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Periodontitis and Atherosclerosis – Mechanisms of Association Through Matrix Metalloproteinase 1 Expression

Zapalenie przyzębia a miażdżyca naczyń – mechanizm związku poprzez ekspresję metaloproteinazy 1

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. The association between periodontitis and atherosclerosis and its complications has long been postulated.
Objectives. The objective of this study was to analyze the expression of MMP-1 in gingival epithelium.
Material and Methods. 29 pieces of periodontal tissues of patients who had died in Sumy Regional Hospital were evaluated for revealing MMP-1 by immunohistochemistry and fluorescent microscopy.
Results. Immunoexpression of MMP-1 (atherosclerotic group) was confirmed by the presence of brown stained cytoplasm epithelial layers and leukocytic infiltration. Immunoexpression of MMP-1 in epithelial layers showed 95.8 ± 2.43% ($P < 0.001$) positive cells. In the lamina propria leukocytic infiltration, immunoreactivity of MMP-1 was classified with a score of 19 in most atherosclerotic cases, that is 41.21 ± 3.86% ($P < 0.05$). Immunoexpression of MMP-1 in epithelial layers showed the result of 35.1 ± 4.89% ($P < 0.05$) positive cells and immunoexpression of MMP-1 in leukocytic infiltration showed 48.23 ± 5.24% ($P < 0.05$). In general, MMP-1 staining seemed to be more intense in the granular cell layer.
Conclusions. MMP-1 plays an important role in the biology of periodontal disease. An increased concentration of MMP-1 in the epithelial layers and leukocytic infiltration of group I patients suggests that the expression of MMP-1 contributes to epithelial invasion of collagen matrix in case of atherosclerosis. This mechanism might contribute to explaining the association between major cardiovascular diseases and oral infections. **(Dent. Med. Probl. 2014, 51, 2, 187–192)**.

Key words: atherosclerosis, immunohistochemistry, MMP-1, periodontitis.

Streszczenie

Wprowadzenie. Od dawna jest postulowany związek między zapaleniem przyzębia a miażdżycą naczyń i jej powikłaniami.
Cel pracy. Analiza ekspresji metaloproteinazy 1 (MMP-1) w nabłonku dziąsłowym i podnabłonkowej tkance łącznej u osób, które zmarły z powodu powikłań miażdżycy naczyń.
Materiał i metody. Zbadano 20 wycinków dziąsła osób, które zmarły z powodu powikłań miażdżycy w szpitalu w Sumie na Ukrainie. Grupę kontrolną stanowiło 9 wycinków dziąsła osób zmarłych z innych przyczyn. Ekspresję MMP-1 oceniano za pomocą badania immunohistochemicznego i badania wykonanego z zastosowaniem mikroskopii fluorescencyjnej.
Wyniki. Immunoekspresja MMP-1 w grupie z miażdżycą była potwierdzona przez brązowe zabarwienie cytoplazmy keratynocytów w nabłonku dziąsłowym i komórkach nacieku leukocytarnego w podnabłonkowej tkance łącznej. Ekspresja MMP-1 w warstwach nabłonka dziąsłowego dotyczyła 95,8 ± 2,43% keratynocytów i była istotnie

większa niż w grupie kontrolnej ($p < 0,001$). Ekspresja MMP-1 na komórkach nacieku leukocytarnego w tkance łącznej była klasyfikowana w 19 przypadkach i wynosiła 41,21 ± 3,86%. W grupie kontrolnej ekspresja MMP-1 w warstwach nabłonka dziąsłowego dotyczyła 35,1 ± 4,89% keratynocytów oraz 48,23 ± 5,24% komórek nacieku leukocytarnego. Ogólnie ekspresja MMP-1 była największa w warstwie ziarnistej nabłonka.
Wnioski. Metaloproteinaza 1 odgrywa istotną rolę w etiologii chorób przyzębia. Nasilona ekspresja MMP-1 w warstwach nabłonka i nacieku leukocytarnego w grupie badanej sugeruje, że ekspresja MMP-1 przyczynia się do inwazji nabłonkowej macierzy kolagenowej w przypadku miażdżycy naczyń. Mechanizm ten mógłby tłumaczyć związek między chorobami sercowo-naczyniowymi a zapaleniem przyzębia. (**Dent. Med. Probl. 2014, 51, 2, 187–192**).

