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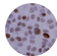

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JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/jre.12380

Biochemical and histopathologic analysis of the effects of periodontitis on left ventricular heart tissues of rats

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Köse O, Arabacı T, Gedikli S, Eminoglu DÖ, Kermen E, Kızıldağ A, Kara A, Ozkanlar S, Yemenoglu H. Biochemical and histopathologic analysis of the effects of periodontitis on left ventricular heart tissues of rats. J Periodont Res 2016; doi: 10.1111/jre.12380. © 2016 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd

Background and Objective: Current epidemiological works have suggested that chronic infections, such as periodontitis, are associated with an increased risk of cardiovascular diseases, including hypertrophy and heart failure. However, mechanisms behind the association are not known. The aim of this study was to evaluate the effects of periodontitis on the serum lipid levels, inflammatory marker levels and left ventricular heart muscle tissues of rats.

Material and Methods: Eighteen male Sprague–Dawley rats were randomly divided into two groups: control (without ligature) and experimental periodontitis (EP; ligatured). Periodontitis was induced by placing ligatures (3.0 silk) at a submarginal position of the lower first molar teeth for 5 wk. Serum samples were collected for biochemical studies (C-reactive protein, interleukin-1β, tumor necrosis factor-α and serum lipids), after which the rats were killed and heart tissue samples were obtained for histopathological and immunological studies (nuclear factor kappa B and β-myosin heavy chain).

Results: Significant increases in C-reactive protein and interleukin-1β levels and no statistically significant increase in tumor necrosis factor-α level were observed in the EP group compared to the control group. In addition, total cholesterol, low-density lipoprotein cholesterol and triglyceride levels were significantly higher in the EP group. Stereological and immunological findings showed that the number of nuclear factor kappa B-p65- and β-myosin heavy chain-positive cardiomyocytes increased significantly in the left ventricular tissue samples of the rats with periodontitis.

Conclusion: Early chronic phase effects of periodontitis on heart tissue are in the form of degenerative and hypotrophic changes. Prolonging the exposure to systemic inflammatory stress may increase the risk of occurrence of hypertrophic changes.

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Key words: animal model; heart failure; inflammation; myosin heavy chain; periodontal disease

Accepted for publication February 11, 2016

Cardiovascular diseases (CVD) are common public health problems and are the reason for about 40% of deaths worldwide (1). In recent years, it has become generally accepted that chronic inflammatory diseases that cause a systemic inflammatory condition can increase the risk of CVD in addition to classical risk factors such as diabetes, smoking, high cholesterol levels and sedentary lifestyle (2–6).

Periodontitis is defined as the destruction of the tooth-supporting tissues because of the dynamic interaction among the specific bacteria, host immune response, environmental factors and genetic factors (7). It appears more often in middle-aged and older individuals and causes local and systemic immune and inflammatory responses (8,9).

Some studies have suggested that periodontitis is a factor in and of itself that might increase the risk of CVD development significantly (3,4,6,10). Although the mechanisms describing the relationship between the mentioned risk increase and periodontitis have not yet been determined, both direct effects on the vascular structures of the periodontopathogenic bacteria involved in the systemic circulation and indirect effects of increased levels of systemic inflammatory markers are accepted mechanisms (11). In addition, attention has been drawn to the notion that chronic systemic inflammatory changes may be more important compared to the direct vascular effects of bacteria (12).

Many studies have demonstrated clearly that periodontitis is a low-grade systemic inflammatory condition characterized by a significant increase in systemic levels of various proinflammatory cytokines (2,6,13) and C-reactive protein (CRP) (5,10,14). Tumor necrosis factor (TNF)-α and interleukin (IL)-1β are the proinflammatory mediators most associated with the transcription factor nuclear factor kappa B (NF-κB) signaling cascade (15). NF-κB has an important role in immune and inflammatory events (16). In unstimulated cells, it is inactive in the cell cytoplasm and is bound to its inhibitor, the inhibitory κB. Many factors, including proinflammatory cytokines, bacterial lipopolysaccharides and reactive oxygen species, can trigger activation of the NF-κB, its translocation into the nucleus and subsequent mRNA expression specific to various mediators and growth factors (16,17).

Many studies have highlighted that chronic activation of NF-κB in cardiac tissue is associated closely with hypertrophy (18–20). Cardiac hypertrophy, which is an adaptive response for maintaining cardiac function, is characterized by an increase in cardiomyocyte dimension (size), and thus, cardiac volume. It leads to heart failure (HF) in a progressive process, which has an important role in the pathogenesis of various CVD (19).

Although the major causes of HF are myocardial infarction, hypertension, cardiomyopathy and valvular heart diseases (21), chronic inflammation is also considered detrimental, and it can lead to cardiac tissue damage, maladaptive remodeling and, ultimately, HF (22). In recent studies, TNF-α and interleukins were shown to be involved in pathological cardiac remodeling and to contribute to the impairment of contractile function, apoptosis and fibrosis (21,23,24).

