ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Evaluation of antioxidant capacity and clinical assessment of patients with chronic periodontitis treated with non-surgical periodontal therapy and adjunctive systemic antibiotherapy

SIMINA BOIA[1)], ŞTEFAN-IOAN STRATUL[1)], MARIUS BOARIU[2)], SORIN URSONIU[3)], SMARANDA LAURA GOŢIA[4)], EUGEN RADU BOIA[5)], CLAUDIA BORZA[6)]

[1)]*Department of Periodontology, Faculty of Dental Medicine, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania*

[2)]*Department of Endodontics, Faculty of Dental Medicine, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania*

[3)]*Department of Public Health, Faculty of Medicine, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania*

[4)]*Department of Physiology, Faculty of Medicine, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania*

[5)]*Department of ENT, Faculty of Medicine, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania*

[6)]*Department of Pathophysiology, Faculty of Medicine, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania*

Abstract

This study aims to evaluate the oxidative stress changes in patients with chronic periodontitis (CP) undergoing non-surgical periodontal therapy alone, compared with non-surgical periodontal therapy with adjunctive systemic antibiotic therapy. Sixteen patients with CP, randomly assigned into two equal groups, were treated either with scaling and root planing (SRP) + Amoxicillin + Metronidazole, each 500 mg, three times daily, for seven days (test group), or with SRP + placebo for seven days (control group). Venous blood and unstimulated saliva samples were collected. Non-surgical periodontal therapy was performed simultaneously with antibiotics administration. Oxidative stress balance was evaluated by measuring derivatives of reactive oxygen metabolites (d-ROMs) and the biological antioxidant potential (BAP) in plasma. After the microscopic evaluation of the pathological aspect of the epithelial cells (ECs), their number, viability and the presence of C-reactive protein (CRP) were reevaluated from saliva at seven days, while reduced glutathione (GSH) level, d-ROMs and BAP at three months. Wilcoxon and Kruskal–Wallis rank-tests were used for statistics. At three months, statistical significant reductions of mean periodontal pocket depth (PPD) and clinical attachment level (CAL) gains (both p=0.01) were found in test group. Full-mouth plaque score (FMPS) decreased statistically significant in control group (p=0.02), d-ROMs decreased statistically significant in test group (mean difference 116.24±107.6 U CARR, p=0.01). Mean GSH, BAP level, number of ECs, their viability and CRP were statistically non-significant. In test group patients, oxidative stress status changed from a very high level to a medium one, suggesting that adjunctive use of antibiotics could have contributed to the reduction of reactive oxygen metabolites, along with significant clinical improvements.

Keywords: periodontitis, antibiotics, epithelial cells, Trypan Blue, oxidative stress.

Introduction

Oxidative stress is incriminated in the pathophysiology of both systemic diseases with a high prevalence, such as hypertension, atherosclerosis, diabetes, and periodontitis [1, 2]. Markers of oxidative stress were highlighted in saliva, which confers it pathogenicity in oral disorders. Oxidative stress is characterized by an imbalance between the production of reactive oxygen species (ROS), and the ability of biological systems to fight these destructive molecules and to induce repairing processes [3, 4].

Antioxidants are defined as substances, which, in low concentrations, when compared to an oxidizable substrate, delay or postpone the oxidation of that substrate [5]. Oxidative stress occurs when there is an imbalance between oxidants and antioxidants, the ROS gaining ground, and generating the destruction of tissues.

Studies have shown that there is a significant decrease in the concentration of antioxidants in saliva of periodontal patients, when compared to healthy individuals, while oxygen-derived free radicals and the products of their reactions play an important role in the pathogenesis of chronic inflammatory disorders, like periodontitis [6].

Glutathione is considered to be an important anti-oxidant that limit cell injury induced by ROS and has an essential role in the control of the inflammatory processes and the redox reactions [7]. Patients with periodontitis display a reduced total antioxidant capacity of the saliva, and lower concentrations of reduced glutathione (GSH) both in serum and in gingival crevicular fluid (GCF) [8].

