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ANALYSIS OF IN SITU MATRIX METALLOPROTEINASE-1 CONCENTRATIONS AND CLINICAL PARAMETERS IN CHRONIC ADULT PERIODONTITIS PATIENTS

АНАЛИЗА НА КОНЦЕНТРАЦИИ НА МАТРИКСМЕТАЛОПРОТЕИНАЗА-1 И КЛИНИЧКИ ПАРАМЕТРИ КАЈ ВОЗРАСНИ ПАЦИЕНТИ СО ХРОНИЧНА ПАРОДОНТОПАТИЈА

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Abstract

Aim: to determine the correlation between the concentration of the collagenase MMP-1 in the inflammatory gingival bounding tissue in patients with a chronic form of periodontal disease with the clinical parameters. **Material and methods:** 32 patients with a chronic form of periodontal disease were monitored, according to the criteria set by AAP 1999. The index of dental plaque Silness-Loe was clinically followed, the index of gingival inflammation Loe-Silness, the index of clinical attachment loss and the Miller-Pelzer index of bone resorption. For setting the concentrations of MMP-1, the quantitative enzyme method was used, with the commercial set Colorimetic SensoLyte MMP-1 ELISA Kit. **Results:** We found the presence of a positive correlation between IDP and the examined MMP-1 (r =0.55). Significantly positive correlation was present between IGI and MMP-1. (r =0.77). The loss of attachment and resorption of the alveolar bone were strongly correlated with the concentrations of MMP-1 (r=0.83 and r=0.76). **Conclusion:** The microorganisms from the biofilm initiate the production of the collagenase MMPs-1 and their concentrations rise with the development of the inflammatory processes, leading to the loss of attachment and resorption of the alveolar bone. **Key words:** MMP-1, chronic periodontal disease, biofilm, inflammation.

Апстракт

Цел: да ги утврдиме корелациите помеѓу концентрациите на ММП-1 во инфламираните гингивални ткивни исечоци кај пациентите со хронична форма на пародонталната болест, со клиничките параметри. **Материјал и метод:** во студијата беа вклучени 32 пациенти со хронична форма на пародонталната болест во согласност со критериумите на ААП од 1999. Клинички ги проследивме индексот на дентален плак по Silness-Loe, индексот на гингивална инфламација по Loe-Silness, клиничкиот губиток на припој (CAL), како и Miller-Pelzer-овиот индекс на ресорпција на алвеоларната коска. За утврдување на концентрациите на ММП-1 беше користен квантитативен ензимски метод со комерцијалниот сет Colorimetic SensoLyte MMP-1 ELISA Kit. **Резултати:** утврдивме присуство на позитивна корелација помеѓу ИДП и испитуваната ММП-1 (r =0.55). Значајна, силно изразена позитивна корелација постоеше помеѓу ИГИ и ММП-1 (r=0.77). Клиничкиот губиток на припој, како и ресорпцијата на алвеоларната коска беа исто така силно позитивно корелирани со концентрациите на ММП-1 кај пациентите со хронична пародонтопатија (r =0.83 and r =0.76). **Заклучок:** микроорганизмите од денталниот плак (биофилмот) ја иницираат продукцијата на ММП-1 и нејзините концентрации се зголемуваат со напредувањето на инфламаторните процеси, доведувајќи до губиток на припој и ресорпција на алвеоларната коска. **Клучни зборови:** ММП-1, хронична пародонтална болест, биофилм, инфламација.

Introduction

Periodontal disease is a common, bacterial induced, chronic inflammatory disease that affects the supporting tissues of the teeth, leading to progressive destruction of connective tissue attachment and the alveolar bone. The bacterial etiology of the disease was discovered and confirmed a long time ago, and the bacteria and pathogenet-

ic mechanisms that lead to progression of the disease were identified[1]. However, tissue destruction as a dominating process is caused by the cascade of proteolytical enzymes originating from bacteria of the biofilm, as well as from the enzymes originating from the host-responsive cells[2,3]. The host initiates and controls the release of enzymes, including matrix-metalloproteinases (MMPs), to allow the tissues to retreat from the microbial destructive lesions. Recently, there has been increasing evidence implicating MMPs as key mediators in the tissue destruction associated with the various forms of periodontal disease, including the progression from gingivitis to periodontitis[4].

Matrix metalloproteinases are a family of 25 genetically various, but structurally related zinc and calcium-dependent endopeptidases that degrade the extracellular matrix (ECM) of interstitial stroma and the basement membrane components[5]. MMPs are expressed by various types of cells, inflammatory cells (monocytes, macrophages, lymphocytes and polymorphonuclear cells), and by resident cells (fibroblasts, epithelial and endothelial cells).