Myosin heavy chain (MHC) is an important regulator protein in the formation of contractile properties of cardiac muscle cells. The isoforms of MHC in mammalian heart tissue have recently been identified as alpha and beta (25). β-MHC was determined to be associated with a reduction in ATPase activity and deceleration in the rate of muscle contraction. Moreover, it has been reported that this protein increases in accordance with the severity of cardiac hypertrophy; therefore, it may be an indicator of cardiac hypertrophy and HF (26,27).

The aim of this experimental study was to investigate how periodontitis affects the structure of heart tissue. In this context, histopathological and immunohistochemical evaluations were performed on left ventricular heart tissue samples. The influence of periodontitis on systemic levels of some inflammatory mediators (TNF-α, IL-1β and CRP) and serum lipid markers was also evaluated.

Material and methods

Animal housing and experimental design

Eighteen male Sprague–Dawley rats weighing 180–200 g were used in the study. The experiments were conducted according to the ethical norms approved by the Local Ethics Committee of Atatürk University for Animal Experiments (ATADEM approval no. 2013/122). All of the animals were housed under standard laboratory conditions (06.00–19.00 h light period, 21 ± 2°C and 58% relative humidity) and given water and fed a normal diet. The rats were divided into two groups ($n = 9$ in each group): control rats (non-ligatured) and rats with experimental periodontitis (EP; the first molar teeth were ligatured with 3-0 sterile silk sutures for periodontitis induction) (2,4,28). After 5 wk of periodontitis induction, the animals were anesthetized with xylazine hydrochloride (10 mg/kg, Rompun; Bayer, İstanbul, Turkey) and ketamine hydrochloride (40 mg/kg, Ketalar; Pfizer, Istanbul, Turkey), and blood samples were collected individually from the inferior vena cava. The rats were then killed with an intracardiac thiopental sodium (pentothal sodium; Abbott, North Chicago, IL, USA) (40 mg/kg) injection, and mandibles and the heart tissues were removed for histological analysis.

Histologic tissue preparation and morphometric analysis

The mandible tissues were fixated for 72 h and then decalcification in 6% nitric acid solution was done for 7 d. The nitric acid solution was refreshed daily, and the tissue decalcification was controlled by a needle during the last 3 d. After decalcification was completed, the tissues were dehydrated in a graded alcohol series, embedded in paraffin wax and sectioned in the buccolingual direction into 5 μm thicknesses using a microtome (Leica RM2125RT; Leica Instruments, Nubloch, Germany). The sections were stained using hematoxylin and eosin for histologic imaging. Measurements

of the mean distance of the cemento-enamel junction–periodontal ligament (CEJ-PL) and the CEJ–alveolar bone crest (CEJ-BC) were obtained for the buccal and lingual sides of first molars using a trinocular light microscope attached to analyzing software (Kameram SLR, 1.4.1.0; Mikro Sistem Ltd., Istanbul, Turkey), as described in previous studies (28,29) (Fig. 1).

Blood sampling and serum biochemical analysis

The collected blood samples were centrifuged at 1500 *g* for 10 min within 1 h after collection. The sera were stored in a freezer at −20°C before being analyzed. The serum activities of total cholesterol (Tcho), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglyceride cholesterol concentrations were analyzed using diagnostic kits (Roche Diagnostics, Mannheim, Germany). The results are expressed as mg/dL.

Serum C-reactive protein analysis

Quantitative measurements of CRP in the serum samples were performed using a commercial rat CRP enzyme-linked immunosorbent assay (ELISA) test kit (BioVendor Co., Modrice, Czech Republic). The tests were performed according to the manufacturer's instructions with an ELISA device (μ-Quant; BioTek Instruments, Winooski, VT, USA). Sample concentrations are expressed as ng/mL.

Serum interleukin-1β and tumor necrosis factor-α analysis

Serum IL-1β and TNF-α concentrations were measured using rat-specific IL-1β and TNF-α ELISA kits (Invitrogen Co., Carlsbad, CA, USA). The analyses were performed according to the manufacturer's instructions. The results are expressed as mean (pg/mL) ± SD of concentration for each animal in the serum sample.

Light microscopy analysis

After the rats were killed, the heart tissues were fixed in 10% neutral buffered formaldehyde for 72 h. The fixed tissues were then dehydrated and embedded in paraffin, and serial sections were cut using a microtome (Leica Instruments). Eighteen slides were obtained for each animal: immunohistochemical staining was used on nine sections and Crossman-modified Mallory's triple staining was used on the remaining nine sections for histopathological and transversal myocyte area calculation. The histological inflammation activity and tissue alterations of both groups were evaluated by high-power light microscope (Nikon Eclipse 50i; Nikon, Kawasaki, Japan). In addition, photomicrographs were taken. Evaluations of all histological and stereological methods were conducted in a blinded fashion throughout the study by one of the authors. For the histopathological evaluations, all inflammation activity and tissue alterations were scored as follows: none = 0, weak = 1, moderate = 2, strong = 3, very strong = 4. The last score consisted of the mean ± SD of the nine counted slides. All of the histologic changes for inflammation, presence or absences of four cell types namely mononuclear cells (lymphocytes and plasma cells) and polymorphonuclear cells (neutrophils and macrophages), were considered for assessment. In addition, presence of some cell lesions including more acidic cytoplasm, condensed nuclei and cytoplasmic shrink were considered as degenerated cell. The morphological criteria and appropriate nomenclature for the histopathological changes were based on a previous study (30).