Periodontitis is an inflammatory oral disease of teeth supporting tissues, manifested through loss of connective tissue attachment, resorption of the alveolar bone, and increased dental mobility, followed by subsequent loss of teeth [9]. Its etiology is multifactorial and involves the development and presence of bacterial biofilm, periodontal pathogens (bacteria which significantly contributed to periodontitis), immune host response and genetic risk

factors [10]. The destruction of tissues is produced by an imbalance between the previously mentioned factors [11]. The periodontium is protected by components of saliva and crevicular fluid, and by oral mucosa epithelial cells (ECs) [12]. ECs form a mechanic barrier, which protects oral mucosa and together with the GCF and the saliva leukocytes, takes part in the local defense mechanisms. An increase in the desquamation rate is associated with a reduction of the mechanic barrier function. ECs with low cell viability (CV) are fragile and may easily release substances that trigger local inflammation [12, 13].

The cellular metabolism and the activation of reactive processes, like inflammation, may induce oxidation reactions, through transfer of electrons or hydrogen atoms between molecules. Products resulting after this process disperse in blood and serve as useful markers of oxidative status [14].

In general, high values of derivatives of reactive oxygen metabolites (d-ROMs) are observed in subjects exposed to risk factors like smoking, alcohol abuse or diseases associated with changes in oxidative balance, like cardiovascular diseases, metabolic syndrome or cancer [14]. The biological antioxidant potential (BAP) test is a photometric test for the determination of biological antioxidant potential. It allows the determination of the blood concentration of antioxidants and provides an overall measure of antioxidants such as bilirubin, uric acid, vitamins C and E, and proteins [14].

The primary goal of non-surgical periodontal therapy is to control microbial periodontal infection by removing bacterial biofilm, calculus and toxins from periodontally-involved root surfaces [15].

This goal is reached by mechanical root instrumentation using ultrasound and hand instruments, alone or in conjunction with various local antimicrobials. The benefits of systemic antibiotic therapy as an adjunct to periodontal non-surgical treatment have been consistently debated in the literature [16–21], and a wide variety of antibiotics has been investigated as an adjunct to mechanical debridement of the periodontal pockets [17–19]. The combination of Amoxicillin (AMX) and Metronidazole (MET) administered after non-surgical periodontal therapy [22] has been shown to be one of the most promising antibiotic protocols in the treatment of periodontitis [20–22].

The aim of this study is to evaluate the oxidative stress changes and pathological aspects of ECs in patients with chronic periodontitis (CP) undergoing non-surgical periodontal therapy alone, compared with non-surgical periodontal therapy with adjunctive systemic antibiotic therapy.

Patients, Materials and Methods

Patient population

From the outpatients of the Department of Periodontology, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania, 16 patients, with at least 12 natural teeth in the oral cavity, clinically distributed in all four quadrants, out of which at least six teeth presented one site with pocket depth (PD) ≥5 mm at baseline and whom have not received periodontal therapy or antibiotic intake in the previous six months, were selected for this study. The ethical approval was obtained from the Research Ethics Committee of the "Victor Babeş" University of Medicine and Pharmacy, Timişoara (Approval No. 06/07.05.2018). The study was conducted over a period of three months (May–August 2018) in accordance to the principles outlined in the Declaration of Helsinki on experimentation involving human subjects. All subjects who participated in this study were informed about the nature and the purpose of the study and each one of them signed an informed written consent regarding the dental procedures and the biological material sampling.

The study population consisted from men and women >30 years old, with clinic and radiographic signs of generalized CP, as described by Armitage (1999) [23].

All patients were investigated clinically [complete dental, internal medicine and ear, nose and throat (ENT) exam] and radiographically at baseline (before therapy). The following clinical parameters were assessed: periodontal pocket depth (PPD), clinical attachment level (CAL), bleeding on probing (BOP), and full-mouth plaque score (FMPS) [24].

After the measurements, full-mouth scaling and root planing (SRP) under local anesthesia was performed. In the test group, additional systemic antimicrobial agents were distributed after SRP sessions. Subjects were clinically and biochemically monitored at baseline and were re-evaluated in the same manner at the three months periodontal recall.

At the end of the non-surgical therapy session, the clinician allocated the patients to one of the two treatment groups, and gave their medications along with instructions for intake:

- Control group: SRP alone + placebo (*N*=8);
- Test group: SRP followed by systemic Amoxicillin and Metronidazole (SRP + AMX + MET) (both 500 mg, three times daily, seven days, *N*=8).

