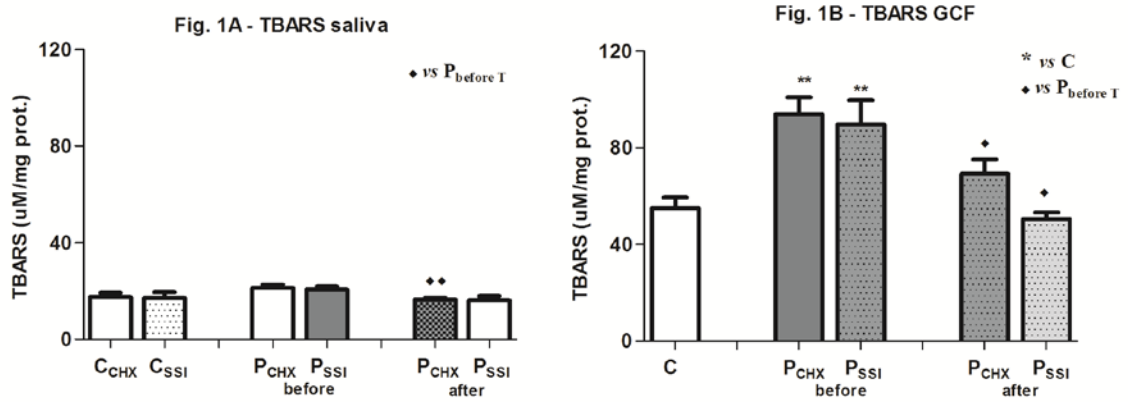

Fig. 1 – A) Thiobarbituric acid products (TBARS) concentration (μmol/mg proteins) in saliva, and B) gingival crevicular fluid (GCF) of the control group (C) with the treatment (T) of chlorhexidine – CHX (C_{CHX}) or silver ions solution – SSI (C_{SSI}), periodontal patients (P) before T with CHX (P_{CHX} before T) or SSI (P_{SSI} before T), as well as P after the treatment with CHX (P_{CHX} after T) or SSI (P_{SSI} after T). Bars in the graph represent mean ± standard deviation (SD). Labels of statistical significance: * – compared to the C before T; ♦ – compared to P_{CHX} before T or P_{SSI} before T treatment; Statistical significance was considered at: *,♦$p < 0.05$, **,♦♦$p < 0.01$, *,♦♦♦$p < 0.001$ (one-way ANOVA, Dunnett's C test).**

Table 3

Thiobarbituric acid reactive substances (TBARS) concentration (μmoL/mg proteins) and total superoxide dismutase (tSOD) activity (U/mg proteins) in the gingival fluid of healthy individuals with the treatment (T) of chlorhexidine – CHX (C_{CHX}) or silver ions solution – SSI (C_{SSI}), periodontal patients (P) before the T with CHX (P_{CHX} before T) or SSI (P_{SSI} before T), as well as P after the T with CHX (P_{CHX} after T) or SSI (P_{SSI} after T)

Parameters	$P_{CHX\ before\ T}$	$P_{CHX\ after}$	C_{CHX}	$P_{SSI\ before}$	$P_{SSI\ after}$	C_{SSI}
TBARS (μM/mg prot.)	93.9 ± 22.6**	69.2 ± 19.1♦	55.2 ± 16.4	89.6 ± 32.1**	50.5 ± 8.8♦	55.2 ± 16.4
tSOD (U/mg prot.)	1210.3 ± 463.5**	881.6 ± 207.6♦	769.9 ± 196.3	1296.0 ± 411.2**	859.6 ± 187.7♦♦	769.9 ± 196.3

All results are represented as mean ± standard deviation (SD). Labels of statistical significance: * – compared to the control group (C); ♦ – compared to P_{CHX} before T or P_{SSI} before T. Statistical significance was considered at: ♦*p* < 0.05, **,♦♦*p* < 0.01 (one-way ANOVA, Dunnett's C test).

