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Influence of experimental periodontitis on cardiac oxidative stress in rats: a biochemical and histomorphometric study

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Background and Objective: The role of oxidative stress in the process of cardiac remodeling, hypertrophy and heart failure is a current topic. The purpose of this experimental study was to investigate the influences of periodontitis on levels of cardiac oxidative stress.

Material and Methods: Twenty rats were separated into two groups: control and experimental periodontitis (EP). Periodontitis was induced by placing a 3.0 silk suture in the cervix of the left and right mandibular first molar teeth for 5 wk. At the end of the experiment, the animals were killed and blood samples and mandibular and ventricular cardiac tissue samples were collected. Levels of alveolar bone loss were determined using measurements performed on histological slices and radiographies. Left ventricular tissue 8-hydroxy-2′-deoxyguanosine, malonylaldehyde, glutathione peroxidase, total oxidant status, total antioxidant status (TAS) levels and serum paraoxonase-1 activity were evaluated biochemically.

Results: Measurements performed on the histological slices and radiographies demonstrated that applying the ligature caused obvious alveolar bone loss. Oxidative damage markers (malonylaldehyde, 8-hydroxy-2′-deoxyguanosine, oxidative stress index: total oxidant status/TAS) were significantly higher, and antioxidant markers (glutathione peroxidase, TAS) were statistically insignificantly higher, in the hearts of rats with EP when compared to the controls. In addition, reduced serum paraoxonase-1 activity was also detected in the EP group.

Conclusion: The pronounced increase in cardiac oxidative stress caused by periodontitis was due to an excessive increase in the production of reactive oxygen species, rather than due to decreased antioxidant capacity. The results indicate that periodontitis-related cardiac oxidative stress might be one of the mechanisms that contribute to the pathological process that leads to heart failure.

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Oxidative stress is the result of changes in the balance between reactive oxygen species (ROS) and antioxidant capacity on behalf of oxidants or against antioxidant capacity (1–3). ROS overproduction causes reversible and irreversible damage in biomolecules (such as membrane lipids, proteins and DNA) that are crucial for the function of tissues and cells (1,4,5).

Current human (2,4) and animal (3,6) studies have put forth that periodontitis is an inflammatory disease characterized by a systemic increase in oxidative stress. In addition, the role of oxidative stress in the pathogenesis of cardiovascular diseases (7,8), in particular, cardiac hypertrophy and heart failure (HF) (9–12), is well known. The role of oxidative stress in the process of cardiac remodeling that results in HF is becoming clearer (10,12–14). ROS impairs contractile functions via its direct effect on the proteins responsible for the contraction–excitation coupling of the myocardium. It activates various hypertrophy signaling kinases and impairs extracellular matrix remodeling by activating matrix metalloproteinases (13,15–17).

In light of this information, we hypothesized that periodontitis, which is a chronic, low-level systemic oxidative disease, might contribute to oxidative stress-related degeneration of the myocardium, which can result in HF. While a few studies have pointed out the negative effects of periodontitis-related systemic oxidative stress on cardiovascular diseases (18–20), the potential effects on heart tissue specifically are not known. As such, in this study, we aimed to investigate the influence of ligature-induced periodontitis on the level of cardiac oxidative stress in left ventricular cardiac samples.

Material and methods

Animal housing

This experimental study, using 20 male Sprague–Dawley rats (weight range: 200–220 g), was conducted at the Medical Experimental Application and Research Center of Atatürk University in accordance with ethical norms accepted by the Experimental Ethics Committee of this university (HADYEK 2013-122). All of the animals were sheltered under standard laboratory conditions (light period: 06:00–19:00 h; temperature: 21 ± 2°C; and relative humidity: 58%) and fed standard rat chow and water throughout the study. The rats were separated into two groups: control and experimental periodontitis (EP).