In order to evaluate the level of oxidative stress, blood samples were taken and transported to the laboratory within one hour after venipuncture, where they were centrifuged and kept at -80^{0}C until the analysis.

The d-ROM test was used to measure the oxidant ability of a plasma sample towards a particular substance (modified aromatic amine) used as an indicator (chromogen) [14] and the BAP test was used for the analysis of the biological antioxidant potential [14].

Unstimulated whole saliva samples were collected for the evaluation of C-reactive protein (CRP), GSH, presence of ECs and CV by using sterile Falcon tubes for 5 minutes in the morning. The samples were collected for biochemical analysis before SRP procedures and at seven days after, when the medication intake was finalized, for the above-mentioned parameters, excepting GSH, which was reassessed at three months. After centrifugation at 2500 rpm, the value of GSH was evaluated from the supernatant trough spectrophotometric method (Jenway Spectrophotometer, UK), after adding Ellman's reagent [5,5'-dithio*bis*-(2-nitrobenzoic acid), DTNB] [25]. From

the supernatant, through an agglutination reaction, the presence of CRP was evaluated using CRP slide (Analyticon Biotechnologies AG, Germany), as well.

Concerning the pathological aspect of the ECs, the microscopic examination was performed with Leica DM750 microscope (Leica Microsystems, Germany) and their appearance was captured with Leica DMshare system (Leica Microsystems, Germany). The number of ECs was assessed from the salivary sediment, using the Bürker counting chamber (BLAUBRAND®, Germany). CV was monitored with the Trypan Blue exclusion assay, which is based on the principle that viable cells possess intact cell membranes that exclude Trypan Blue dye, whereas non-viable cells do not, therefore the dye penetrates and colors the cytoplasm in blue [26].

The Wilcoxon rank-test was used for the intra-group statistical analysis of the two saliva determinations, and the Kruskal–Wallis rank-test for the inter-group testing.

Results

Mean age of the patients in control group was 50.62± 6.39 years old and in test group 37.62±5.31 years old.

Both PPD and CAL changes presented a statistical significance (p=0.01), showing reductions at the three months reevaluation meaning that the primary outcome of the periodontal therapy was achieved (Table 1, Figures 1 and 2).

The FMPS decreased in both groups, but statistically significantly only in control group (p=0.02) (Figure 3).

Table 1 – ***Clinical results. Mean values ± SD for the investigated clinical parameters (PPD, CAL, BOP, FMPS) at baseline and at three months***

Clinical variables	Control group (SRP + placebo, seven days, *N*=8)	Test group (SRP + AMX + MET, seven days, *N*=8)	Inter-group comparison control–test *p*-value control–test
PPD [mm]			
Baseline	2.8525±0.3629935	3.7±0.621059	0.02*
Three months	2.675±0.2815772	2.95±0.8280786	n.s.
Intra-group comparison *p*-value baseline–three months	n.s.	0.01*	
CAL [mm]			
Baseline	3.175±0.7343607	4.4125±1.159356	0.02*
Three months	2.8875±0.4998214	3.6875±1.388151	n.s.
Intra-group comparison *p*-value baseline–three months	n.s.	0.01*	
BOP [%]			
Baseline	23.5±11.35	29.75±13.38	n.s.
Three months	18.5±13.29	12.75±10.2	n.s.
Intra-group comparison *p*-value baseline–three months	n.s.	n.s.	
FMPS [%]			
Baseline	31.5±21.0238	44.75±26.93776	n.s.
Three months	18.125±14.50554	32.75±27.9732	n.s.
Intra-group comparison *p*-value baseline–three months	0.02*	n.s.	

SD: Standard deviation; SRP: Scaling and root planing; AMX: Amoxicillin; MET: Metronidazole; PPD: Periodontal pocket dept; CAL: Clinical attachment level; BOP: Bleeding on probing; FMPS: Full-mouth plaque score [24]. *Statistically significant p values; n.s.: Not significant.

