

Analysis of hematological and oxidative stress parameters in the evaluation of experimentally induced periapical lesions

¹Antonela M. Berar, ²Dragomir C. David, ¹Liana Lascu, ³Luminita Matros, ⁴Radu S. Campian

¹Department of Prosthodontics, Faculty of Dentistry, "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; ²National Institute of Public Health-Regional Centre for Public Health, Cluj-Napoca, Romania; ³Department of Microbiology, "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; ⁴Department of Oral Rehabilitation, Faculty of Dentistry, "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.

Abstract. Aim: To evaluate hematological and oxidative stress parameters in animal models associated with experimentally induced periapical lesions (PL) in molar tooth and to investigate the influence of inflammatory periapical lesions on these parameters at different intervals. Material and method: 100 Adult Wistar rats was divided into four groups: group I, control rats with no lesions; group II, rats with induced periapical lesions and no pathology; group III, diabetic rats with periapical lesions; and group IV, ovariectomized rats with periapical lesions under bisphosphonates therapy. The periapical lesions in groups II, III and IV were induced in all animals by exposure of the pulp in right first mandibular molar to the oral microorganisms. The blood samples were collected from all groups; the group I was used as the control for the rest of other groups at time zero (T0), while in groups II, III, IV, the collection of blood samples was made by retro-orbital sinus puncture at 14 (T1), 30 (T2) and 60 (T3) days following pulp exposure. Hematological parameters (total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, erythrocytes, hemoglobin, hematocrit and platelets) and oxidative stress markers (malondialdehyde-MDA and glutathione-GSH) were determined. The presence of periapical lesions was confirmed radiographically in experimental animals. Results: Statistically significant differences between all groups were found following evaluation of hematological and oxidative stress parameters: total leukocytes $p=0.043$, neutrophils $p=0.034$, eosinophils $p=0.022$, platelets $p=0.0001$, erythrocytes $p=0.001$, hemoglobin $p=0.016$, as well as oxidative stress markers: MDA $p<0.001$, GSH $p=0.022$. There were no statistically significant differences regarding lymphocytes and monocytes between these groups, $p>0.05$. A statistically significant increase in neutrophils count was observed in group II compared to the control group I ($p=0.036$) at 60 days (T3), in group III compared to group I at 30 days (T2) ($p=0.004$), and in group IV at 14 days (T1) compared to group I ($p=0.01$). Conclusion: The animal model of experimentally induced PL in molar teeth influences the investigated hematological and oxidative stress parameters. The presence of PL was associated with variations in these parameters' levels in the peripheral blood.

Key Words: periapical lesions, hematological parameters, oxidative stress markers.

Copyright: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Corresponding Authors: A. M. Berar, email: antonela_berar@yahoo.com

Introduction

Periapical lesion (PL) is an inflammatory process caused by bacterial infection of the root canal system, which develops around the apex of the necrotic or infected teeth. The endodontic environment provides a selective habitat for the development of a mixed, predominantly anaerobic flora (Munir et al 2011). By colonizing the necrotic root canals, bacteria induce damage to periradicular tissues and give rise to inflammatory changes (Siqueira & Rôças 2007). The nature and extension of PL is determined by the pathogenicity of bacteria and the host defense response. PL generally occurs following interaction between microbial factors and the host defense system at the interface of the infected radicular pulp and periodontal ligament. This dynamic process leads to local inflammation, resorption of hard tissues and development of periapical lesions (Nair 2004). The host response against bacterial infection involves the inflammatory cells, the

activation of chemical mediators of inflammation, which are important in the pathogenesis of periapical tissue destruction. The severity of the inflammatory reaction in the periapical area depends on the host's defensive ability and biological status. Periapical reaction has two stages: acute and chronic, according to the evolution of the inflammatory process. During the acute stage, mediators of inflammation and pro-inflammatory cytokines are released from periapical tissues into bloodstream in response to the bacterial infection of root canal systems (Li et al 2000). In the later stage, the persistent microbial infection within the root canal system of the affected teeth can induce periodic low-grade bacteremia, which contributes to elevated pro-inflammatory cytokines leading to systemic manifestation and alteration of general status (Verdi 2013). Additionally, bacterial products, including toxins, are absorbed into the systemic circulation and stimulate host defense.