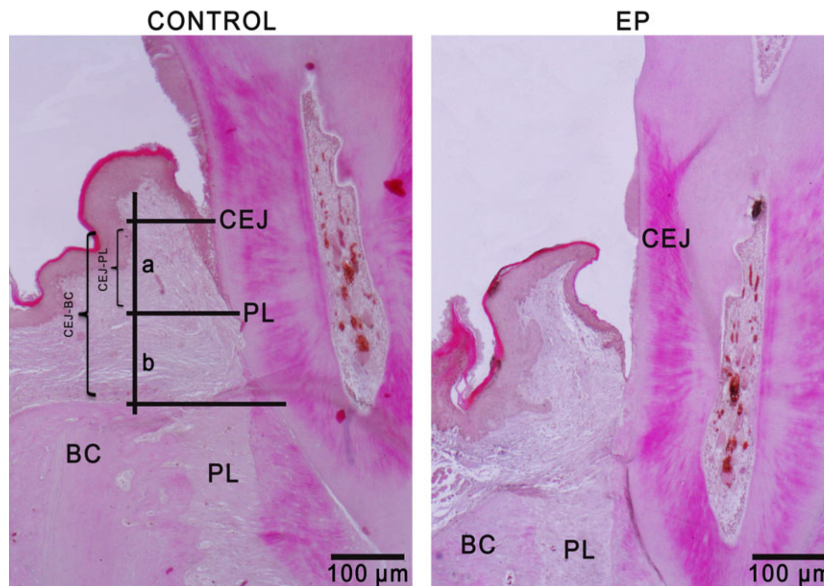

Fig. 1. Representative histologic view for mandible tissues of control and EP groups. a, CEJ-PL distance; a+b, CEJ-BC distance; BC, bone crest; CEJ, cemento-enamel junction; EP, experimental periodontitis; PL, periodontal ligament. Hematoxylin–eosin staining.

Immunohistochemical analysis

NF-κB-p65- and β-MHC-positive cells were determined by the immunohistochemical method (streptavidin–biotin–peroxidase staining), wherein they appear brown in color. Anti-NF-κB primary antibody (dilution: 1/50; Santa Cruz Biotechnology, Dallas, TX, USA) and biotinylated secondary antibody (DAKO-Universal LSAB Kit-K0690; Dako, Carpinteria, CA, USA) were used for NF-κB-p65 immunohistochemical staining. Anti-β-MHC (Abcam Biotechnology, Cambridge, UK) rat-specific primary antibody (dilution: 1/100) and biotinylated secondary antibody (Dako) were also used for β-MHC immunohistochemical staining. The antibody binding sites were visualized with DAB (3,3′-diaminobenzidine tetrahydrochloride; Sigma Chemical, St. Louis, MO, USA).

Calculation of β-myosin heavy chain cell density

All of the slides β-MHC-positivity analysis was performed on three sections of heart tissue from each rat using a conventional trinocular light microscope (Nikon). Semiquantitative analyses were performed in this study. The positive cell intensity was scored as follows: none = 0; weak = 1; moderate = 2; strong = 3; very strong = 4. Totally, three sections from each animal for both groups were evaluated and scores were noted and mean of all scores for each group was accepted as the last score. The last score expressed as mean ± SD.

Stereological analysis

Calculation of nuclear factor-kappa B-p65-positive cells—An NF-κB-positive cell count of every heart tissue sampled by the fractionator was calculated using the optic fractionator frame method (31) via the stereo investigator system. The cells were counted and calculated using a 40× Leica Plan Apo objective (NA = 1.40; Leica Microsystems, Wetzlar, Germany), which allowed accurate recognition.

The density of the NF-κB-positive cells was estimated according to the following formula: PCD = NPC /(CFA × NSS) where PCD is positive numerical cell density per μm² area, NPC is number of positive cells, CFA is counting frame area (XY) (μm²) and NSS is number of sampling sites. The stereology results are expressed as immune reactive cells per 1000 μm^2 area.

Calculation of cardiomyocyte transverse area and myocyte nucleus volume—Serial sections were used to calculate the cardiomyocyte transverse area and myocyte nucleus volume. They were obtained in the transverse plane without randomness in orientation, and determinations of the myocyte transverse area and cardiomyocyte nucleus volume were conducted as described previously (32,33). The cardiomyocyte transverse area and nucleus volume of each segment of heart tissue were calculated using the nucleator method via the stereo investigator system (Microbrightfield StereoInvestigator software v. 9.0; Microbrightfield, Williston, VT, USA) according to above procedure.