Słowa kluczowe: miażdżyca naczyń, zapalenie przyzębia, immunohistochemia, MMP-1.

Periodontal disease involves inflammation of the periodontium and is accompanied by apical migration of junctional epithelium, leading to the destruction of connective tissue attachment and alveolar bone loss [1].

Colonization of endogenous gram-positive and gram-negative periodontal bacteria, including *Porphyromonas gingivalis*, *Aggregatibacter actinomycetem comitans*, *Tannerella forsythia* and *Treponema denticola*, appears to be the primary initiator of the disease [2]. These products include endotoxins, cytokines and protein toxins [3]. These molecules penetrate the gingival epithelium and initiate a host response that eventually results in the development of periodontal disease.

As the biofilm continues to proliferate, soluble compounds penetrate the sulcular epithelium. This, in turn, signals the gingival epithelium to produce chemical mediators including interleukins, prostaglandins, and tumor necrosis factor [4]. Bacterial factors either result in degradation of host tissues or cause the release of MMPs from epithelial tissue cells leading to collagen destruction.

Matrix metalloproteinases (MMPs) secreted by cervical and ovarian cancer, especially MMP-2 and MMP-9, play crucial roles in tumor invasion and metastasizing. Among the MMPs, type I collagenase (MMP-1 or interstitial collagenase) degrades the fibrillar collagens and thus is important for the tumour traversing the extracellular space [5].

The MMP-1 are zinc-dependent endopeptidas that are collectively capable of degrading almost all components of the extracellular space [6]. MMP-1 is also involved in various pathologic processes, such as inflammation and degenerative diseases [7].

Ryo Tamamura et al. (2005) investigated the localization of 6 chains and MMPs in normal oral mucosal tissue, precancerous lesions, early squamous cell carcinoma immimohistochemically. In epithelial dysplasia, MMPs were detected continuously along basement membrane [8]. However, the mechanism of MMP-1 activation was not elucidated in relation to atherosclerosis with periodontal disease and degradation of extracellular periosteum or periodontal membrane matrix.

On the one hand, periodontal disease is a complication of atherosclerosis. The presence of periodontal disease in atherosclerotic patient is a serious health hazard leading to severe atherosclerosis and alveolar bone loss [9, 10].

On the other hand, periodontal bacteria induce cross-reactions on vascular epithelium resulting in vascular inflammation and atherosclerosis [11].

The authors analyzed the presence and periodontal tissue localization of MMP-1 by immunohistochemistry, for the purpose of evaluating the autolytic degradation of collagen under the influence of atherosclerosis. Finally, the authors underlined the importance of immunohistochemistry diagnosis to prevent possible damages to the teeth and adjacent bone.

Material and Methods

The study sample consisted of periodontal tissues of patients who died in the Sumy Regional Hospital and was investigated for MMP-1 antibodies. Each case was then studied by immunohistochemistry to evaluate some inflammatory, endothelial and stromal markers. The subjects were divided into 2 equal groups:

Patient's Group (Group I): included 20 people who had died from complications of atherosclerosis. MMP-1 was expressed with a score of 19 in most cases of atherosclerosis. Diagnosis was made at autopsy on changes in the aorta (Fig. 1).

Appearance of positive factors was detected semiquantitatively by counting of positive structures in visual field (MMP-1: 0–5% – few, 5–20% – moderate, 20–40% – numerous, 40–100% – abundance positive structures in visual field).

Arteriosclerosis is the thickening of media and intima of the arteries (aorta) seen as a result of aging. The changes are non-selective and affect most of the arteries. These are possibly induced by stress and strain on vessel wall during life.