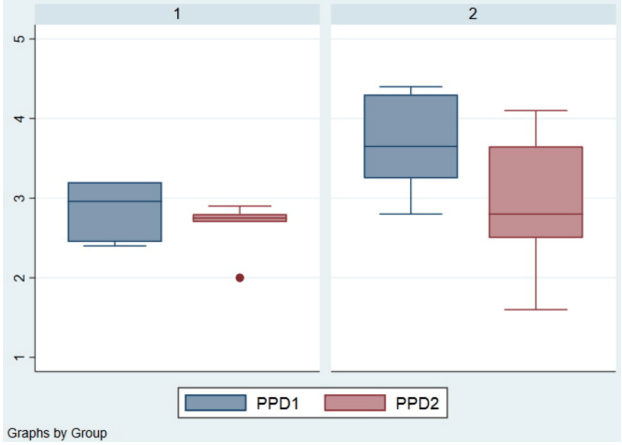

Figure 1 – ***Periodontal pocket depth variation (baseline – PPD1, three months recall – PPD2) in test (1) and control (2) groups.***

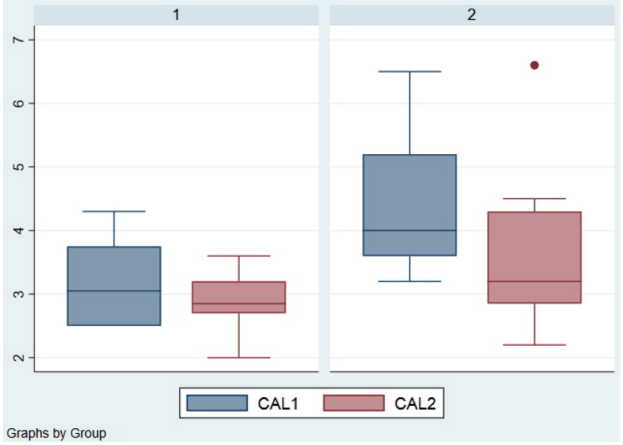

Figure 2 – ***Clinical attachment level variation (baseline – CAL1, three months recall – CAL2) in test (1) and control (2) groups.***

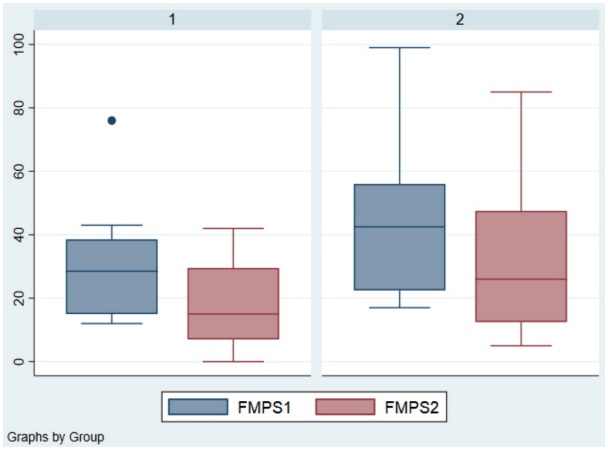

Figure 3 – ***Full mouth plaque scores variation (baseline – FMPS1, three months recall – FMPS2) in test (1) and control (2) group.***

The BOP in control group decreased from 23.5±11.35% to 18.5±13.29% and in test group from 29.75±13.38% to 12.75±10.2%, fact that reveals that antibiotics have a greater impact in the inflammation control (Table 1).

At seven days, the CV in control group decreased from 74.62±6.43% to 73.5±9.35% and in test group increased from 75.12±5.24% to 78.12±5.43% (Table 2). It is presented how Trypan Blue staining is absorbed through the degraded membrane of the dead cells (Figures 4–6).

The number of ECs increased in control group from 1460±974.35/μL to 1582.5±781.26/μL and decreased in test group from 1875±777.81/μL to 1516.25±426.41/μL (Table 2).

Detectable CRP levels remained in more patients in the control group (seven out of eight) than in the test group (four out of eight).