Statistical analysis

Because the data did not represent a normal distribution and the coefficient variables were >20%, differences between the two groups were tested with the Mann–Whitney test using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). All data are expressed as mean average ± SD; $p < 0.05$ was considered significant.

Results

Comparison of attachment and alveolar bone losses

Table 1 shows the comparison of morphometric measurements between the groups. Buccal and lingual CEJ-PL and CEJ-BC distances were significantly higher in the EP group compared to the control rats ($p < 0.05$).

Biochemical results

The biochemical analysis showed that the lipid profiles were mostly different between the groups: Tcho, LDL, triglycerides and HDL/LDL concentrations were significantly higher in the EP group than in the control group ($p < 0.05$). Increase in serum HDL concentration was not statistically significant compared to the controls ($p = 0.140$). In addition, IL-1β and CRP levels were significantly higher in the EP group compared to the control group ($p = 0.021$), but there was no significant difference in TNF-α level ($p = 0.150$). All of the biochemical results are presented in Table 2.

Histopathological results

Histological changes in the hearts of the control and EP groups were graded and are summarized in

Table 1. Comparison of attachment levels and alveolar bone losses between the groups

	Control	EP
CEJ-PL (a, μm)		
Buccal	74.41 ± 13.80^{a}	228.08 ± 26.28^{b}
Lingual	67.60 ± 9.27^{a}	235.12 ± 21.93^{b}
CEJ-BC (a+b, μm)		
Buccal	114.51 ± 16.03^{a}	392.05 ± 25.02^{b}
Lingual	120.32 ± 19.30^{a}	382.12 ± 19.21^{b}

a, cemento-enamel junction–periodontal ligament (CEJ-PL); a+b, cemento-enamel junction –alveolar bone crest (CEJ-BC); EP, experimental periodontitis. Values are expressed as mean ± SD. Differences in histometric analysis results between groups were tested by the Mann–Whitney test. ^{a,b}Superscript letters in the same line indicate significant differences between groups; $p < 0.05$.

Table 2. Concentrations of serum lipids and inflammatory parameters in the control and EP groups

	Control (n = 9)	EP (n = 9)	p value
Tcho (mg/dL)	57.14 ± 9.92^{a}	95.50 ± 4.12^{b}	0.013
HDL (mg/dL)	46.25 ± 7.52^{a}	52.75 ± 3.20^{a}	0.140
LDL (mg/dL)	6.22 ± 1.92^{a}	18.54 ± 1.75^{b}	0.013
Triglycerides (mg/dL)	55.32 ± 13.13^{a}	85.34 ± 17.33^{b}	0.024
HDL/LDL	7.54 ± 1.05^{a}	2.82 ± 0.81^{b}	0.016
CRP (ng/mL)	2748.52 ± 440.71^{a}	6143.25 ± 360.41^{b}	0.027
IL-1β (pg/mL)	64.81 ± 11.70^{a}	104.40 ± 18.97^{b}	0.021
TNF-α (pg/mL)	1.53 ± 0.46^{a}	2.73 ± 0.39^{a}	0.150

CRP, C-reactive protein; EP, experimental periodontitis; HDL, high-density lipoprotein; IL, interleukin; LDL, low-density lipoprotein; Tcho, total cholesterol; TNF, tumor necrosis factor. All results expressed as mean ± SD. ^{a,b}Superscript letters in the same line indicate significant differences between groups; Mann–Whitney test was used ($p < 0.05$).

Table 3. Myocardial tissues of the control group displayed a normal structure and morphology (Fig. 2A and 2B), whereas heart tissues of the EP group had significantly degenerated cells and inflammatory cell infiltration, as well as histopathological changes, including cell degeneration, collagen accumulation and inflammatory cells around the arterioles. Capillary endothelial changes and perivascular fibrosis were also observed in the myocardial tissues of the EP group (Fig. 2C, D, E). These quantitative findings confirm the morphometric results and suggest that the myocardial tissue damage of the EP group might affect cardiac structure and function (Table 3).

Immunohistochemical assessment of nuclear factor-kappa B-p65-positive cells

Figure 3A shows the comparison between groups of the NF-κB-p65-positive cells. The immunohistochemical evaluations showed that NF-κB-positive cell counts were significantly higher in the EP group compared to the control group (p = 0.001) (Fig. 3B).

Immunohistochemical assessment of β-myosin heavy chain-positive cells

The positivity analyses were performed by an experienced histologist in their field who were unaware of the study groups. Positivity of β-MHC was seen as diffuse in myofibrils of cardiomyocytes. Intensity of β-MHC–

Table 3. Histopathologic and immunohistochemical evaluations for the control and EP groups

Groups	Cell degenerations	Inflammatory cell infiltrates	Increase of interstitial tissue	β-MHC-positive cells intensity
Control	0.55 ± 0.68^a	0.22 ± 0.41^a	0.66 ± 0.47^a	1.03 ± 0.46^a
EP	2.88 ± 0.56^b	2.77 ± 0.62^b	3 ± 0.47^b	3.42 ± 0.57^b

EP, experimental periodontitis; MHC, myosin heavy chain.Intensity was scored as follows: none = 0; weak = 1; moderate = 2; strong = 3; very strong = 4. All results expressed as mean ± SD. ^{a,b}Superscript letters in the same line indicate significant differences between groups. Mann–Whitney test was performed for statistical analysis. $p < 0.05$.