The changes are as under: 1) Fibroelastosis: The intima and media are thickened due to an increase in elastic and collagen tissue. 2) Elastic reduplication: The internal elastic lamina is split or reduplicated so that two wavy layers are seen.

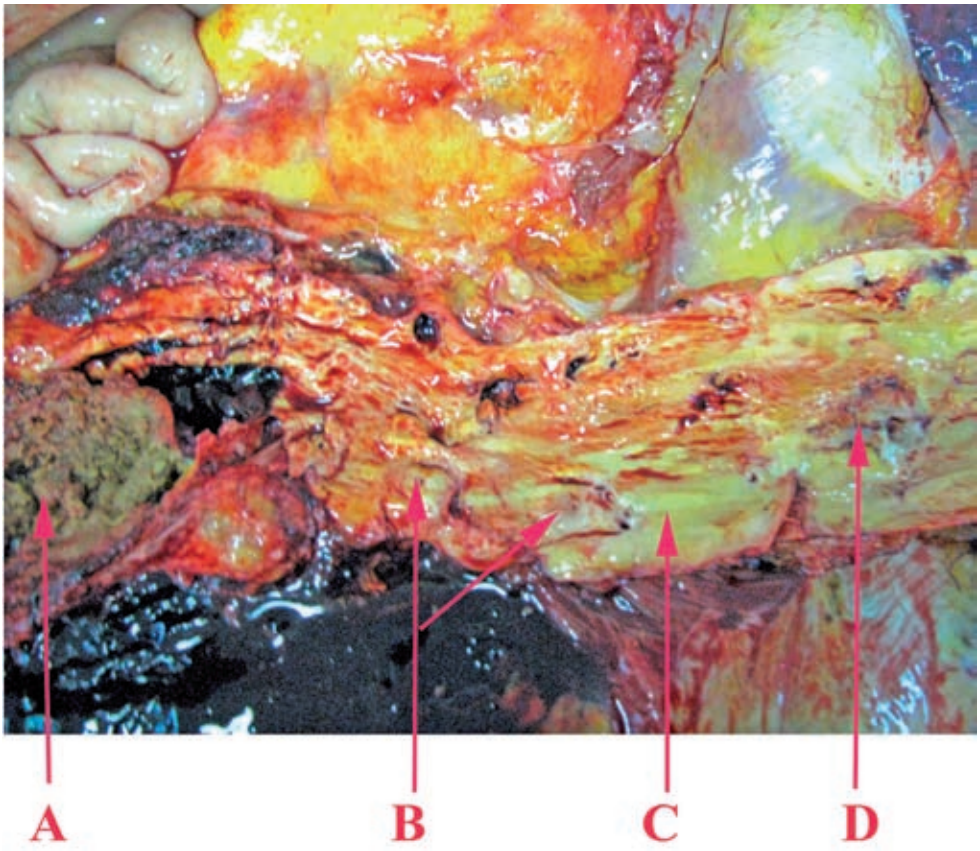

Fig. 1. Atherosclerotic changes in aorta A – areas of endothelial damage, B – atheromatous plaque, C – early lesion, D – ulcerated plaque

Ryc. 1. Zmiany miażdżycowe w aorcie: A – obszar zniszczenia nabłonkowego, B – płytka miażdżycowa, C – zmiany wczesne, D – płytka z owrzodzeniem

Control Group (Group II): included 9 patients with various diagnoses (not atherosclerotic ones)

Paraffin sections were prepared for acridine orange staining by mounting on the slides, dried on a hot plate, and then immersed into 3 sets of xylene for 2 min each followed by 3 sets of absolute ethanol for 5 min and finally rinsed with tap water. The aim was to remove the wax and dehydrate the sections. Slides (paraffin) were placed into acridine orange staining solution for 15 min, and rinsed with phosphate-buffered saline (PBS). Then the slide was soaked in 0.1% calcium chloride solution for 3 min and was washed with PBS once again. Cover glass was mounted for observation under a fluorescence microscope to observe and read the result.