Induction of periodontitis

Periodontitis was induced in the rats using a procedure similar to those used in our previous studies (6,21–24) and other EP studies (3,25). The rats in the EP group were anesthetized with xylazine hydrochloride (10 mg/kg bodyweight) (Rompun, Bayer, Istanbul, Turkey) and ketamine hydrochloride (40 mg/ kg bodyweight) (Ketalar, Pfizer, Istanbul, Turkey), and the mandibular right and left first molar teeth were ligated with a 3.0 silk suture. The ligatures were retained in the submarginal position for 5 wk to provoke the development of plaque accumulation, inflammation and, finally, periodontitis.

Blood and tissue sampling

On the last day of the fifth week, the rats were anesthetized with the medications mentioned above and intracardiac blood samples were collected via a needle and syringe. The rats were then killed with a lethal injection of pentobarbital (50 mg/kg). The first molar teeth and the mandibular tissue surrounding the neighboring tissues were dissected out. The right mandibular tissue samples were transferred to containers with 10% neutral formaldehyde solution for light microscopic analysis. Radiographic evaluations were performed on the left mandibular tissue samples. The left ventricular heart tissue was also removed, weighed, washed with cold saline, quickly blotted with filter paper and immediately frozen at −80°C for biochemical analysis. Serum samples were obtained by centrifuging the blood at 1500 *g* for 10 min within 1 h of collection. The samples were stored at −80°C before they were analyzed. Histopathological and biochemical examinations were performed by two expert researchers who were unaware of the study group allocations.

Histometric and radiographic analyses

Histometric and radiographic analyses were carried out to determine and compare the extent of alveolar bone loss. Alveolar bone loss on the buccal and lingual sides of the right first molar teeth was determined histomorphometrically. The removed mandibular tissues were fixed in 10% neutral buffered formalin for 3 d and then decalcified in 6% nitric acid for 14 d. These tissues were then dehydrated, embedded in paraffin and sectioned along the molars in a bucco-lingual plane by microtome, for hematoxylin–eosin staining. In each mandibular first molar, histometric analyses were performed on eight 5 μm thick systematically selected slices from among all sections. Alveolar bone loss (distance between the cemento-enamel junction and the alveolar bone crest) was determined using a trinocular light microscope integrated with analyzing software (Kameram SLR; Mikro Sistem Ltd., Istanbul, Turkey) (Fig. 1). Levels of loss in the mesial and distal sides of the left first molar teeth were measured with analyses performed on the radiographies. Three points were considered, as shown in Fig. 2: (a) the alveolar crest on the distal/mesial side of the tooth; (b) apex of the distal/mesial root; and (c) peak of the distal/mesial cusp. The linear distances between points *a–b* and *b–c* were evaluated to calculate the periodontal bone support using the following formula: $a{-}b/b{-}c \times 100$. The histometric and radiographic analysis procedures were described in detail in previous studies (6,21–25).