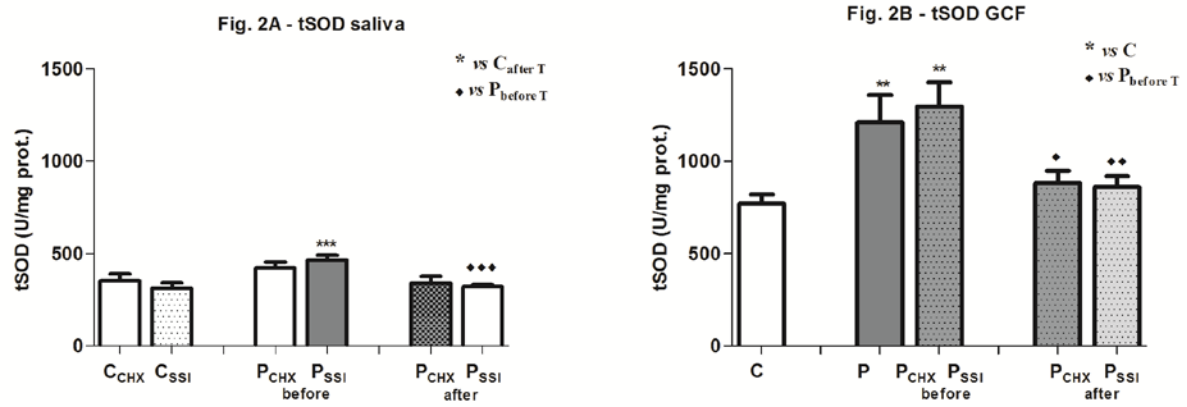

Fig. 2 – A) total superoxide dismutase – SOD activity (tSOD; U/mg proteins) in saliva, and B) gingival crevicular fluid (GCF) of the control group (C) with treatment (T) of chlorhexidine – CHX (C_{CHX}) or silver ions solution – SSI (C_{SSI}), periodontal patients (P) before T with CHX (P_{CHX} before T) or SSI (P_{SSI} before T), as well as P after T with CHX (P_{CHX} after T) or SSI (P_{SSI} after T). Bars in the graph represent mean ± standard deviation (SD). Labels of statistical significance: * – compared to the control group before T; ♦ – compared to P_{CHX} before T or P_{SSI} before T. Statistical significance was considered at: *,♦*p* < 0.05, **,♦♦*p* < 0.01, *,♦♦♦*p* < 0.001 (one-way ANOVA, Dunnett's C test).**

In the group of periodontal patients before the SSI treatment, tSOD activity was significantly higher ($p < 0.001$) in the saliva compared to that in the C group (Figure 2A). On the contrary, tSOD activity decreased in the saliva of patients after the SSI treatment compared to the values before the SSI treatment (Figure 2A; $p < 0.001$). In both periodontal groups before the antiseptic treatment (CHX or SSI), tSOD activity was increased in the GCF (Figure 2B) compared to the control values. The activity of tSOD significantly decreased in GCF after both antiseptic treatments compared to the values before the treatment.

Discussion

Increased TBARS concentration and SOD activity in the saliva and GCF of periodontal patients indicate prominent cell and tissue impairment. In the literature, there is no human study on the oxidative stress biomarkers that appear as an outcome of antiseptic effects in periodontal tissues. Therefore, our study is the first on this issue.

Oxidant/antioxidant balance differs across oral environmental compartments like hard dental tissue, saliva, and GCF [28, 29]. Patients with periodontal disease are more susceptible to an imbalance of antioxidant-oxidative stress conditions [30]. ROS-related tissue destruction should be determined *via* the final product of LPO, such as MDA since ROS have an extremely short life and are not easy to detect.

Membrane fluidity is a crucial factor that determines cellular communication, membrane elasticity, and biological transport of proteins and lipids. In the presence of reducing equivalents, microsomal membrane fluidity is declined, and the membrane becomes rigid and vulnerable to oxidative injury. Lipid peroxidation has been implicated in the pathogenesis of periodontal disease [7]. ROS can attack polyunsaturated fatty acids and form various LPO products such as MDA, which is one of the most frequently used indicators of LPO and may be a potential biomarker indicating oxidative stress. An increase in salivary levels of MDA is an important indicator of periodontal tissue destruction and of great importance as it provides an impression on the common periodontal health. Similar to our previous study, Tsai et al. [31] also reported that LPO significantly correlated with clinical parameters of periodontal disease. Our previous study revealed comparable results as we obtained lower clinical parameters during periodontal treatment with both antiseptics [20].

In saliva samples, no difference in TBARS concentration was found among patients and healthy controls (Figure 1A). The GCF appears to be a more reliable source for identifying periodontal disease. If a disruption of the balance between ROS and antioxidants occurs, it may play a role in the progress of oral inflammatory diseases, as increased TBARS concentration in GCF evokes an enhancement in LPO level in the periodontium and the oral environment in periodontitis (Figure 1B).