Immunostaining for MMP-1 was performed on formalin-fixed (pH 7.4), paraffin-embedded thyroid tissue sections using mouse monoclonal anti-MMP-1 (Thermo Fisher Scientific UK). Briefly, 4 μm thick tissue sections were dewaxed in xylene and were brought to water through graded alcohols. Antigen retrieval was performed by microwaving slides in 10 mM citrate buffer (pH 6.2) for 30 min at high power, according to the manufacturer's instructions. To remove the endogenous peroxidase activity, sections were then treated with freshly prepared 1.0% hydrogen peroxide in the dark for 30 min at 37°C. Non-specific antibody binding was blocked by dint of blocking serum. The sections were incubated at 37°C for 30 min, with primary antibodies against MMP-1 diluted 1:100 in phosphate buffered saline (PBS) pH 7.2, after 3 washings with PBS. Anti-(mouse IgG)-horseradish peroxidase conjugate (1:40 000 dilution) was used for the detection of the MMP-1 primary antibodies, sections were then incubated for 20 min at 37°C. The color was developed by DAB.

Data was analyzed using the program Origin Version 8. The Student method was used to perform simple comparative analysis. The variables were regarded as normally distributed.

Results

In Fig. 2 the authors observed an enhanced expression of MMP-1 in epithelial layers and leukocytic infiltration. In group I, MMP-1 expression extended to the lamina propria as inflammation progressed. MMP-1 increased activity could explain the change of collagen (Fig. 4) quality and quantity, since its preferred substrates are the collagens.

The immunoexpression of MMP-1 (Group I) was confirmed by the presence of brown stained cytoplasm epithelial layers and leukocytic infiltration. In general, MMP-1 staining was more intense in the basal cell layer. As far as immunoexpression of MMP-1 in epithelial layers is concerned, 95.8 ± 2.43% ($P < 0.001$) appeared to be positive

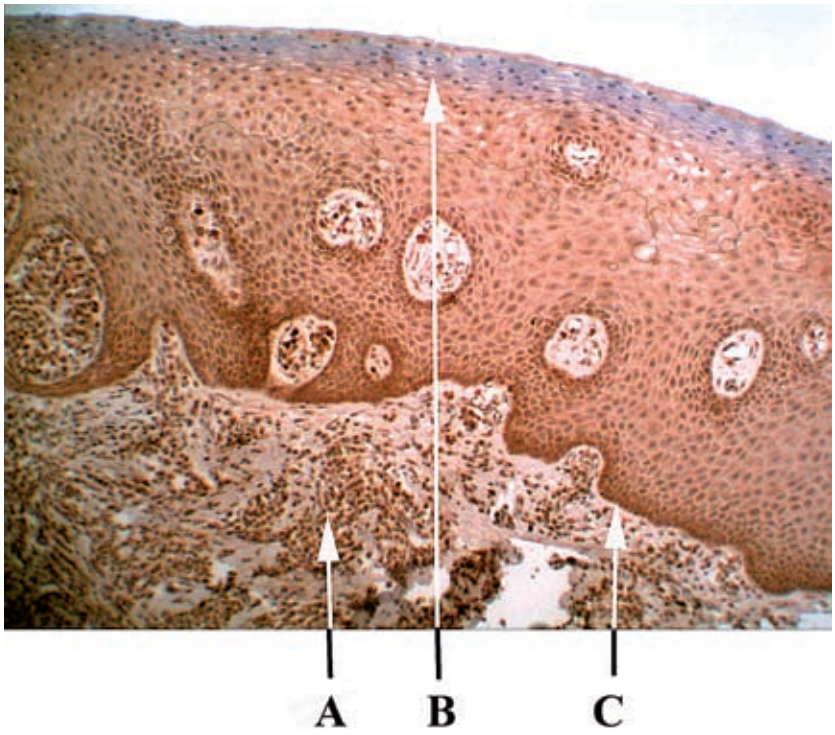

Fig. 2. Expression of MMP-1 in gingival, group I (×100 magnification) A – leukocytic infiltration, B – granular layer of the epithelium, C – basal cells layer