Some studies have demonstrated a relationship between PL and several systemic conditions such as diabetes mellitus or cardiovascular disease (Chapple *et al* 2007). Diabetes mellitus may be associated with the development of more severe forms of periapical lesions. It has been demonstrated that metabolic conditions in type 2 diabetes may enhance the development of periradicular lesions (Iwama *et al* 2003). During periapical lesion progression, the alveolar bone resorption is triggered by several inflammatory mediators (IL-1, IL-6, TNF- α) released by leucocytes, macrophages. In this study, we used an ovariectomized rat model induced osteoporosis and treated with bisphosphonates. The ovariectomy causes the reduced alveolar bone density in female rats (Anbinder *et al* 2006; Omi & Ezawa 1995) and the estrogen deficiency induced osteoporosis in the female rats with similar response to that in a post-menopausal women. In addition, the oxidative stress markers contribute to the development of postmenopausal osteoporosis by increasing the bone resorption (Cervellati 2013). It has been demonstrated that antiresorptive therapy with bisphosphonates has beneficial effects in the management of alveolar bone destruction associated with periodontal destruction (Reddy 1995). Zoledronic acid is used in the systemic treatment of osteoporosis due to their ability to reduce bone resorption (Roux 2004).

Oxidative stress is induced by an imbalance between excessive reactive oxygen species (ROS) production and antioxidant mechanisms. The presence of local inflammation and bone resorption in periapical area may lead to an increased production of free radicals and ROS (Basu *et al* 2001; D' Aiuto *et al* 2010). Increased ROS levels cause oxidative damage to proteins, lipids and DNA nucleic acid bases, contributing to the development of inflammatory process (Dalle *et al* 2006).

The aim of the study was to analyze the systemic effects induced by periapical lesions in experimental rat models by measuring hematological and oxidative stress parameters. In this study, we present an animal model of inflammatory periapical lesion in which the systemic effects of local periapical inflammation on hematological parameters were evaluated. In parallel, oxidative stress markers (malondialdehyde MDA and glutathione GSH) were used for the quantification of the oxidative stress level and antioxidant activity.

Material and methods

Four groups of adult Wistar rats (body weight 220-250 grams, age 24 weeks) were used within this study. The animals were kept in plastic well-aerated cages (6 per cage), in a climate-controlled room (21 to 24°C) with a 12 h light/12 h dark cycle. The animals had free access to water and were fed balanced food for laboratory rats (daily intake 20 g/animal). The study was carried out in the Experimental Research Laboratory of the Department of Physiology of UMF Cluj-Napoca. The experimental protocol was approved by the Medical Ethics Committee of the "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca (Protocol No. 421/17.12.2014).

Initially, the experimental animals were divided into four groups, as detailed below:

Group I (control group) included 10 healthy rats that received no intervention or treatment, but these animals were kept in the same environment as other groups.

Group II included 30 healthy animals with experimentally induced PL in the right first mandibular molar, which received no treatment for the entire experimental period of the study.

Group III included 30 experimentally induced diabetic rats. Diabetes was induced by administration of an intraperitoneal injection of streptozotocin (Sigma Aldrich Chemie GmbH Munich, Germany), 4 mg/kg body weight. Blood glucose monitoring was performed in all diabetic rats and the levels of glucose were measured using the Accutrend Plus® Glucometer (Roche Diagnostics GmbH, Germany), with blood obtained from the rat's tail vein (Lenzen 2008)

Group IV included 30 ovariectomized female rats treated with subcutaneous injections of zoledronic acid monohydrate (Sigma Aldrich Chemie GmbH Munich, Germany), at a dose of 0.25 mg/kg-1. The bilateral ovariectomy in the female rats were performed as previously described by Anbinder 2006. The first injection with zoledronic acid was administered 7 days after ovariectomy and at three-day intervals, during the entire experimental period.

For the induction of periapical lesions, at time 0, the experimental animals were anesthetized with a mixture of 0.1 ml ketamine hydrochloride (Vetased, SC Pasteur-Filipesti SRL) and 0.05 ml xylazine hydrochloride (Alfasan International BV, Netherlands) per 100 mg body weight, administered intramuscularly. PL was induced by preparing a class I occlusal cavity in the right first mandibular molar, using a round bur to open the pulp chamber. Then, the cavity was left open to the oral environment for bacterial contamination (Tanni-Ishii 1994). The presence of PL was confirmed radiographically.