Biochemical analyses

Ventricular tissue analyses—Before dissection, all tissues were rinsed with phosphate-buffered saline solution. The 25 mg tissues were homogenized
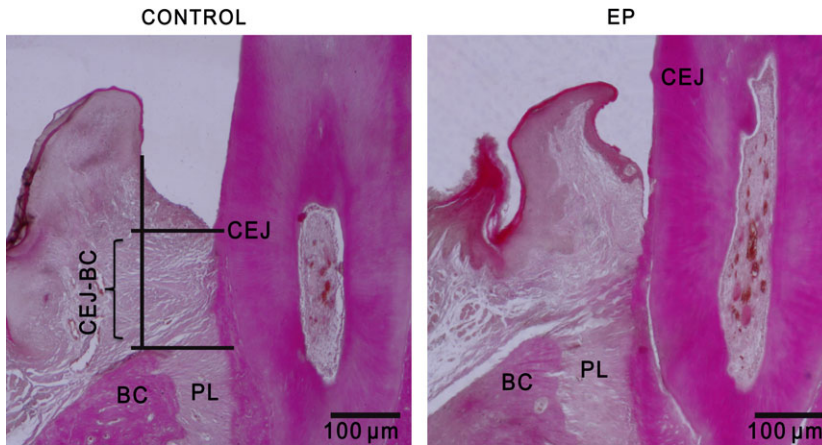
Fig. 1. Histologic appearance of the control and EP groups. BC, bone crest; CEJ, cemento-enamel junction; CEJ-BC, cemento-enamel junction-alveolar bone crest distance; EP, experimental periodontitis; PL, periodontal ligament, hematoxylin–eosin staining.
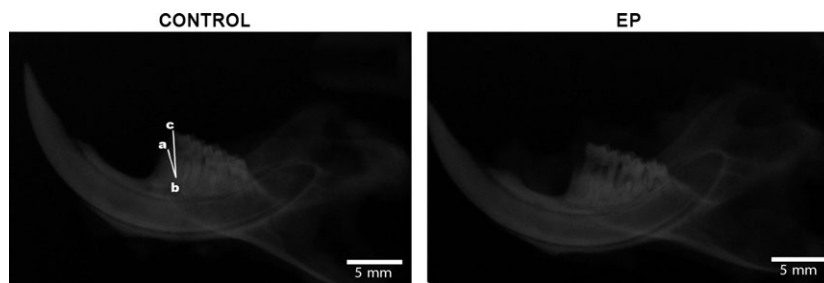

Fig. 2. Assessment of medial and distal periodontal bone support percentages for the control and EP groups. (a) Crest bone on the distal/mesial side of the tooth; (b) apex of the distal/mesial roots; and (c) tip of the distal/mesial cusp of the tooth. EP, experimental periodontitis.

in ice-cold 2 mL phosphate buffers (50 mM, pH 7.4) that were appropriate for the variable to be measured. The tissue homogenates were centrifuged at 5000 g for 20 min at 4°C and the supernatants were extracted to analyze malonylaldehyde (MDA), 8-hydroxy-2′-deoxyguanosine (8-OHdG), glutathione peroxidase (GSH-Px), total oxidant status (TOS), total antioxidant status (TAS) and protein concentration. The protein concentration of the supernatant was measured using the method described by Bradford (26). All tissue results were expressed by dividing into grams of protein.

Determination of tissue malonylaldehyde, 8-hydroxy-2′-deoxyguanosine and glutathione peroxidase levels—The method described by Ohkawa *et al.* (27) was used to measure MDA levels. GSH-Px was also measured using a colorimetric kit (Cayman Chemical Company, Ann Arbor, MI, USA) that determined GSH-Px activity indirectly via a coupled reaction with glutathione reductase–red oxidized glutathione, according to the manufacturer's protocols. The results are expressed as mean U/g protein ± standard deviation (SD). Levels of 8-OHdG were determined using a commercial enzyme-linked immunosorbent assay kit (Sigma Chemical Co., St. Louis, MO, USA). The results are expressed as mean ng/g protein ± SD.

Determination of tissue total oxidant status and total antioxidant status levels and calculation of oxidative stress index—The TOS and TAS levels of the supernatants were determined using a novel automated measurement method and commercially available kits (Rel Assay Diagnostics, Gaziantep, Turkey), both developed by Erel (28,29). The results are expressed as μmol hydrogen peroxide (H_2O_2) equivalent/L and μmol Trolox equivalent/L, respectively. The percentage ratio of TOS to TAS was used as the oxidative stress index (OSI). OSI was calculated as TOS (in μmol/L) divided by TAS (μmol Trolox equivalent/L) divided by 100 (29).

Serum analysis

Serum paraoxonase (PON)-1 activity was determined spectrophotometrically at 412 nm at 37°C using a commercially available kit (Rel Assay Diagnostics) according to the manufacturer's instructions. PON-1 activity was expressed as U/L serum.

Statistical analysis

As all of the data presented a normal distribution and the coefficient variation was less than 20%, intergroup differences were tested by analysis of variance and Duncan's *post-hoc* test, using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). All data were expressed as mean average ± SD ($p < 0.05$ was considered significant).