Ryc. 2. Ekspresja dziąsłowa MMP-1 w grupie badanej (powiększenie 100 razy): A – naciek leukocytarny podnabłonkowej tkanki łącznej, B – warstwa ziarnista nabłonka, C – komórki warstwy podstawnej

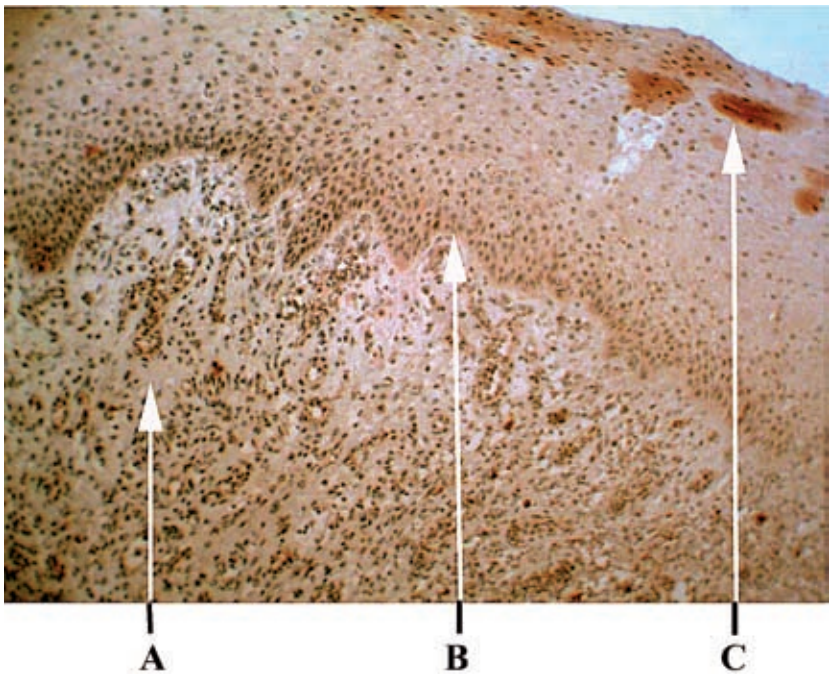

Fig. 3. Expression of MMP-1 in gingiva, group II (×100 magnification) A – leukocytic infiltration, B – basal cells of the epithelium, C – granular cells layer

Ryc. 3. Ekspresja dziąsłowa MMP-1 w grupie kontrolnej (powiększenie 100 razy): A – naciek leukocytarny tkanki łącznej, B – komórki podstawne nabłonka, C – komórki warstwy ziarnistej

cells. In the lamina propria leukocytic infiltration, immunoreactivity of MMP-1 was 41.21 ± 3.86% ($P < 0.05$).

MMP-1 was expressed in group II at the invasion front in all specimens analyzed. The immunoexpression of MMP-1 (Fig. 3) in epithelial layers showed the result of 35.1 ± 4.89% ($P < 0.05$) positive cells and immunoexpression of MMP-1 in leukocytic infiltration showed 48.23 ± 5.24% ($P < 0.05$). In general, MMP-1 staining seemed to be more intense in the granular cell layer. Figure 5 explains the change of collagen in group II,

Discussion

Oral pathogens like *Porphyromonas gingivalis* can infect epithelial cells [12]. Furthermore, exposure of cultured epithelial cells to this pathogen is associated with epithelial activation and expression of cell adhesion molecules [13]. Activation of MMP-1 is likely to be a result of atherosclerosis and periodontal infection.