A total of 1.5-2 ml blood samples were collected from the group I at time 0 (T0) of the experiment (used as control at T0 for the other groups), at 14 (T1), 30 (T2) and 60 (T3) days after the lesion induction under general anesthesia by retro-orbital sinus puncture. The blood was stored into tubes with ethylene diaminetetraacetic acid (EDTA) as anticoagulant. Thirty minutes after blood collection the animals were checked for post-operative complications and periorbital lesions (Parasuraman *et al* 2010). After blood collection, the animals were sacrificed with a ketamine overdose.

Hematological analysis was carried out in the Laboratory of the Department of Pathophysiology of the Faculty of Veterinary Medicine. Complete blood count was performed with the Abacus Junior Vet automatic analyzer (Diatron Messtechnik, Hungary). Blood smears were stained using Diff Quick and Giemsa staining. The following hematological parameters were assessed: total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, erythrocytes, hemoglobin, hematocrit and platelets.

Oxidative stress parameters were determined in the Oxidative Stress Laboratory of the Department of Physiology, UMF Cluj-Napoca. Oxidative stress was quantified by measuring the concentration of malondialdehyde (MDA), and antioxidant activity of reduced glutathione (GSH) using a Perkin Elmer spectrophotometer. The concentration of MDA and GSH was expressed as nmol/ml.

Measurement of MDA concentration: Malondialdehyde (MDA) was determined using a fluorimetric method with 2-thiobarbituric acid 10 mM in K₂HPO₄ 75 mM pH3. After cooling, the reaction product was extracted into n-butanol. Its concentration was determined in the organic phase after separation by

centrifugation. The measurement of emission intensity was calculated at 534 nm by the synchronous fluorescence technique at a wavelength difference between excitation and emission ($\Delta\lambda$) of 14 nm (Conti et al 1991).

Measurement of GSH level: The antioxidant capacity was quantified using reduced glutathione GSH level. A volume of plasma was mixed with 10% tricarboxylic acid (TCA), and after 10 minutes it was centrifuged. The supernatant was separated and 1.7 ml phosphate buffer pH 8 and 1 ml o-phthalaldehyde were added. After 15 minutes, the emission intensity at 420 nm was measured at an excitation of 350 nm (Hu 1994).

Statistically analysis was performed using Start Direct v 2.7.2 software and the Excel application (Microsoft Office 2007). All values are presented as median, standard deviation (SD), minimum and maximum. The comparisons between the groups were performed in the case of normal distribution data using Anova test or non-parametric Kruskal-Wallis test in the case of non-uniform distribution values. In the case of two paired samples, the Student's t test was applied for normal distribution data, and the non-parametric Wilcoxon test was used for non-uniform distribution values. The Student's t test for unpaired samples was used for normal distribution data, and the non-parametric Mann-Whitney test was for non-uniform distribution values.

Results

The digital radiographs of right hemimandibles had shown the presence of PL in the right first mandibular molar starting with day 14 after the induction of these lesions (Fig. 1). The analysis of radiographic images detected no significant differences in lesion's size between the experimental groups.

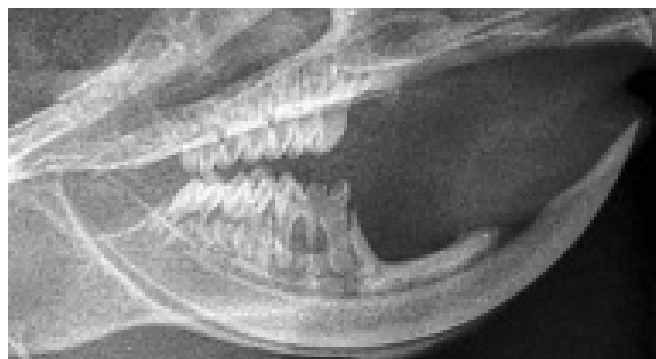


Fig. 1. Radiographs in induced periapical lesions on the first right mandibular molar

After experimental induction of periapical lesions in the molar teeth of the rats, the differences in the systemic response to periapical inflammation were found between the experimental groups and the control group for almost all hematological parameters and oxidative stress parameters. An increase in the number of total leukocytes was found at 60 days (T3) in group II compared to group I, with statistical significance ($p=0.014$), in group III compared to group I ($p=0.19$), while in group IV, there was a decrease of total leukocytes compared to the control group, without statistically significant differences ($p=0.71$). A decrease in total leukocytes count was recorded in group IV with respect to group II ($p=0.026$) (Table 1).