After three months, GSH mean values decreased in the control group from 68.68±75.37 μmol/L to 65.14±66.71 μmol/L and in test group from 48.73±33.89 μmol/L to 46.46±21.59 μmol/L (Table 2).

d-ROMs and BAP values have changed in the following manner:

- Control group: both d-ROMs and BAP increased (d-ROMs from 448.94±128.42 U CARR to 458.91±137.11 U CARR and BAP from 1783.3±510.04 μmol/L to 2319.9 μmol/L) (Table 2, Figure 7);
- Test group: both d-ROMs and BAP decreased (d-ROMs from 491.83±134.85 U CARR to 375.58±126.06 U CARR, p=0.01, and BAP from 2246.18±918.35 μmol/L to 1890.16±582.71 μmol/L) (Table 2, Figure 7).

Table 2 – ***Biochemical results. Mean values ± SD for each investigated parameter at baseline and at seven days for CV, EC and at three months for GSH, d-ROMs and BAP***

Biochemical variables	Control goup (SRP + placebo, seven days, *N*=8)	Test group (SRP + AMX + MET, seven days, *N*=8)	Inter-group comparison control–test *p*-value control–test
CV [%]			
Baseline	74.62±6.43	75.12±5.24	n.s.
Seven days	73.5±9.35	78.12±5.43	n.s.
Intra-group comparison *p*-value baseline–seven days	n.s.	n.s.	
EC [N/μL]			
Baseline	1460±974.35	1875±777.81	n.s.
Seven days	1582.5±781.26	1516.25±426.41	n.s.
Intra-group comparison *p*-value baseline–seven days	n.s.	n.s.	
d-ROMs test [U CARR]			
Baseline	448.94±128.42	491.83±134.85	n.s.
Three months	458.91±137.11	375.58±126.06	n.s.
Intra-group comparison *p*-value baseline–three months	n.s.	0.01*	
BAP test [μmol/L]			
Baseline	1783.3±510.04	2246.18±918.35	n.s.
Three months	2319.9	1890.16±582.71	n.s.
Intra-group comparison *p*-value baseline–three months	n.s.	n.s.	
GSH [μmol/L]			
Baseline	68.68±75.37	48.73±33.89	n.s.
Three months	65.14±66.71	46.46±21.59	n.s.
Intra-group comparison *p*-value baseline–three months	n.s.	n.s.	

SD: Standard deviation; CV: Cell viability; EC: Epithelial cells; GSH: Reduced glutathione; d-ROMs: Derivatives of reactive oxygen metabolites; U CARR: Caratelli Units; BAP: Biological antioxidant potential; SRP: Scaling and root planing; AMX: Amoxicillin; MET: Metronidazole. *Statistically significant *p* values; n.s.: Not significant.

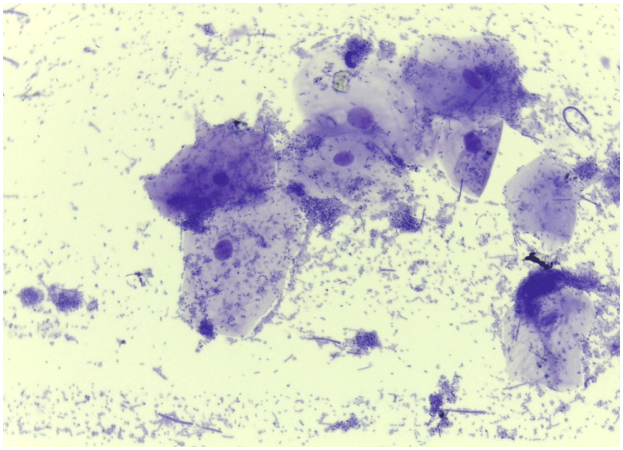

Figure 4 – ***Salivary smear: the dead epithelial cells are dark blue and the viable cells are light blue. Trypan Blue staining, ×400.***

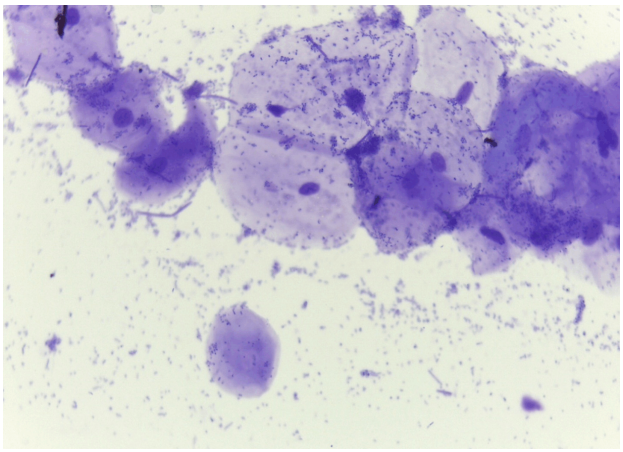

Figure 5 – ***Salivary smear: the dead squamous epithelial cells from superficial and intermediate layers of the buccal epithelium appeared dark blue, while the viable cells stained light blue. Trypan Blue staining, ×400.***