Type I collagen, responsible for strength and rigidity of connective tissue, is the main bone organic matrix component [14]; and MMP-1 is one of the proteases that can degrade the triple-helical

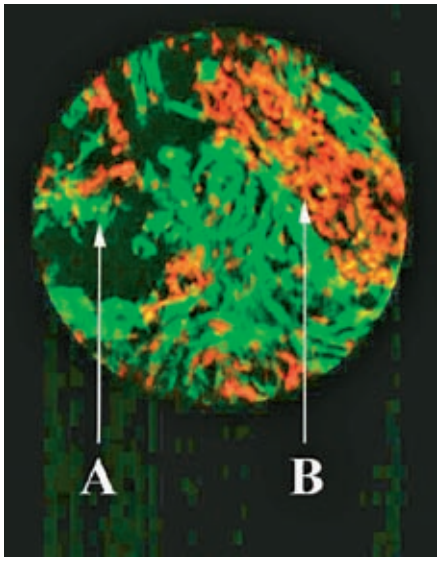

Fig. 4. Collagen changes in gingiva. Acridine orange, group I (× 150 magnification): A – collagen resorption, B – microbial-leukocytic infiltration

Ryc. 4. Zmiany kolagenu w dziąśle. Oranż akrydynowy w grupie badanej (powiększenie 150 razy): A – rozpad kolagenu, B – naciek leukocytarno-bakteryjny

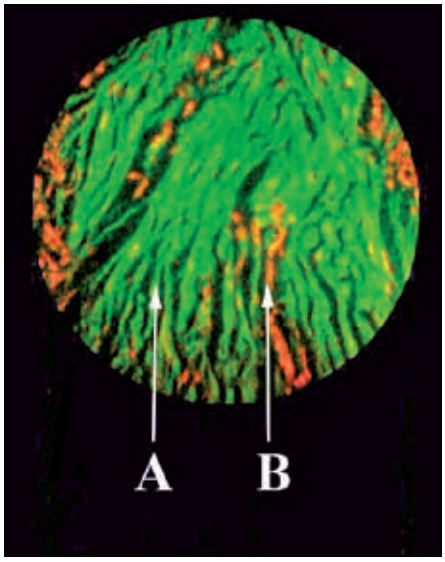

Fig. 5. Collagen changes in gingiva. Acridine orange group II (× 150 magnification): A – collagen, B – leukocytic infiltration

Ryc. 5. Zmiany kolagenu w dziąśle. Oranż akrydynowy w grupie kontrolnej (powiększenie 150 razy): A – kolagen, B – naciek leukocytarny

domain of type I fibrillar collagen [15]. The presence of MMP-1 in epithelial layers may be associated with the degradation of the organic bone matrix [14]. The present study showed that epithelial layers and leukocytic infiltration produced MMP-1. However, the expression was higher in the parenchyma. It is believed that these stromal enzymes (bone acidic glycoprotein-75) potentiate the action of MMPs produced by the parenchyma; and we think that this fact reinforces periodontal disease [16, 17].

Inflammation could act via endothelial and epithelial dysfunction that represents the first step of atherosclerotic disease. Periodontal disease is associated with a hemodynamic deterioration of the arterial wall, probably due to the inflammation. In turn, low wall shear stress could cause a worsening of atherosclerosis directly or as a result of progressive inflammation [18, 19].

Conclusion

In conclusion, we can say that MMP-1 plays an important role in the biology of periodontal disease. An increased concentration of MMP-1 in epithelial layers and leukocytic infiltration of group I patients (diagnosed with atherosclerosis) suggests that the expression of MMP-1 contributes to epithelial invasion of collagen matrix. The mechanism might also suggest the association between cardiovascular diseases and oral infections.