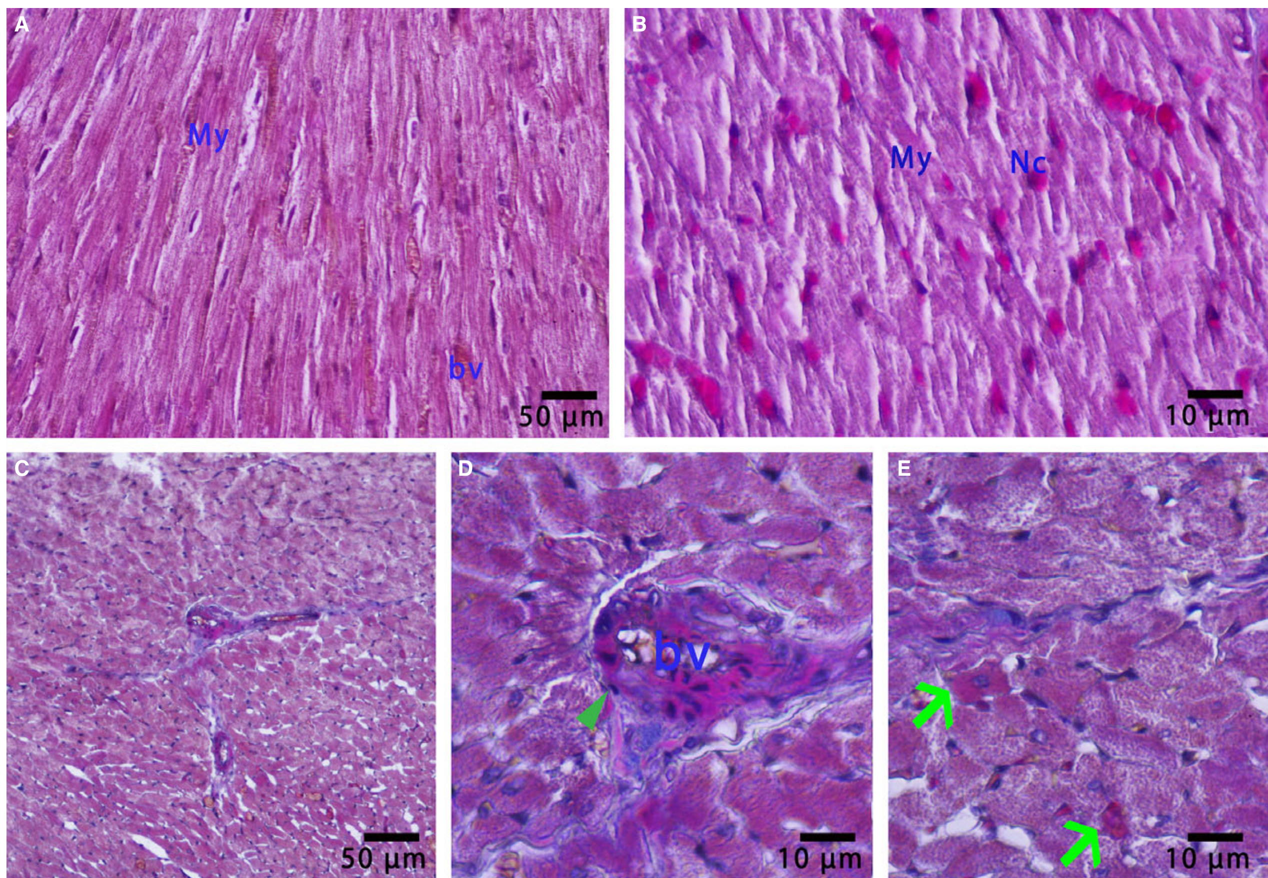

Fig. 2. Micrograph of myocardial section of the control and experimental periodontitis groups, bv, blood vessel; green arrowhead, inflammatory cells; green open arrows, degenerated cells; My, cardiac myocyte; Nc, cardiac myocyte nucleus. Crossman's modified Mallory triple staining.

positivity was higher in the EP group sections compared to the control group sections (Table 3, Fig. 4).

Average transverse area and nucleus volume of ventricular cardiomyocytes

In the stereological analysis, statistically significant differences were found between the cardiomyocyte cytoplasm areas of the control and EP groups ($p = 0.002$). Cardiomyocyte nucleus volumes were higher in the EP group, but this difference was not statistically significant ($p = 0.398$). Average transverse area and volume of cardiomyocyte nuclei in the control and EP groups are shown in Fig. 5.