MMP-1 is synthesized and secreted by the tissue fibroblasts and macrophages in the chronic inflammation, but it has also been associated with normal tissue remodelling[6]. In vitro MMP-1 is expressed in numerous cells, such as hondrocytes, osteoblasts, keratocytes and various tumour cells[7,8]. MMP-1 can initiate extracellular matrix destruction and cooperates with other MMPs in collagen degradation[9,10]. MMP-1 (and MMP-13) are the key enzymes responsible for degradation of type I and III collagen.

The **aim** of this study was to evaluate the correlations between concentrations of MMP-1 in the inflammatory gingival bounding tissue at patients with a chronic form of periodontal disease with the clinical parameters.

Material and methods

In order to realize this goal, at the Clinic of Oral Pathology and Periodontology were studied 32 patients of both sexes, male and female, aged from 21 to 65 years, with moderate to severe expression of a chronic form of periodontal disease: pocket depth over 4mm according to the criteria proposed by the AAP, 1999. In all subjects, after their history was taken, we conducted a clinical examination to determine the level of periodontal destruction. We noted the dental plaque by using index IDP - Silness-Loe; gingival inflammation by using index IGI - Loe-Silness; clinical attachment loss – CAL, and index of bone resorption by Miller-Pelzer. All subjects were healthy and none of them had used antibiotics within the 6 months preceding the study. The test material was taken with the incision of inflamed gingival tissue during conventional treatment – curettage of the periodontal pockets. Gingival biopsies contained epithelium, connective tissue, proliferated sulcular epithelium and pathologically altered epithelium from the bottom of the pockets. Samples were stored in sterile plastic tubes, then in the shortest period of time were frozen and kept at - 80^0 C until further laboratory analysis, but not for more than 6 months. For setting the concentrations of MMP-1 a quantitative enzymatic method was used, the commercial SensoLyte MMP- 1 ELISA Kit Colorimetric from AnaSpec Inc, and with the help of this we obtained a quick, safe and sensitive determination of concentrations of MMP - 1 in the gingival tissue substrate.

The test material (samples) was frozen and kept in the Laboratory of the Faculty of Pharmacy in Skopje. The laboratory assays were performed at the Institute of Molecular Biology and Genetics at the Faculty of Natural Sciences and Mathematics at the "Ss. Cyril and Methodius" University, Skopje. Obtained data were statistically processed using Statistics 6.1 computer programs for Windows.

The protocol for the collection of samples from human subjects was approved by the ethical committee for medical-dental investigations of the Faculty of Dentistry at the Ss. Cyril and Methodius University, Skopje, according to the Helsinki Declaration on Human rights.

Results and discussion

Although research investigations into the pathogenesis of periodontal disease traditionally focused on the impact of bacterial infection, in the last two decades more attention has been directed towards the study of host response factors (mechanisms) that determine the disease[11]. Molecular and cellular studies on the pathogenesis of the disease confirm that biofilm is a primary etiologic factor, but the disease still occurs as a result of the interaction between specific pathogens and sensitive immune and immune-inflammatory host-responses[12], which are modulated by a number of intrinsic (genetic) and externally induced factors[13].

Table 1 shows mean values of clinical parameters - IDP, IGI, CAL, the index of bone resorption and concentration of MMPs-1 in inflamed gingival samples in patients with a chronic form of periodontal disease. The mean value for the index of dental plaque (IDP) ranged from 1 to 3 (2.37 ± 0.61). The gingival inflammation index (IGI) ranged from 1 to 3, mean 2.31±0.59. Clinical attachment loss (CAL) ranged from 4 to 7mm; the average value was 5.25 ± 1.18 mm. The average value for the index of bone resorption was 3 ± 0.92mm, ranging from

2 to 4mm. Concentration of MMP-1 ranged from 301.36 to 1456.76 pg/100 microg. protein in inflamed gingival tissues, the average value was 749.02 ± 338.03 pg/100 microg.protein.