Table 1. Comparative analysis for total leucocytes values ($\times 10^9/l$) in studied groups

| Groups | Time | Median | SD | Min | Max |
|--------|------|--------|------|------|-------|
| I | T0 | 9.39 | 1.98 | 5.49 | 12.01 |
| | T1 | 11.62 | 2.08 | 8.4 | 14.34 |
| II | T2 | 10.16 | 2.68 | 5.64 | 14.77 |
| | T3 | 12.81 | 3.36 | 8.28 | 18.41 |
| III | T1 | 9.26 | 2.58 | 4.63 | 12.88 |
| | T2 | 11.13 | 3.44 | 6.49 | 15.25 |
| | T3 | 11.76 | 3.14 | 8.78 | 16.78 |
| IV | T1 | 11.13 | 2.68 | 7.43 | 15.9 |
| | T2 | 10.24 | 2.47 | 7.26 | 14.7 |
| | T3 | 8.87 | 3.91 | 0.66 | 13.59 |

Regarding the mean absolute values of neutrophils in group II, there was a statistically significant increase compared to the control group I ($p=0.036$) at 60 days (T3). In group III, neutrophils significant increased compared to group I at 30 days (T2) ($p=0.004$), and in group IV at 14 days (T1), the mean absolute value of neutrophils increased compared to group I ($p=0.010$). In group IV, a slight decrease in neutrophils count was observed when compared to group II ($p=0.028$) (Table 2).

Table 2. Comparative analysis for neutrophils values ($\times 10^9/l$) in studied groups

| Groups | Time | Median | DS | Min | Max |
|--------|------|--------|------|------|------|
| I | T0 | 2.47 | 0.3 | 2.1 | 3.01 |
| | T1 | 3.17 | 0.92 | 1.87 | 5.16 |
| II | T2 | 2.23 | 0.7 | 1.23 | 3.65 |
| | T3 | 4.09 | 2.06 | 1.4 | 8.83 |
| III | T1 | 3.02 | 1.36 | 1.94 | 5.38 |
| | T2 | 4.26 | 2.3 | 2.24 | 8.59 |
| | T3 | 2.85 | 0.74 | 1.75 | 4.03 |
| IV | T1 | 4.47 | 1.98 | 1.56 | 7.49 |
| | T2 | 3.47 | 1.32 | 2.15 | 6.9 |
| | T3 | 2.28 | 0.9 | 0.17 | 2.99 |

There were statistically significant increases of lymphocytes in group II compared to group I ($p=0.034$), statistically insignificant increases in group III compared to group I ($p=0.37$), while group IV had similar values to group I. Also, a decrease in the mean value of eosinophils in groups II, III and IV compared to group I was found ($p=0.54$; $p=0.001$, and $p=0.008$) (Table 3).

There was an increase in the value of platelets in group II, compared to group I, with statistical significance ($p=0.006$), in group III compared to group I, without statistical significance ($p=0.92$). Regarding the mean value of erythrocytes, a statistically significant increase was found in group II compared to group I ($p=0.0001$). The mean value of hemoglobin and hematocrit values also increased in group II compared to group I, with statistical significance (Hb $156.2 \pm 6.90g/l$ vs $141.4 \pm 13.21g/l$, $p=0.001$ and Ht $50.99 \pm 1.54\%$ vs $45.95 \pm 3.45\%$, $p=0.001$).