There are several explanations for higher TBARS concentrations in GCF compared to the saliva of periodontal patients (Tables 2 and 3). Some investigations proposed that elevated GCF flow was associated with enlarged PMNL levels, which sequentially contributed to peroxidase improvement through the activity of myeloperoxidase. Secondly, enhancement in TBARS concentration in GCF may partially be a sign of intensified LPO in the periodontium itself, contrary to increased amounts of bacterial products. TBARS levels in GCF, which were higher than those in saliva in our study, defined that a local increase in the LPO process was more prominent in the periodontal region and was more significant than the systemic increase.

Development of oxidative stress in GCF of patients with periodontal disease is characterized by ROS overproduction, depletion of reducing equivalent sourced, and oxidative injury of biomolecules accompanied by lipids [32]. All these results correspond to those in our study. In addition, we revealed the improved reduction of TBARS level in saliva after the CHX treatment (22%), as well as after both antiseptics in GCF of periodontal patients, which suggests that applied antiseptics had beneficial effects in lowering lipid components and preventing oxidative stress improvement in chronic periodontitis (Figures 1A and 1B).

The antioxidative defense system performs a notable part as a scavenger of ROS in the process of their removal. Changes in the activities of antioxidative enzymes may indicate an increase in reactive oxygen and the regular function disturbance of antioxidative defense systems. The enzymatic antioxidant activities were significantly higher in these patients, which suggests that the scavenging of disproportionately generated LPO products at the inflammatory sites possibly induces an increased enzymatic antioxidant activity [33]. In addition to diminished energy production, the products of anaerobic glucose metabolism lead to the formation of ROS, which disrupts the deterioration and periodontal tissue destruction. Total SOD activity, as an indicator of oxidative stress, was higher in periodontal patients in saliva in relation to the activity of SOD in the control group, indicating that the periodontal environment induced the synthesis of SOD (Figure 2A). In accordance with our results, Novakovic et al. [8] also found that patients with periodontitis had higher tSOD in saliva compared to controls. These findings suggest that an increase in SOD activity may be accompanied by an early inflammatory syndrome, while its improvement arises in response to pathological progression. The reduction of tSOD activity was noticed only in the P_{SSI} group (32%) after the treatment ($p < 0.001$) compared to the periodontal group before the SSI treatment in saliva (Figure 2A).

Furthermore, our study observed higher tSOD activity in GCF of periodontal patients compared to controls (Table 3). The human periodontal ligament acquires the SOD, which proposes biological protection against ROS. Bacterial lipopolysaccharides also stimulate superoxide release from gingival fibroblasts, suggesting that the induction of SOD may correspond to an important protective mechanism of the fibroblasts in inflammation. Increased SOD activity in inflamed gingiva may reveal increased superoxide radical generation by PMNL occupying the diseased sites. Antiseptics therapy can reduce the disease-causing microbes associated with oral plaque, which may assist in inflammatory reduction. The reason for reduced tSOD activity (Figure 2) in treated groups (CHX, SSI) may be attributed to the lack of the substrate, such as superoxide anion, which reacts easily with nitrogen monoxide to form harmful peroxynitrite anion and this reaction, involved in the acetylation of amino acids, is accomplished by gram-negative anaerobes [34].

Our results explained that antioxidants like SOD in GCF of periodontal patients before the antiseptic treatment credibly expose periodontal response and the tissue response to treatment. In our study, we revealed a positive Spearman correlation between tSOD activity before and after CHX treatment ($r = 0.7400$, $p < 0.01$), as well as between tSOD activity before antiseptic treatments in GCF of periodontal patients. This positive correlation analysis could be just a result of the preserved antioxidative defense system.

The efficacy of two different antiseptics in decreasing the inflammatory impact on the periodontal tissues subsequently improves clinical parameters and systemic biochemical oxidative stress and inflammatory markers. The current study proposed that GCF has better diagnostic potential than saliva. TBARS may be a better marker for gingival inflammation rather than periodontitis. The major changes in oxidant/antioxidant status were registered in periodontal groups when increased TBARS concentration was accompanied by increased tSOD activity.

Conclusion

This is the first paper that achieved the antioxidative effects of antiseptics with SSI on periodontal patients. Utilized antiseptics with antioxidant capacity can reduce periodontal disease initiated by tissue damage, which is of remarkable clinical application in dentistry. We showed that the assessment of saliva, as well as GCF oxidative status, might represent a useful method for evaluating the applied antiseptics in patients with chronic periodontitis.

Conflict of interest

No conflict of interest exists for any of the authors of this article.

Acknowledgments

This work was supported by the University of Defence in Belgrade, Serbia (Project No. MFVMA/04/19-21) and the Ministry of Education, Science, and Technological Development, Republic of Serbia (Project No. III41018).

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Received on September 21, 2020
Revised on November 12, 2020
Accepted on December 7, 2020
Online First December 2020