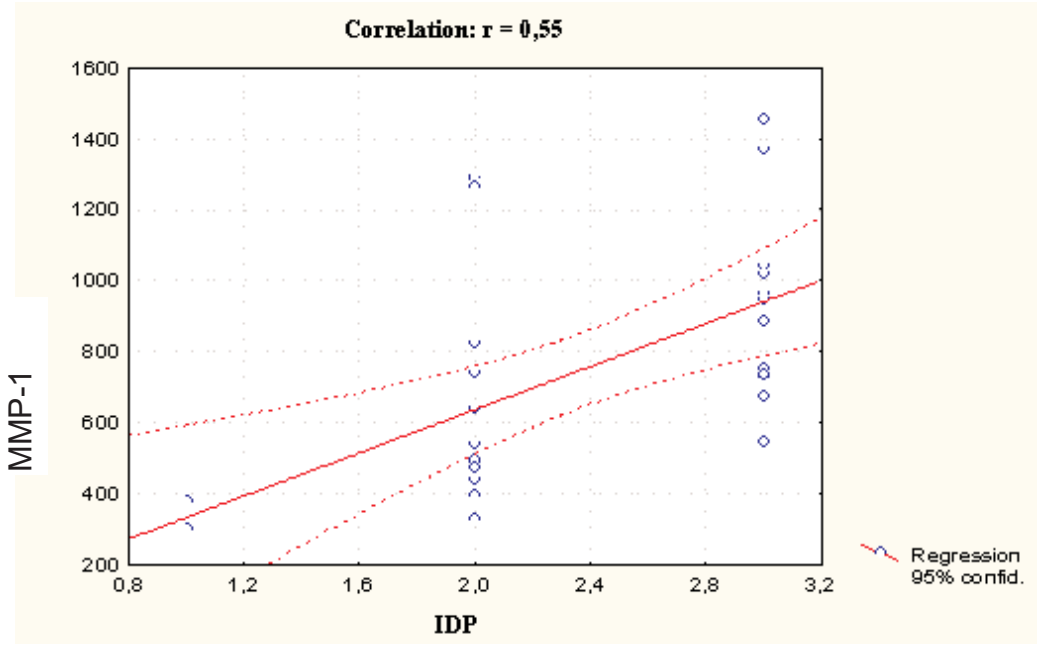
Table 1. Average values of examined clinical parameters IDP, IGI, CAL and the index of bone resorption and concentration of MMPs-1 in patients with a chronic form of periodontal disease

Parameters	mean	SD	min	max
IDP	2.37	0.61	1	3
IGI	2.31	0.59	1	3
CAL	5.25	1.18	4	7
Bone resorp. index	3	0.92	2	4
MMPs-1	749.02	338.03	301.36	1456.76

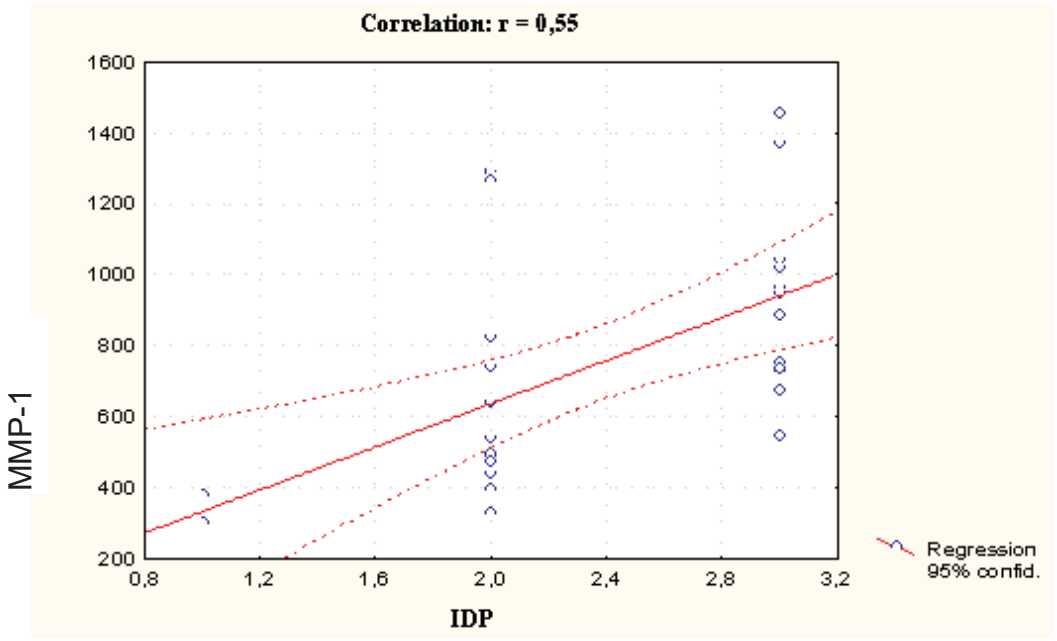
Analysing the correlations between the concentration of MMP-1 in inflamed gingival tissues and clinical parameters, we noted a significant positive correlation between the mean values of the index of dental plaque and MMP-1 (r =0.55). This means that large amounts of dental plaque cause increased concentrations of examined MMP-1. Numerous studies suggest that the interaction of bacteria present in the biofilms with inflammatory cells (monocytes/macrophages, polymorphonuclear cells), and with residential cells (fibroblasts) results in the release of proteases from the host-cells. Bacterial adhesion activates secretion of proinflammatory mediators, such as (IL-1α, IL-1β, TNF-α) from epithelial cells, which diffuse into connective tissue and stimulate host-cells to produce and release matrix metalloproteinase[14]. Hence, increased levels of interstitial collagenase were detected in inflamed human gingival tissues, gingival fluid and inflammatory exudates from gingiva[15]. MMP-1 that is present in these tissues is proven to be converted from a latent to an active, destructive form of this enzyme during the inflammatory processes in periodontal disease. Domeij and co-workers[16] suggest that gingival fibroblasts are capable of producing MMP-1 (and MMP-3), as a result of IL-1β and TNF-α stimulation.