Table 3. Comparative analysis for lymphocytes values ($\times 10^9/l$) in studied groups

| Groups | Time | Median | DS | Min | Max |
|--------|------|--------|------|------|-------|
| I | T0 | 5.74 | 1.2 | 3.23 | 7.35 |
| | T1 | 6.98 | 2.39 | 3.15 | 10.78 |
| II | T2 | 6.66 | 2.87 | 3.27 | 10.93 |
| | T3 | 6.3 | 1.24 | 4.11 | 8.1 |
| III | T1 | 5.13 | 1.76 | 2.22 | 7.47 |
| | T2 | 5.78 | 2.53 | 2.34 | 10.12 |
| | T3 | 7.76 | 2.41 | 4.88 | 11.66 |
| IV | T1 | 5.56 | 1.45 | 2.72 | 8.1 |
| | T2 | 5.66 | 1.33 | 3.77 | 8.73 |
| | T3 | 5.49 | 2.55 | 0.4 | 9.24 |

Statistically significantly higher MDA values were obtained at 60 days (T3) in group II compared to group I ($p < 0.001$), in group III compared to group I ($p = 0.001$), and in group IV compared to group I ($p = 0.004$). A statistically significant decrease was recorded between group III and II ($p = 0.006$), respectively group IV and II ($p = 0.002$) (Table 4). Regarding GSH values, group III had statistically significantly higher values compared to group I ($p < 0.0001$). A statistically significant decrease was observed between groups IV and III, ($p = 0.035$) (Table 5).

Table 4. Comparative analysis for MDA values (nmol/ml) in studied groups

| Groups | Time | Median | DS | Min | Max |
|--------|------|--------|------|------|------|
| I | T0 | 1.52 | 0.27 | 1.14 | 1.99 |
| | T1 | 3.29 | 1.12 | 1.71 | 5.15 |
| II | T2 | 3.22 | 0.71 | 2 | 4.4 |
| | T3 | 3.12 | 0.62 | 2 | 4.27 |
| III | T1 | 2.99 | 0.58 | 2.17 | 3.87 |
| | T2 | 2.52 | 0.44 | 2.04 | 3.27 |
| | T3 | 2.29 | 0.56 | 1.45 | 3.14 |
| IV | T1 | 2.95 | 0.72 | 2 | 4.1 |
| | T2 | 2.73 | 1.09 | 1.26 | 4.77 |
| | T3 | 2.2 | 0.56 | 1.48 | 2.98 |

Table 5. Comparative analysis for GSH values (nmol/ml) in studied groups

| Groups | Time | Median | DS | Min | Max |
|--------|------|--------|------|------|------|
| I | T0 | 5.16 | 0.48 | 4.03 | 5.76 |
| | T1 | 4.24 | 1.15 | 2.61 | 6.4 |
| II | T2 | 4.7 | 0.97 | 3 | 6.15 |
| | T3 | 5.22 | 2.28 | 2.3 | 7.64 |
| III | T1 | 5.12 | 0.94 | 4.03 | 6.95 |
| | T2 | 5.83 | 1.84 | 3.68 | 8.46 |
| | T3 | 6.85 | 1.21 | 5.7 | 9.4 |
| IV | T1 | 3.31 | 0.46 | 2.47 | 3.99 |
| | T2 | 3.54 | 1.05 | 2.4 | 5.76 |
| | T3 | 5.74 | 1.3 | 4.3 | 8.43 |

For the statistical analysis between groups, statistically significant differences were observed at 60 days (T3) between all groups for total leucocytes ($p = 0.043$), neutrophils ($p = 0.034$), eosinophils ($p = 0.022$), platelets ($p = 0.0001$), erythrocytes ($p = 0.001$), hemoglobin $p = 0.016$ and MDA ($p < 0.001$), GSH ($p = 0.022$).

The mean absolute value of neutrophils recorded statistically significant differences in group II between 30 days (T2) and 60 days (T3) ($p = 0.039$), in group III between 14 days (T1) and 30 days (T2) ($p = 0.048$) and in group IV between 14 days (T1) and 60 days (T3), between 30 days (T2) and 60 days (T3) ($p = 0.009$, $p = 0.005$). Mean absolute values of lymphocytes emphasised statistically significant differences in group III between 14 days (T1) and 60 days (T3) ($p = 0.015$).

The MDA values recorded significant differences in group III between 30 days (T2) and 60 days (T3) ($p = 0.021$). The GSH values were significant in group III between 14 days (T1) and 60 days (T3) ($p = 0.019$), in group IV between 14 days (T1) and 60 days (T3) and between 30 days (T2) and 60 days (T3) ($p = 0.0001$, $p = 0.002$).