References

[1] Hwalee Y., Sam Na H., Yeon Jeong S., Hee Jeong S., Park H.P., Chung J.: Comparison of inflammatory microRNA expression in healthy and periodontitis tissues. Biocell 2011, 35, 43–49.
[2] Feng Z., Weinberg A.: Role of bacteria in health and disease of periodontal tissues. Periodontol. 2000, 2006, 40, 50–76.
[3] Kornman K.S., Page R.C., Tonetti M.S.: The host response to the microbial challenge in periodontitis: assembling the players. Periodontol. 2000, 14, 33–53.
[4] Scannapieco F.A.: Periodontal inflammation: from gingivitis to systemic disease. Compend. Cont. Educ. Dent. 2004, 25, 16–25.
[5] Monteagudo C., Merino M.J., San-Juan J., Liotta L.A., Stetler-Stevenson W.G.: Immunohistochemical distribution of type IV collagenase in normal, benign, and malignant breast tissue. Am. J. Pathol. 1990, 136, 585–592.
[6] Birkedal-Hansen H., Moore W.G., Bodden M.K., Windsor L.J., Birkedal-Hansen B., DeCarlo A., Engler J.A.: Matrix metalloproteinases: a review. Crit. Rev. Oral. Biol. Med. 1993, 4, 197–250.
[7] Kostamo K., Sorsa T., Leino M., Tervahartiala T., Alenius H., Richardson M. Toskala E.: *In vivo* relationship between collagenase-2 and interleukin-8 but not tumour necrosis factor-alpha in chronic rhinosinusitis with nasal polyposis. Allergy 2005, 60, 1275–1279.

[8] Tamamura R., Nagatsuka H., Gnnduz M., Ge L., Naitoh I., Satlo Y., Yabe A., Nagai N.: Immunohistochemical study of collagen type IV alpha chains and MMP-2, -9 at the basement membrane in oral carcinogenesis. J. Hard Tissue Biol. Proc. 2005, 14, 237–238.
[9] Rashid M.N., Fuentes F., Touchon R.C., Wehner P.S.: Obesity and the risk for cardiovascular disease. Prev. Cardiol. 2003, 6, 42–47.
[10] Sheiham A., Stelle J.G., Marcenes W., Finch S., Walls A.W.G.: The relationship between oral health status and body mass index among older people. A national survey of older people in Great Britain. Br. Dent. J. 2002, 192, 703–706.
[11] Reyes L., Herrera D., Kozarov E., Roldá S., Progulske-Fox A.: Periodontal bacterial invasion and infection: contribution to atherosclerotic pathology. J. Periodontol. 2013, 84, 30–50.
[12] Deshpande R.G., Khan M.B., Genco C.A.: Invasion of aortic and heart endothelial cells by *Porphyromonas gingivalis*. Infect. Immun. 1998, 66, 5337–5343.
[13] Khlgatian M., Nassar H., Chou H.H., Gibson F.C., Genco C.A.: Fimbria dependent activation of cell adhesion molecule expression in *Porphyromonas gingivalis* infected endothelial cells. Infect Immun. 2002, 70, 257–267.
[14] Delaisse J.M., Engsig M.T., Everts V., Ovejero M.C., Ferreras M., Lund L.: Proteinases in bone resorption: obvious and less obvious roles. Clin. Chim. Acta. 2000, 291, 223–234.
[15] Ala-Aho R., Kahari V.M.: Collagenases in cancer. Bioch. 2005, 87, 273–286.
[16] Kumar M.S., Vamsi G., Sripriya R., Sehgal P.K.: Expression of matrix metalloproteinases (MMP-8 and -9) in chronic periodontitis patients with and without diabetes mellitus. J. Periodontol. 2006, 77, 1803–1808.
[17] Kremer E.A., Chen Y., Suzuki K., Nagase H., Gorski J.P.: Hydroxyapatite induces autolytic degradation and inactivation of matrix metalloproteinase-1 and -3. J. Bone Min. Res. 1998, 13, 1890–1902.
[18] Beck J.D., Elter J.R., Heiss G.: Relationship of periodontal disease to carotid artery intimamedia wall thickness: the atherosclerosis risk in communities (ARIC) study. Arterioscler. Thromb. Vasc. Biol. 2001, 21, 1816–1822.
[19] Mercanoglu F., Oflaz H., Oz O.: Endothelial dysfunction in patients with chronic periodontitis and its improvement after initial periodontal therapy. J. Periodontol. 2004, 75, 1694–1700.

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