Graph 1 shows the correlation between the dental plaque index and MMP-1 in patients with a chronic form of periodontal disease. Pearson's coefficient of correlation suggests that there is a significant positive correlation between these parameters (r=0.55).

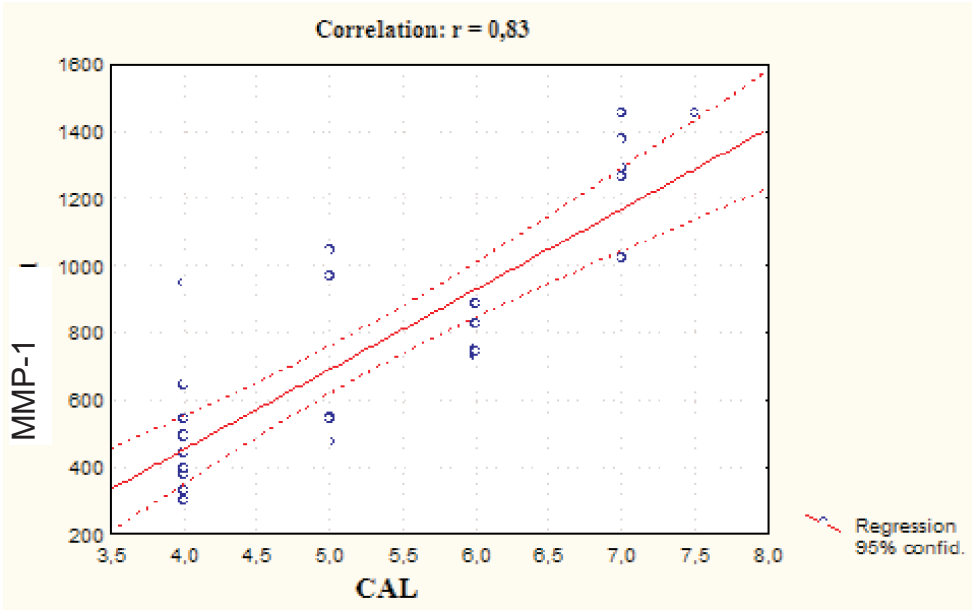
We noted a significant positive correlation between the IGI and MMP-1 in patients with chronic periodontitis (r=0.77). These results suggest that the intensification of gingival inflammation leads to increased values of MMP-1. Our findings comply with the results of Alfant *et al.*[17]. In their studies they detected a statistically significant reduction of the collagenases MMP-1 (-8,-13) 6 weeks after periodontal therapy, and they observed a



Graph 1. *Correlation between IDP and MMP-1 in patients with chronic periodontitis*



Graph 2. *Correlation between IGI and MMP-1 in patients with chronic periodontitis*