Discussions

The hemogram or complete blood count is frequently used as a broad screening test for various diseases states including anemia, infection and inflammatory process. Hematological parameters are used to detect changes in the number of white blood cells, red blood cells and platelets. Similarly to inflammation located in other parts of the body, periapical inflammation is a biological defense response triggered by various etiological, mainly microbial agents present inside the root canals of teeth with necrotic or infected pulp. Pathogenic bacteria and their products, as well as inflammatory mediators produced in periapical tissues enter the bloodstream, causing systemic effects. As described by Martinez et al (2007) periapical tissue destruction is caused by endogenous inflammatory mediators released during interaction between bacteria and host cells, mainly polymorphonuclear neutrophils, lymphocytes, plasma cells, and macrophages. The local inflammatory infiltrate mainly formed by activated macrophages and lymphocytes initiate periapical inflammation, eventually leading to progressive bone resorption (Nair 2004). The transient bacteremia can cause systemic effects, especially in immunocompromised hosts (chemotherapy, diabetes, corticosteroids, elderly (Newman 1996).

Leukocytes, particularly neutrophils, are the first body line defense of cells which against infection. In early stages, one of the systemic effects of inflammation is leukocytosis, with an increased level of neutrophils. Neutrophils are recruited in higher numbers during the inflammatory process; they are mobilized from bloodstream and migrate to the site of periapical inflammation for exerting their functions of phagocytosis, destruction and elimination of pathogenic microorganisms. Neutrophils, macrophages and lymphocytes produce pro inflammatory cytokines, reactive oxygen species that modulate local inflammatory response and trigger the systemic response. In our study, total leukocyte count increased in group II in all experimental periods, in groups III at 30, 60 days and in group IV at 14 and 30 days. The absolute number of neutrophils obtained from peripheral blood showed an increase in all groups of animals with induced PLs compared to the control group. Our findings are in agreement with other studies, in which acute inflammatory disease, particularly due to bacterial infection, shows elevated

levels of neutrophils in the blood (Ryan & Majno 1977). An increased peripheral leukocyte count has been also associated with type 2 diabetes (Ford 2002; Orshita 2004).

Blood platelets are also involved in the induction of the inflammatory process; they play a role in the antimicrobial defense of the body and in tissue repair processes. They are recruited at the site of the lesion and release a series of substances that trigger chemoattraction of leukocytes (platelet activating factor, macrophage inflammatory protein and stimulate fibroblast proliferation). Platelets mediate the adhesion of leukocytes between them, as well as their adhesion to endothelial cells. In this study, inflammation located in the periapical region was associated with thrombocytosis; statistically significant differences in platelet numbers were found in group II compared to group I ($1482.9 \pm 346.65 \times 10^9/l$ vs $1187.4 \pm 155.48 \times 10^9/l$, $p=0.005$), in group III compared to group I ($1641.5 \pm 980.15 \times 10^9/l$ vs $1187.4 \pm 155.48 \times 10^9/l$, $p<0.0001$). The increase in the number of platelets in the experimental groups is correlated with the evolution of the inflammatory process, being part of the body's response to microbial aggression.

In this study, the presence of PL is associated with a high level of systemic oxidative stress. The activation of neutrophils produces ROS that cause tissue destruction through several mechanisms: lipid peroxidation by activation of cyclooxygenase and lipoxygenase, and stimulation of proinflammatory cytokines released from monocytes and macrophages. The high level of ROS determines oxidative stress (D'Aiuto *et al* 2010).

The effects of ROS were quantified by the measurement of serum malondialdehyde (MDA) concentration in the experimental groups and the control group. MDA significantly increased in all experimental groups compared to the control group at all evaluation times, which indicates the presence of oxidative stress. The production of ROS following activation of polymorphonuclear leukocytes may contribute to the progression of the inflammatory lesions. The oxidant imbalance in the periapical region might contribute to progression of asymptomatic periapical lesions (Dezerega *et al* 2012).