Discussion

The biochemical findings of the current study are generally in line with those of previous studies, demonstrating that EP led to increased inflammatory mediator levels and lipid profile in serum. In addition, the histopathological and immunological findings indicate that EP causes significant changes in the morphology and physiology of ventricular myocytes.

The first step in the pathogenesis of CVD is atherosclerotic changes. Atherosclerosis is a dynamic and progressive disease that can be defined as the combination of endothelial dysfunction and inflammation (34). Studies have also drawn attention to the significant association between periodontitis and endothelial dysfunction and systemic inflammation in humans (2,35). Our findings indicating that EP caused a significant increase in the levels of serum CRP (2,5,10,36) and IL-1β (28,37) are consistent with those of previous studies. It is also important to note that serum CRP level may be an important indicator in terms of risk of CVD, specifically myocardial infarction (10,38,39). In addition, it has been clearly shown that periodontal treatment causes a decrease in serum CRP levels (10,40).

However, our findings also indicate that EP caused a slight increase in serum TNF-α levels. Although this finding is generally compatible with those of animal studies with similar designs (4,28), some studies detected a significant increase in TNF-α level (41). Human studies have reported varying results (6,42). Popa *et al.* (43) reported that the effects of TNF-α on glucose and lipid metabolism are more significant compared to the inflammatory effects on endothelial cells. In this context, it may be considered that TNF-α induced by periodontal infection has limited atherogenic effects (6). In a recent study, Miyajima *et al.* (4) found no significant changes in TNF-α, CRP or IL-6 serum levels in rats with EP. However, they found that TNF-α and IL-6 mRNA expression increased 2.7- and 2.6-fold, respectively, in circulating mononuclear cells. That study also showed that EP caused a significant increase in TNF-α-related mRNA expression in aortic tissue.

The effects of periodontitis on serum lipid profiles were also evaluated in the current study, and periodontitis was determined to be associated with hyperlipidemia. Our findings indicating that EP caused a

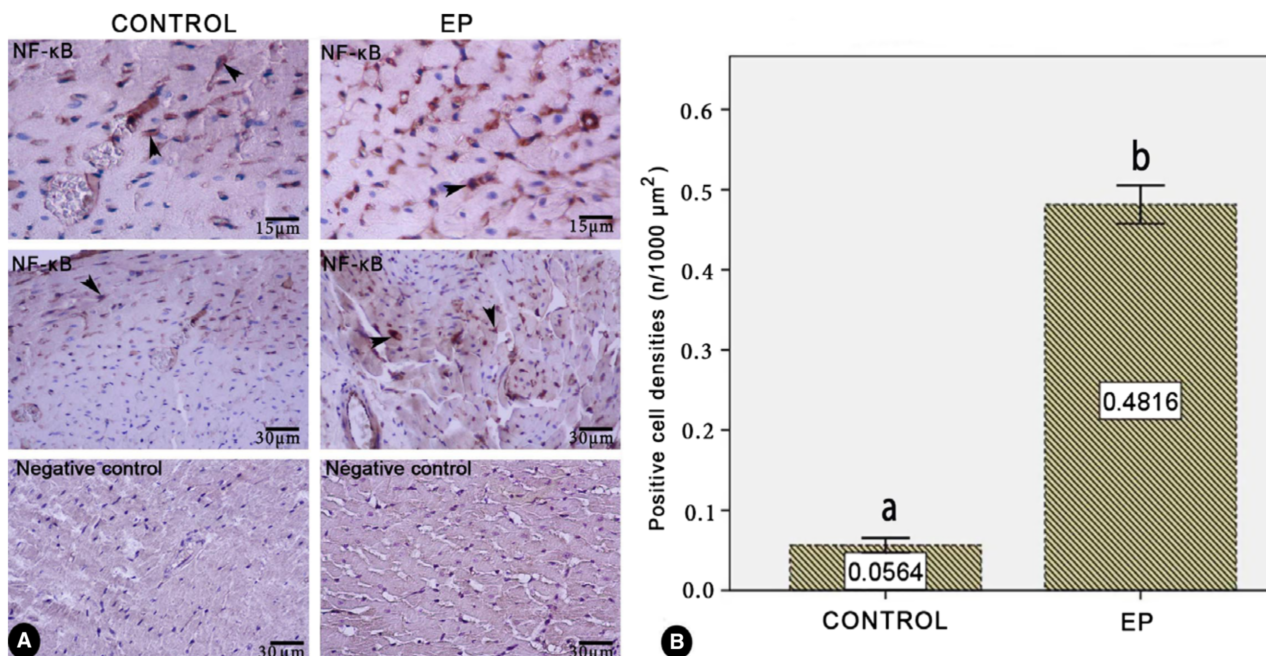

Fig. 3. Immunohistochemical NF-κB-p65 staining results. (A) Myocardial section of the control and EP groups; the immune positive cells were intensively seen in gradual magnifications. Negative control; not primer antibody used sections of control and EP groups. Arrows show NF-κB-positive cells. Streptavidin–biotin peroxidase staining. (B) Comparisons of NF-κB-positive cell densities for both groups. NF-κB-positive cell error bars show SDs and the footnote letters (ab) indicate significant differences between groups (Mann–Whitney test, $p < 0.05$). EP, experimental periodontitis; NF-κB, nuclear factor kappa B.