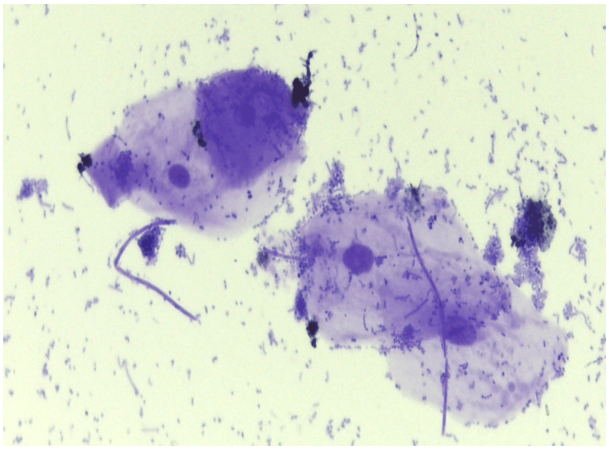

Figure 6 – ***Salivary smear: dark blue squamous dead epithelial cells from superficial layer of buccal epithelium and light blue viable cells of intermediate layer of buccal epithelium in a background of small bacteria and* Candida *spp. hyphae. Trypan Blue staining, ×400.***

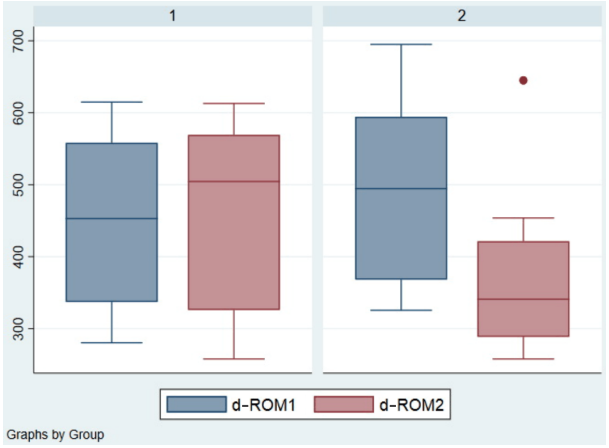

Figure 7 – ***Reactive oxygen metabolites variation (baseline – d-ROM1, three months recall – d-ROM2) in test (1) and control (2) group.***

Discussions

This study estimates and compares the levels of oxidative stress along with the evaluation of pathological aspects of ECs from the oral mucosa in patients with CP undergoing systemic antibiotic treatment adjunctive to non-surgical periodontal therapy.

Complete dental, internal medicine and ENT exam was performed in each patient in order to rule out associated pathology [27–31].

As Thomson *et al.* (1999) [32] and Squier & Kremer (2001) [33] presented in their research, physiological desquamation of the oral mucosa occurs in order to maintain the thickness and integrity of the natural epithelial barrier and provides a significant quantity of exfoliated ECs in the saliva. Their turnover time is faster than for cells from other locations, which may have important implications in the rehabilitation of the tissues from damage and important relevance in the pathogenesis of gingival inflammation. Based on these facts, in our study, the evaluation of the number of ECs and their viability in the saliva, at the end of the systemic antibiotic adjunctive treatment was justified. We observed the decrease of the shed ECs in test group, in association with the increase of the CV, fact that may suggest the restoration of the integrity of the epithelial oral barrier in patients of the test group.