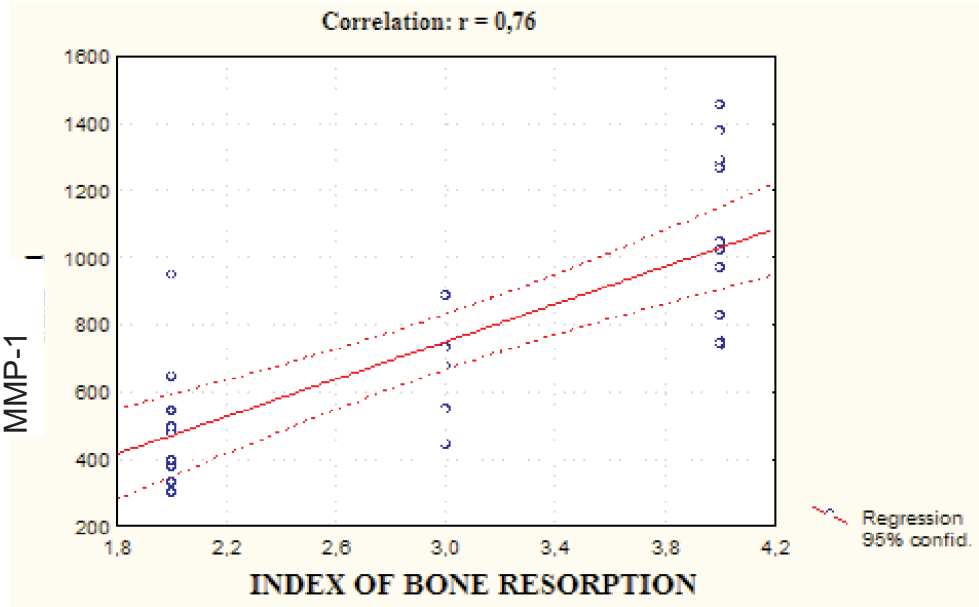


Graph 3. *Correlation between CAL and MMP-1 in patients with chronic periodontitis*

post-therapy reduction in gingival bleeding index, indicating reductions in gingival inflammation. Similar results were noted by Buduneli, Tuter and Sorsa[18,19,20] in their previous studies, investigating MMP changes associated with treatment of chronic periodontitis. Some other authors suggest that tissue extracts and cultured tissue samples from inflamed gingiva increase the collagenase activity, compared with healthy gingiva[21].

Graph 2 shows the correlation between the IGI (index of gingival inflammation) and MMP-1 in patients with chronic periodontitis. Pearson's coefficient of correlation suggests that there is a significant, strong, positive correlation between these parameters (r =0.77).

The loss of attachment was strongly, positively correlated with the concentrations of examined MMP-1 in patients with a chronic form of periodontal disease



Graph 4. *Correlation between the index of bone resorption and MMP-1 in patients with a chronic form of periodontal disease*

(r=0.83). This means that increasing levels of MMP-1 in inflamed gingival tissues lead to a severe loss of epithelial attachment. Chronic periodontal inflammation is characterized by increased apical proliferation and migration of gingival sulcular epithelial cells. This results in degradation of periodontal ligament collagen fibers and finally irreversible loss of tooth attachment. In periodontal diseases, inflamed gingival sulcular epithelium exhibits local loss of integration, evidently associated with epithelial migration into underlying periodontal connective tissue[22].

Graph 3 shows the correlation between CAL (clinical attachment loss) and MMP-1 in patients with chronic periodontitis. Pearson's coefficient of correlation suggests that there is a significant, very strong positive correlation between these parameters (r = 0.83).

Pearson's coefficient of correlation suggests that the resorption of the alveolar bone is strongly, positively correlated with the concentrations of examined MMP-1 in patients with a chronic form of periodontal disease (r=0.76). Our findings agree with Hayami *et al.*[23]. In their studies they indicate that inhibition of collagenases with dexamethasone increases the formation of mineralized nodules in the PDL. During the periodontal disease, destruction and loss of extracellular matrix (ECM) occurs which is mediated by MMP originating from inflammatory and resident cells present in the periodontal ligament complex. PDL cells are crucial for the regeneration of lost periodontal and mineralized tissues, through processes due to differentiation of precursor cells into osteoblasts. Hajami *et al.*[24] suggest that with the presence of elevated levels of MMP-1 (or MMP-13), such differentiation can be inhibited, because of the reduced amount of osteoblasts which are able to mend and repair the bone tissue. Moreover, they suggest further *in vivo* studies on animal models, aiming to get more information about the mechanisms that involve MMP-1 and/or MMP-13 in the inhibition of osteoblastogenesis and limited bone repair.

Graph 4 shows the correlation between the bone resorption index and the concentration of MMP-1 in patients with chronic periodontitis. Pearson's coefficient of correlation suggests that there is a significant positive correlation between these parameters (r = 0.76).

Conclusion

The microorganisms from the biofilm initiate the production of the collagenase-MMP-1 and their concentrations rise with the development of the inflammatory processes, leading to the loss of attachment and resorption of the alveolar bone.

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