Results

Histometric and radiographic results

Histometric evaluations demonstrated that levels of alveolar bone loss (distances between the cemento-enamel junction and alveolar bone crest) were significantly higher in the EP group when compared to the control group ($p < 0.05$) (Fig. 1; Table 1). In the radiographic measurements, mesial periodontal bone support and distal periodontal bone support in the EP group were significantly lower than in the control group ($p < 0.05$) (Fig. 2; Table 1).

Biochemical results

Heart ventricular tissue oxidative stress biomarker levels—MDA and 8-OHdG levels were higher in the EP group than in the control group ($p < 0.05$) while GSH-Px levels were statistically insignificantly higher ($p > 0.05$). TOS levels and the OSI of the cardiac tissues

Table 1. Comparison of the alveolar bone losses between the groups

	Histometric analysis: CEJ-BC (μm)		Radiographic analysis: PBS (%)	
	Buccal	Lingual	Mesial	Distal
Control	94.27 ± 8.20^a	105.05 ± 10.02^a	61.30 ± 7.74^a	62.82 ± 8.20^a
EP	381.86 ± 32.71^b	375.44 ± 26.31^b	44.70 ± 6.08^b	40.40 ± 7.02^b

CEJ-BC, the distance between the cemento-enamel junction and the alveolar bone crest; EP, experimental periodontitis; PBS, periodontal bone support. Results are expressed in mean ± SD. Different superscript letters (a, b) in the same column indicate significant differences between groups. ANOVA and *post-hoc* Duncan test were performed ($p < 0.05$).

were higher in the EP group when compared to the control group ($p < 0.05$). TAS levels were statistically insignificantly higher in the rats with periodontitis ($p > 0.05$) (Table 2).

Serum paraoxonase-1 activity—Serum PON-1 activity was lower in the EP group compared to the control group ($p < 0.05$) (Table 2).

Discussion

The present study shows that in rats with ligature-induced periodontitis, the level of oxidative stress in the left ventricular cardiac tissue increased significantly, while local antioxidant capacity was not affected significantly. To the best of our knowledge, only one other study found in the literature has investigated the effects of periodontal inflammation on cardiac oxidative stress. Tomofuji *et al.* (30) reported that in Wistar rats with bacterial lipopolysaccharide-induced periodontitis, cardiac 8-OHdG levels increased more than twofold. The authors found that 8-OHdG levels increased not only in cardiac tissue, but also in liver, kidney and brain

tissue. It is clear that this particularly valuable experimental study did not aim to evaluate broadly the effects of periodontitis on cardiac oxidative stress levels. Moreover, it was not made clear from which part of the heart the samples used in the biochemical evaluations were collected. In the present study, periodontitis was induced by applying a ligature to the first molar teeth bilaterally at a sub-marginal position for a period of 5 wk. Because various studies (10,13,31) have pointed out the changes in ventricular tissue observed in the early stages of HF pathogenesis, we specifically collected tissue samples from the left ventricle to perform extensive evaluations of oxidative stress levels.

Markers used in this experimental study to evaluate levels of oxidative stress were MDA, 8-OHdG, GSH-Px and OSI. MDA and 8-OHdG are end products that reflect the extent of oxidative damage to membrane lipids and DNA, respectively (3). Various studies have pointed out the close relationship between systemic levels of these markers and HF (32–34). Kobayashi *et al.* (32) reported a significant correlation

between urinary 8-OHdG levels and HF-related cardiac functional parameters, and Suzuki *et al.* (33) stated that serum 8-OHdG level might be a significant indicator of HF risk. The significantly elevated levels of MDA and 8-OHdG in the EP group in this study demonstrate that periodontitis causes serious oxidative damage in the cellular membranes and genetic materials of cardiac tissue, which is consistent with the findings of Tomofuji *et al.* (30).