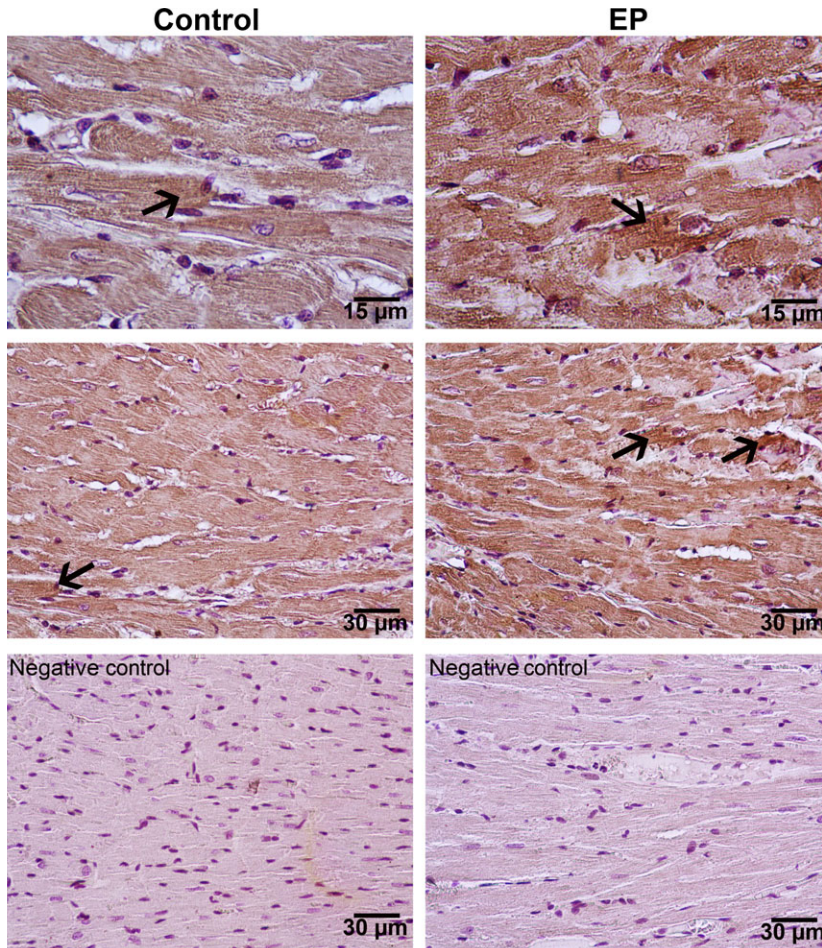

Fig. 4. Immunohistochemical staining for the β-MHC for the control and EP groups. Arrows show β-MHC positivity in cardiac myofibrils. Negative control; not primer antibody used sections of control and EP groups. Streptavidin–biotin peroxidase staining. EP, experimental periodontitis.

statistically significant increase in Tcho and LDL levels and a slight increase in HDL level are compatible with the study conducted by Machado *et al.* (44). However, the increase in triglyceride level was determined more significant in our study. Human studies have also drawn attention to the relationship between periodontitis and a significant deterioration in serum lipid profiles (14,45). It has been shown that various proinflammatory cytokines released by active monocytes in the periodontal area are inhibited by the production of the lipoprotein lipase enzyme. This condition leads to lipid metabolism disorders that are characterized by an increase in serum Tcho and LDL levels (46). In this context, our findings support the view that periodontitis may increase the risk of CVD by affecting lipid profiles associated with an increase in CRP levels (14). Furthermore, it has been demonstrated that hyperlipidemia increases susceptibility to infection by negatively affecting immune system cells (47). The lipid profile changes may provoke periodontitis-mediated endothelial and atherosclerotic changes via various indirect mechanisms.

We also evaluated the effects of EP on left ventricular muscle tissue, specifically on myocyte morphology and physiology. Our histopathological findings after 35 d of EP included inflammatory cell infiltration, degeneration of myocytes, collagen accumulation, capillary endothelial changes and perivascular fibrosis. Studies with similar designs reported that EP caused significant inflammatory cell infiltration in the aortic wall (4) and the atrium (48). Miyajima *et al.* (4) demonstrated that periodontitis-activated monocytes and macrophages adhered to the aortic endothelial cells by increasing p65 NF-κB-mediated vascular cell adhesion molecule-1 expression. The authors also evaluated the number of inflammatory cells adhering to the endothelium and ultimately determined a ~1.7-fold increase in rats with periodontitis compared to control rats. Activated monocytes/macrophages are likely to adhere to ventricular endothelial cells via a similar mechanism and then proceed to the muscle tissue. Various cytokines and growth factors are released intensely by these cells, are converted into macrophages and direct the histopathological changes mentioned above. In addition, our stereological findings indicating that the cardiomyocyte cytoplasmic areas decreased significantly in the rats with periodontitis refer to hypotrophic changes in accordance with our histopathological findings. The volumetric decreases in cardiomyocytes might be a result of periodontitis-mediated inflammatory stress provoked by NF-κB activation. Yu *et al.* (48) found hypertrophy, particularly in myocytes in the left atrium, during histopathological evaluations performed 90 d after EP in dogs. Interestingly, they did not observe significant hypertrophy in ventricular myocytes. In this context, it can be considered that periodontitis-related histopathological changes in heart tissue that are degenerative and hypotrophic changes in the early stage and hypertrophic changes in the chronic stage might change in relation to exposure to inflammatory stress.