Serum glutathione plays a role in antioxidant defense in animals, protecting from the negative effects of free radicals and reactive oxygen species (Wu *et al* 2004). In the group of ovariectomized female rats treated with bisphosphonates, the MDA level was increased compared to the control group I, with statistically significant differences ($p=0.004$), and GSH level was reduced, with statistically significant differences ($p=0.0007$). Our results are supported by the observations of other studies that report high oxidative stress levels and low antioxidant levels after menopause (Goy *et al* 2015). The involvement of oxidative stress in type 2 diabetes has also been demonstrated (Helmerson *et al* 2004). It has been suggested that oxidative stress might be a later process in the development of diabetes, possibly secondary to inflammation that occurs in the pathogenesis of diabetes. A recent study found increased oxidative stress values in patients with chronic apical periodontitis (Inchingolo *et al* 2014).

Conclusion

Experimentally induced periapical lesions in the animal model caused alterations of hematological parameters (total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils and platelets) in peripheral blood. Oxidative stress markers also underwent

changes during the development of the inflammatory process located in the periapical region. The systemic effects of inflammatory periapical lesions are influenced by the stage of inflammation. The changes in hematological and oxidative stress values could provide quantifiable measures of the host response to the experimentally induced periapical inflammation.

Acknowledgements

This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/138776.

References

- Anbinder AL, Prado FD, Prado MD, Balducci, Racha RF. The influence of ovariectomy, simvastatin and sodium aledronate on alveolar bone in rats. *Braz Oral Res* 2007;21(3):247-252.
- Basu S, Michaëlsson K, Olofsson H, Johansson S, Melhus H. Association between oxidative stress and bone mineral density. *Biochem Biophys Res Commun* 2001;288(1):275-279.
- Cervellati C, Bonaccorsi G, Cremonini E, Romani A, Fila E, Castaldini MC, *et al*. Oxidative stress and bone resorption interplay as a possible trigger for postmenopausal osteoporosis. *Biomed Res Int* 2014;569563:1-8.
- Chapple I, Milward M, Dietrich T. The prevalence of inflammatory periodontitis is negatively associated with serum antioxidant concentrations. *J Nutr* 2007;137(3):657-64.
- Conti M, Moran PC, Levillain P, Lemomier A. Improved fluorimetric determination of malondialdehyde. *Clin Chem* 1991;37(7):1273-1275.
- D'Aiuto F, Nibali L, Parkar M, Patel K, Suvan J, Donos N. Oxidative stress, systemic inflammation, and severe periodontitis. *J Dent Res* 2010;89(11):1241-1246.
- Dalle Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem* 2006;52(4):601-623.
- Dezerega A, Madrid S, Mundi V, Valenzuela MA, Garrido M, Paredes R, *et al*. Pro-oxidant status and matrix metalloproteinases in apical lesions and gingival crevicular fluid as potential biomarkers for asymptomatic apical periodontitis and endodontic treatment response. *J Inflamm* 2012;9(1):1-9.
- Ford ES. Leukocyte count, erythrocyte sedimentation rate, and diabetes incidence in a national sample of US adults. *Am J Epidemiol* 2002;155(1):57-64.
- Goy B, Atmaca M, Aslan M, Ucler R, Alay M, Seven I, *et al*. Relationship between serum DHEAS and oxidative stress levels of body mass index in healthy postmenopausal women. *Redox Rep* 2015 [Epub ahead of print]
- Helmerson J, Vessby B, Larsson A, Basu S. Association of Type 2 Diabetes with Cyclooxygenase-Mediated Inflammation and Oxidative Stress in an Elderly Population. *Circulation* 2004;109(14):1729-34.
- Hu ML. Measurement of protein thiol groups and glutathione in plasma. In: *Methods in Enzymology* vol.233, Academic Press, Inc. 1994, pp.380-384.
- Inchingolo F, Marrelli M, Annibali S, Cristalli MP, Dipalma G, *et al*. Influence of endodontic treatment on systemic oxidative stress. *Int J Med Sci* 2014;11:1-6.
- Iwama A, Nishigaki N, Nakamura K, Imaizumi I, Shibata N, Yamasaki M, *et al*. The effect of high sugar intake on the development of periradicular lesions in rats with type 2 diabetes. *J Dent Res* 2003;82(4):322-325.
- Lenzen S. The mechanisms of alloxan-and streptozotocin-induced diabetes. *Diabetologia* 2008;51(2):216-226.

- Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic Diseases Caused by Oral Infection. *Clin Microbiol Rev* 2000;13(4):547-558.
- Martinez ZR, Naruishi K, Yamashiro K, Myokai F, Yamada T, Matsuura K, et al. Gene Profiles during Root Canal Treatment in Experimental Rat Periapical Lesions. *J Endod* 2007;33(8):936-943.
- Munir BM, Iqbal N, Tirmazi SM, Majeed HA. Bone Healing of cyst-like periapical lesion following surgical endodontics: a case report. *Pakistan Oral and Dental Journal* 2011;31(2):436-438.
- Nair PNR. Pathogenesis of apical periodontitis and the causes of endodontic failures. *Crit Rev Oral Biol Med* 2004;15(6):348-381.
- Newman H N. Focal infection. *J Dent Res* 1996;75(12):1912-1919.
- Omi N, Ezawa I. The effect of ovariectomy on bone metabolism in rats. *Bone* 1995;17(4 Suppl):163-168.
- Orshita K, Yamane K, Hanafusa M, Mori H, Mito K, Okubo M, Hora H, Kohno N. Elevated white blood cell count in subjects with impaired glucose tolerance. *Diabetes Care* 2004;27:491-496.
- Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother* 2010;1(2):87-93.
- Reddy MS, Weatherford TW 3rd, Smith CA, West BD, Jeffcoat MK, Jacks TM. Jaks Alendronate Treatment of Naturally-Occurring Periodontitis in Beagle Dogs. *J Periodontol* 1995;66:211-217.
- Roux C, Garnero P, Thomas T, Sabatier JP, Orcel P, Audran M. Recommendations for monitoring antiresorptive therapies in postmenopausal osteoporosis. *Joint Bone Spine* 2005;72(1):26-31.
- Ryan GB, Majno G. Acute inflammation. A review. *Am J Pathol* 1977;86(1):183-276.
- Siqueira JF, Rôças IN. Bacterial pathogenesis and mediators in apical periodontitis. *Braz Dent J* 2007;18:267-280.
- Tani-Ishii N, Wang CY, Tanner A, Stashenko P. Changes in root canal microbiota during the development of rat periapical lesions. *Oral Microbiol Immunol* 1994;9(3):129-135.
- Virdi HK. Hematological parameters-a diagnostic mirror for periodontitis. *Indian Journal of Dental Sciences* 2003; 2(5):45-48
- Wu G, Fang Y-Z, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. *J Nutr* 2004;134(3):489-92.

Authors

- Antonela M. Berar, Departement of Prosthodontics, "Iuliu Hatieganu" University of Medicine and Pharmacy, 32 Clinicilor Street, 400006, Cluj-Napoca, Romania, EU, email: antonela_berar@yahoo.com
- Dragomir-Cosmin David, National Institute of Public Health-Regional Centre for Public Health, 6 Pasteur Street, 400349, Cluj-Napoca, Romania, EU, email: davidcosmin18@yahoo.com.
- Liana Lascu, Department of Prosthodontics "Iuliu Hatieganu" University of Medicine and Pharmacy, 32 Clinicilor Street, 400006, Cluj-Napoca, Romania, EU, email: liana.lascu@um-fcluj.ro.
- Luminita Matros, Department of Microbiology, "Iuliu Hatieganu" University of Medicine and Pharmacy, 6 Pasteur Street, 400349, Cluj-Napoca, Romania, EU, email: Matros.Luminita@umfcluj.ro.
- Radu S. Campian, Department of Oral Rehabilitation, "Iuliu Hatieganu" University of Medicine and Pharmacy, 15 Victor Babes Street, 400012, Cluj-Napoca, Romania, EU, email: rcampian@email.com.

Citation

Berar AM, David DC, Lascu L, Matros L, Campian RS. Analysis of hematological and oxidative stress parameters in the evaluation of experimentally induced periapical lesions. *HVM Bioflux* 2015;7(3):162-167.

Editor

Stefan C. Vesa

Received

9 June 2015

Accepted

29 June 2015

Published Online

29 June 2015

Funding

None reported

Conflicts/ Competing Interests

None reported