Although specific oxidative degradation products, such as MDA and 8-OHdG, are measured in the evaluation of oxidative stress level, they fail to demonstrate the final oxidative status of the tissue. Similarly, evaluations of enzymatic or non-enzymatic antioxidant levels do not adequately reflect antioxidant effectiveness. Recent studies have reported that TOS and TAS measurements reveal oxidative and antioxidant statuses clearly (2,3,28,29). It has been reported that TAS, which is a practical and reliable method, reflects not only the effectiveness of known antioxidants, but also that of antioxidants that have not yet been discovered (2,29). OSI, a current parameter that is a proportional value between TOS and TAS, was recommended by Erel (29) for revealing the level of oxidative stress more precisely and practically. It is influenced by both ROS production and antioxidant status (2,3,29). Our findings, showing that periodontitis causes a significant increase in levels of TOS and OSI and a statistically insignificant increase in TAS levels, indicate that the pro-oxidative effect of periodontitis on cardiac tissue might be particularly related to its role in provoking ROS production.

GSH-Px is an enzyme that is found in large amounts in cardiac tissue, particularly in cytosolic and mito-chondrial compartments in which ROS production is extensive. It has more important functions in the antioxidant protection of this tissue as compared to other enzymatic antioxidants (10,35). It is also a key antioxidant that catalyzes the reduction of H_2O_2 and hydroperoxides (10). Moreover, Shiomi *et al.* (36) reported that mice with GSH-Px gene overexpression were more resistant to myocardial oxidative stress, remodeling and

Table 2. Comparison of the biochemical results between the groups

	Control	EP
MDA (tissue: nmol MDA/g protein)	25.424 ± 5.210^a	33.411 ± 7.151^b
8-OHdG (tissue: ng/g protein)	0.511 ± 0.107^a	0.860 ± 0.153^b
GSH-Px (tissue: U/g protein)	42.21 ± 12.55^a	45.88 ± 14.20^a
TOS (tissue: μmol H_2O_2 equiv/g protein)	8.224 ± 4.64^a	12.451 ± 4.48^b
TAS (tissue: mmol Trolox equiv/g protein)	0.886 ± 0.325^a	0.897 ± 0.462^a
OSI (ratio)	1.304 ± 0.714^a	1.935 ± 0.754^b
PON-1 activity (serum: U/L)	78.42 ± 14.33^a	62.61 ± 18.02^b

EP, experimental periodontitis; GSH-Px, glutathione peroxidase; MDA, malonylaldehyde; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; OSI, oxidative stress index; PON-1, paraoxonase-1; TAS, total antioxidant status; TOS, total oxidant status. Results are expressed in mean ± SD. Different superscript letters (a, b) in the same line indicate significant differences between groups. ANOVA and *Post-Hoc* Duncan Test were performed ($p < 0.05$).

ultimately, HF. In this study, we determined that GSH-Px levels were slightly higher in the cardiac tissue of the rats with periodontitis. Similarly, TAS levels were statistically insignificantly higher in the rats with periodontitis. This slight increase in antioxidant activity might be related to an adaptive response. The study by Tsutsui *et al.* (10,37) supports our findings that oxidative stress in HF is the result of a diffuse increase in ROS production rather than a decrease in antioxidant capacity.

Although the present study clearly revealed that periodontitis caused an increase in oxidative stress in the left ventricle, the underlying mechanisms were not investigated, which represents an evident weakness in the study. Tomofuji *et al.* (30) showed that lipid peroxides that are extensively released into circulation from periodontal tissues might trigger oxidative DNA damage in the cardiac tissue of rats with lipopolysaccharide-induced periodontitis. It was also clearly demonstrated that ligature-induced periodontitis led to endothelial dysfunction (38) and inflammation (39) in the aorta. Miyajima *et al.* (39) revealed that there was a significant increase in the expression levels of p65 nuclear factor-kappa B and vascular cell adhesion molecule-1 in the aortic endothelial cells of rats with periodontitis compared to the control group, resulting in the invasion of these periodontitis-activated monocytes/macrophages into the vascular wall. Furthermore, it was reported in that study that periodontitis increased tumor necrosis factor-alpha-associated mRNA expression in the aorta. It is likely that periodontitis enables inflammatory cells to penetrate into deeper tissues by causing ventricular endothelial dysfunction, similar to that found in the aorta, and that this condition triggers a complex inflammatory response, including ROS overproduction. Duarte *et al.* (40) demonstrated that the composition of the microbial plaque deposited on the ligature that was placed on the cervix of the teeth to provoke the formation of periodontitis greatly resembled that which is found in humans. Moreover,