In the context of the presence of oxidative stress, the enzymatic and non-enzymatic systems, which preserve the antioxidant status under physiological conditions, become overwhelmed. This phenomenon occurs because there is a metabolic disorder due to an imbalance caused by either the low capacity of the antioxidant defense system or the excessive generation of oxygen metabolites. Because it plays an important role in the non-enzymatic antioxidant defense system, GSH is a specific antioxidant which was reported by Gümüş *et al.* (2009) [34] and D'Aiuto *et al.* (2010) [35] to have significantly lower values in saliva of patients with CP, fact also confirmed by our findings. Similar results were obtained by Savita *et al.* (2015) [36] at the three months reevaluation of salivary GHS and by Öngöz *et al.* (2016) [37] at one month. Its decrease may be due its consumption in the mechanisms of the neutralization of free radicals as a scavenger. The GSH reduction tendency in our findings may be the result of its use in the local antioxidant systems.

In the research of Vassalle *et al.* (2012) [2] and Chapple & Matthews (2007) [38], it is presented that the ROS, by initiating free-chain chain reactions, have a destructive potential on a very wide range of tissues, and their presence is due to a deficiency in the homeostatic balance, when the antioxidant defense systems become overwhelmed, in accordance with our study data.

Related to our research, the studies of Tsai *et al.* (2005) [39], Akalin *et al.* (2007) [40], Konopka *et al.* (2007) [41] and Chapple *et al.* [42] carried on human subjects have highlighted the fact that periodontitis is associated with a systemic state of oxidative stress level by inducing a minor local inflammatory status. In association with a low total antioxidant capacity, a direct correlation among the features of periodontal disease, the systemic inflammatory status and oxidative stress may be stated. The fact that periodontal disease generates oxidative stress formation, or, conversely, that it may be a result of oxidative stress is an aspect that remains insufficiently proven and further research is needed.

Our findings are comparable to those of Tamaki *et al.* (2008) [43] carried on CP patients in the maintenance phase of periodontal therapy to whom oxidative stress balance was evaluated. These results show that CAL values were positively associated with plasma d-ROMs levels but not with BAP levels, aspect confirmed by our findings. This might indicate that plasma d-ROMs levels could influence the clinical outcome in periodontal disease, however, the involvement of blood total antioxidant level in its progression remaining insignificant.

Also similar to our study is the one study published by Tamaki *et al.* (2009) [44], who showed that non-surgical periodontal treatment improved both clinical periodontal parameters and plasma d-ROMs, at a two-month re-evaluation, suggesting that there is a close relationship between periodontal conditions and systemic oxidative status.

In our study, mean age of the patients in control group (where the BAP increased) was higher than in test group (where the BAP decreased). These findings do not confirm the literature which shows that there is a reduction of the BAP in the elderly [14], suggesting a direct correlation with a reduced activity of the plasmatic antioxidant barrier.

Another finding in our study that is supported by authors like Ehmke *et al.* (2005) [45] and Feres *et al.* (2012) [46] is that better clinical results were found measuring mean full-mouth PPD or CAL as endpoint, in the evaluation of the outcome provided by the use of adjuvant antibiotic medication, compared to the treatment with SRP alone. Also, in other recent placebo-controlled clinical trials like those of Cosgarea *et al.* (2016) [20] and Cosgarea *et al.* (2017) [21], antibiotic groups exhibited improved clinical results than those with placebo medication after SRP, confirming the present research results.

One of the limitations of the present study is the relatively small number of enrolled subjects. A more comprehensive research, including a larger number of participants, is needed in order to verify our conclusions. Further and more extensive studies are required before the BAP and d-ROMs evaluation can be used as a routine and standardized technique in the daily practice of monitoring periodontitis patients.

Conclusions

In patients whom received periodontal therapy combined with adjunctive antibiotic therapy, oxidative stress status decreased from a very high level to a medium one. The reduction of reactive oxygen metabolites levels could be attributed to the adjunctive use of antibiotics. The number of desquamated ECs was reduced, and significant improved clinical results were observed after antibiotic treatment.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

Supported by internal funds from "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania: Doctoral Grant No. 13901/19.11.2014; Research Grant No. PII-C5-TC-2017-07-769/17.01.2017.

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Corresponding author
Marius Boariu, Lecturer, DMD, PhD, Department of Endodontics, Faculty of Dental Medicine, "Victor Babeş" University of Medicine and Pharmacy, 9 Revoluţiei 1989 Avenue, 300049 Timişoara, Romania; Phone +40722–701 871, e-mail: boarium@yahoo.com

Received: September 1, 2018

Accepted: December 20, 2018