NF-κB has three basic cardiac effects, determined by two factors (activation time and cellular content): hypoxia/ischemia followed by acute cardioprotection, hypertrophy and chronic cytotoxicity (19). Many studies have drawn attention to the relationship between the increase in NF-κB-p65-dependent transcriptional

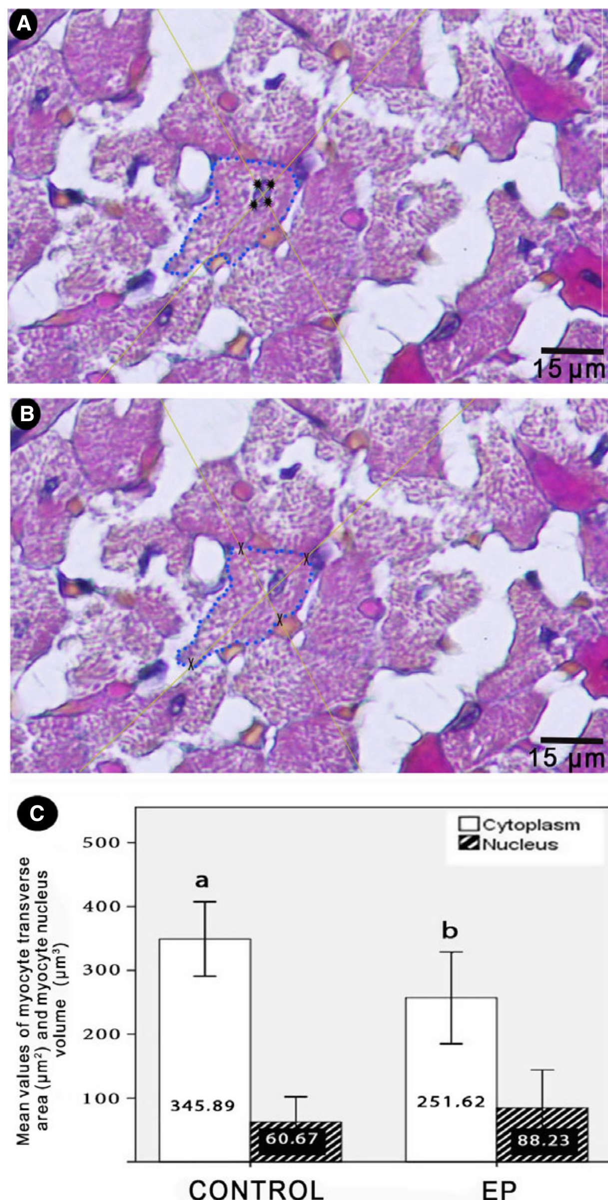

Fig. 5. Heart tissue from rats. (A) Snapshot of estimation of cytoplasmic area of ventricular cardiomyocytes. (B) Snapshot of estimation of nucleus volumes of ventricular cardiomyocytes. (C) Average transverse area and nuclei volume of ventricular cardiomyocytes for control and EP groups. Error bars shows SDs and the footnote letters (ab) indicate significant differences between groups (Mann–Whitney test, $p < 0.05$). EP, experimental periodontitis.

activity and cardiac hypertrophy (20,49). Li *et al.* (18) reported that in ventricular myocyte cell culture isoproterenol-induced cardiac hypertrophy caused an increase in NF-κB activation as well as the gene expression of β-MHC, which is considered a cardiac hypertrophy biomarker. Periodontitis is a chronic source of stress for cardiomyocytes, as it is a chronic systemic inflammatory condition. Our findings indicating that the number of NF-κB-positive and β-MHC-positive myocytes increased significantly in rats with periodontitis may suggest an increase in the risk of ventricular hypertrophy and eventual HF in the later stages.

The biochemical findings of the current study support those of previous studies and show that periodontitis might increase the risk of CVD initiating and/or provoking endothelial and atherosclerotic changes. Our histopathological findings suggest that the effects of periodontitis in the early chronic phase on ventricular tissue are in the form of degenerative and hypotrophic changes. The increase in NF-κB and β-MHC activities can be a leading indicator of hypertrophic changes. It is clear that long-term and comprehensive studies are required to reveal the effects of periodontitis on cardiac tissue and the mechanisms of action.

Acknowledgement and conflict and interest

We have no conflicts of interest.

The study was self-funded by us and our institution.

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