Hokamura *et al.* (41) found that intravenous administration of *Porphyromonas gingivalis* dramatically induced aortic intimal hyperplasia in the mouse model. In this context, it is evident that there is a need for further studies aiming to determine the direct and/or indirect contributions of plaque bacteria in periodontitis-related inflammatory and oxidative alterations in cardiac tissue.

In the present study, we also evaluated the effects of periodontitis on serum PON-1 activity and we concluded that the mentioned activity was clearly lower in the rats with periodontitis. PON-1, a multitasking protein, is one of three members of the paraoxonase enzyme family (PON-1, PON-2 and PON-3). PON-1 protects low- and high-density lipoproteins from lipid peroxidation by catalyzing the degradation of oxidized lipids contained in the oxidized lipoproteins (42). It also protects cells against oxidative damage (43). Previous studies have shown that PON-1 plays a vital role in limiting atherosclerosis, vascular inflammation and oxidative stress (44,45). In this regard, the significant decrease in PON-1 activity in rats with periodontitis might be one of the mechanisms that provoke an increase in cardiac oxidative stress. This view is supported by the Guns *et al.* (46) study, which showed that the PON-1 gene transferred reduced vascular oxidative stress in mice with pre-existing atherosclerosis.

The findings of this study support our hypothesis that a significant increase in oxidative stress in cardiac tissue caused by ligature-induced periodontal destruction is the result of ROS production rather than a decrease in antioxidant capacity. However, the present study has some limitations. First, alveolar bone loss is a late outcome of periodontal disease. It was an important deficiency of the present study that the possible effects of many host response events on cardiac oxidative parameters occurring due to the early stages of periodontitis pathogenesis were not evaluated in a comprehensive manner. In addition, because only male rats were included in the study, the

obtained findings cannot be considered valid for both sexes. As such, there is an obvious need for further and more detailed studies to demonstrate the mechanism by which periodontitis influences cardiac oxidative stress. Although the findings of this animal study should not be correlated directly with periodontitis in humans, it is obvious that periodontal treatment is essential in limiting oxidative degeneration that might lead to HF.

Conflict of interest

We have no conflict of interests.

References

1. Chapple IL, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontol 2000* 2007;**43**:160–232.
2. Baltacioglu E, Yuva P, Aydin G *et al.* Lipid peroxidation levels and total oxidant/antioxidant status in serum and saliva from patients with chronic and aggressive periodontitis. Oxidative stress index: a new biomarker for periodontal disease? *J Periodontol* 2014;**85**:1432–1441.
3. Yagan A, Kesim S, Liman N. Effect of low-dose doxycycline on serum oxidative status, gingival antioxidant levels, and alveolar bone loss in experimental periodontitis in rats. *J Periodontol* 2014;**85**:478–489.
4. Akalin FA, Baltacioglu E, Alver A, Karabulut E. Lipid peroxidation levels and total oxidant status in serum, saliva and gingival crevicular fluid in patients with chronic periodontitis. *J Clin Periodontol* 2007;**34**:558–565.
5. Chapple IL, Brock GR, Milward MR, Ling N, Matthews JB. Compromised GCF total antioxidant capacity in periodontitis: cause or effect? *J Clin Periodontol* 2007;**34**:103–110.
6. Kose O, Arabaci T, Kara A *et al.* Effects of melatonin on oxidative stress index and alveolar bone loss in diabetic rats with periodontitis. *J Periodontol* 2016;**87**: 82–90.

7. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 2001; **104**:2673–2678.

8. Ramond A, Godin-Ribuot D, Ribuot C et al. Oxidative stress mediates cardiac infarction aggravation induced by intermittent hypoxia. *Fundam Clin Pharmacol* 2013;**27**:252–261.

9. Ahmed Z, Tang WH. Pharmacologic strategies to target oxidative stress in heart failure. *Curr Heart Fail Rep* 2012;**9**:14–22.

10. Tsutsui H, Kinugawa S, Matsushima S. Oxidative stress and heart failure. *Am J Physiol Heart Circ Physiol* 2011;**301**: 2181–2190.

11. Karabacak M, Dogan A, Tayyar S, Bas HA. Oxidative stress status increase in patients with nonischemic heart failure. *Med Princ Pract* 2014;**23**:532–537.

12. Munzel T, Gori T, Keaney JF Jr, Maack C, Daiber A. Pathophysiological role of oxidative stress in systolic and diastolic heart failure and its therapeutic implications. *Eur Heart J* 2015;**36**:2555–2564.

13. Tsutsui H, Kinugawa S, Matsushima S. Mitochondrial oxidative stress and dysfunction in myocardial remodelling. *Cardiovasc Res* 2009;**81**:449–456.

14. Takimoto E, Kass DA. Role of oxidative stress in cardiac hypertrophy and remodeling. *Hypertension* 2007;**49**:241–248.

15. Sabri A, Hughie HH, Lucchesi PA. Regulation of hypertrophic and apoptotic signaling pathways by reactive oxygen species in cardiac myocytes. *Antioxid Redox Signal* 2003;**5**:731–740.

16. Tham YK, Bernardo BC, Ooi JY, Weeks KL, McMullen JR. Pathophysiology of cardiac hypertrophy and heart failure: signaling pathways and novel therapeutic targets. *Arch Toxicol* 2015; **89**:1401–1438.

17. Tsutsui T, Tsutamoto T, Wada A et al. Plasma oxidized low-density lipoprotein as a prognostic predictor in patients with chronic congestive heart failure. *J Am Coll Cardiol* 2002;**39**:957–962.

18. Nibali L, Rizzo M, Li Volti G, D'Aiuto F. Lipid subclasses profiles and oxidative stress in aggressive periodontitis before and after treatment. *J Periodontal Res* 2015;**50**:890–896.

19. Itabe H. Oxidized low-density lipoprotein as a biomarker of in vivo oxidative stress: from atherosclerosis to periodontitis. *J Clin Biochem Nutr* 2012;**51**:1–8.

20. Bullon P, Newman HN, Battino M. Obesity, diabetes mellitus, atherosclerosis and chronic periodontitis: a shared pathology via oxidative stress and mitochondrial dysfunction? *Periodontol 2000* 2014;**64**:139–153.

21. Arabaci T, Kermen E, Ozkanlar S et al. Therapeutic effects of melatonin on alveolar bone resorption after experimental periodontitis in rats: a biochemical and immunohistochemical study. *J Periodontol* 2015;**86**:874–881.

22. Akman S, Canakci V, Kara A, Tozoglu U, Arabaci T, Dagsuyu IM. Therapeutic effects of alpha lipoic acid and vitamin C on alveolar bone resorption after experimental periodontitis in rats: a biochemical, histochemical, and stereologic study. *J Periodontol* 2013;**84**:666–674.

23. Kara A, Akman S, Ozkanlar S et al. Immune modulatory and antioxidant effects of melatonin in experimental periodontitis in rats. *Free Radic Biol Med* 2013;**55**:21–26.

24. Kose O, Arabaci T, Kermen E et al. Effects of alpha-lipoic acid and its combined use with vitamin C on periodontal tissues and markers of oxidative stress in rats with experimental periodontitis. *Oxid Antioxid Med Sci* 2015;**4**:91–96.

25. Longhini R, Aparecida de Oliveira P, Sasso-Cerri E, Cerri PS. Cimetidine reduces alveolar bone loss in induced periodontitis in rat molars. *J Periodontol* 2014;**85**:1115–1125.

26. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;**72**:248–254.

27. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;**95**:351–358.

28. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem* 2004;**37**:112–119.

29. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 2005;**38**:1103–1111.

30. Tomofuji T, Ekuni D, Irie K et al. Relationships between periodontal inflammation, lipid peroxide and oxidative damage of multiple organs in rats. *Biomed Res* 2011;**32**:343–349.

31. Meta-analysis Global Group in Chronic Heart Failure (MAGGIC). The survival of patients with heart failure with preserved or reduced left ventricular ejection fraction: an individual patient data meta-analysis. *Eur Heart J* 2012;**33**:1750–1757.

32. Kobayashi S, Susa T, Tanaka T et al. Urinary 8-hydroxy-2′-deoxyguanosine reflects symptomatic status and severity of systolic dysfunction in patients with chronic heart failure. *Eur J Heart Fail* 2011;**13**:29–36.

33. Suzuki S, Shishido T, Ishino M et al. 8-Hydroxy-2′-deoxyguanosine is a prognostic mediator for cardiac event. *Eur J Clin Invest* 2011;**41**:759–766.

34. Takeishi Y. Biomarkers in heart failure. *Int Heart J* 2014;**55**:474–481.

35. Matsushima S, Kinugawa S, Ide T et al. Overexpression of glutathione peroxidase attenuates myocardial remodeling and preserves diastolic function in diabetic heart. *Am J Physiol Heart Circ Physiol* 2006;**291**:2237–2245.

36. Shiomi T, Tsutsui H, Matsusaka H et al. Overexpression of glutathione peroxidase prevents left ventricular remodeling and failure after myocardial infarction in mice. *Circulation* 2004;**109**:544–549.

37. Tsutsui H, Ide T, Shiomi T et al. 8-oxo-dGTPase, which prevents oxidative stress-induced DNA damage, increases in the mitochondria from failing hearts. *Circulation* 2001;**104**:2883–2885.

38. Brito LC, DalBo S, Striechen TM et al. Experimental periodontitis promotes transient vascular inflammation and endothelial dysfunction. *Arch Oral Biol* 2013;**58**:1187–1198.

39. Miyajima S, Naruse K, Kobayashi Y et al. Periodontitis-activated monocytes/macrophages cause aortic inflammation. *Sci Rep* 2014;**4**:5171.

40. Duarte PM, Tezolin KR, Figueiredo LC, Feres M, Bastos MF. Microbial profile of ligature-induced periodontitis in rats. *Arch Oral Biol* 2010;**55**:142–147.

41. Hokamura K, Inaba H, Nakano K et al. Molecular analysis of aortic intimal hyperplasia caused by Porphyromonas gingivalis infection in mice with endothelial damage. *J Periodontal Res* 2010;**45**:337–344.

42. Watson AD, Berliner JA, Hama SY et al. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. *J Clin Invest* 1995;**96**:2882–2891.

43. Rosenblat M, Aviram M. Paraoxonases role in the prevention of cardiovascular diseases. *BioFactors* 2009;**35**:98–104.

44. Seo D, Goldschmidt-Clermont P. The paraoxonase gene family and atherosclerosis. *Curr Atheroscler Rep* 2009;**11**:182–187.

45. Tward A, Xia YR, Wang XP et al. Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice. *Circulation* 2002;**106**:484–490.

46. Guns PJ, Van Assche T, Verreth W et al. Paraoxonase 1 gene transfer lowers vascular oxidative stress and improves vasomotor function in apolipoprotein E-deficient mice with pre-existing atherosclerosis. *Br J Pharmacol* 2008;**153**:508–516.