

THE LEVEL OF MATRIX METALLOPROTEINASE-8 IN THE GINGIVAL CREVICULAR FLUID – DIAGNOSTIC AND PROGNOSTIC IMPLICATIONS IN PERIODONTAL DISEASE

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Keywords: matrix metalloproteinase-8 (MMP-8), gingival crevicular fluid (GCF), periodontal disease

Abstract: The biochemical composition of healthy and diseased periodontal connective tissues is determined by the extension of the degradation and synthesis of matrix components. Objective: The aim of our study was to comparatively assess the level of matrix metalloproteinase-8 (MMP-8) in the gingival crevicular fluid (GCF) in patients with periodontal disease with the levels found in patients free of periodontal disease. Materials and methods: The filter paper intended for sampling was placed to the maximum depth of the gingival sulcus. To perform the ELISA test, the GCF samples were assessed in a dilution of 1:20. Results: There was a statistically significant difference between the concentration levels of MMP-8 in the GCF in the group of patients with chronic periodontitis or gingivitis and the subjects in the control group. Conclusion The concentration of MMP-8 in GCF may be a particularly useful indicator for monitoring the therapeutic response in periodontal disease.

INTRODUCTION

The biochemical composition of healthy and diseased periodontal connective tissues is determined by the extension of the degradation and synthesis of matrix components.(1) Degradation is a normal feature of periodontal connective tissues. The gingival cells and those of the periodontal ligament have a higher recovery rate than other tissues, even in adults, a rate which becomes excessive when inflammation is present.

The major change of the biochemical constituents of connective tissue in periodontal disease is the loss of collagen due to its degradation. There are at least four mechanisms by which the extracellular matrix can be degraded and modified during development of marginal periodontitis: chronic enzymes released by the host and bacterial cells, phagocytosis of the matrix components, the synthesis of reactive oxygen species, and the release of a wide range of cytokines and other inflammatory mediators.

The type of matrix metalloproteinase produced by the periodontal ligament cells is influenced by some of the cytokines present in the periodontal tissue, including IL-1, TNF- α , PgE.(2) The products of the microbial plaque also stimulate the production of matrix metalloproteinase by a variety of cells.

The main type of collagenase detected in inflamed periodontal tissue is collagenase 2 (MMP-8), also known as polymorphonuclear leukocyte collagenase type because it is expressed by polymorphonuclear leukocytes. Vilella et al.(3) studied the relationship between the gingival crevicular fluid (GCF) collagenase activity and the status of periodontal disease, thus demonstrating a high collagenolytic activity correlated with the severity of periodontal damage and with the depth of the pockets.

Collagenase in the gingival extracts, in GCF, and in the polymorphonuclear one preferentially degrade collagen type I and II, while the collagenases in gingival cell cultures preferentially degrade collagen type I and III, suggesting that these collagenases have different origins: the first ones are of

polymorphonuclear origin, while the latter are of fibroblast origin.(4)

PURPOSE

The aim of our study was to comparatively assess the level of matrix metalloproteinase-8 (MMP-8) in GCF in patients with periodontal disease with the levels found in patients free of periodontal disease.

METHODS

Our study included 39 subjects, patients who presented to the Department of Periodontology of the Faculty of Dentistry within the University of Medicine and Pharmacy of Tirgu-Mures and who meet the following criteria:

- non-smoker,
- no major inflammatory diseases,
- no periodontal or antibiotic treatment one month before the study.

After initial clinical examination by recording clinical indicators and X-ray examination, the patients were included in one of the study groups based on periodontal diagnosis:

- group I: chronic marginal gingivitis (11 subjects),
- group II: chronic marginal periodontitis (12 subjects),
- group III, the control group: free of periodontal disease (16 subjects).

All patients were informed about the protocol and informed consent was obtained.

In the first two groups, from patients in whom we found clinical signs of periodontal problems, GCF samples were collected from the mesio-buccal region of the gingival sulcus of a randomly chosen tooth. From patients in the control group, sampling was performed in the mesio-buccal region of the gingival sulcus of the inferior canine. The surface of each tooth from which sampling was performed was gently dried with an air jet so as not to cause gum bleeding, and the teeth were kept dry by isolation with cotton rolls.

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Article received on 12.10.2014 and accepted for publication on 07.01.2015
ACTA MEDICA TRANSILVANICA March 2015;20(1):104-106

CLINICAL ASPECTS

The filter paper intended for sampling was placed to the maximum depth of the gingival sulcus and was kept there for 3 minutes. Samples containing traces of blood were discarded.

Elution of the absorbed fluid on the filter paper was performed for each sample in 75µl 50 mM Tris-HCl, pH 7.8, containing 0.2 M NaCl and 1 mM CaCl₂ for 2 hours at 22°C under vibration, according to the procedure described by Emingil et al. (2006). GCF eluted samples were frozen at -20°C until the ELISA test. In order to perform the ELISA test, the GCF samples were assessed in a dilution of 1:20.

The determined values (µg/l) of MMP-8 in the GCF were then statistically analyzed by GraphPad InStat software v.3.10. To evaluate the relationship between the clinical entities (healthy, gingivitis, periodontitis) and MMP-8 levels in GCF we used the nonparametric ANOVA Kursk-Wallis test. Differences of $p < 0.05$ were considered statistically significant.

RESULTS

The determined values of MMP-8 in GCF in the three groups investigated are shown in figure no. 1 and table no. 1.

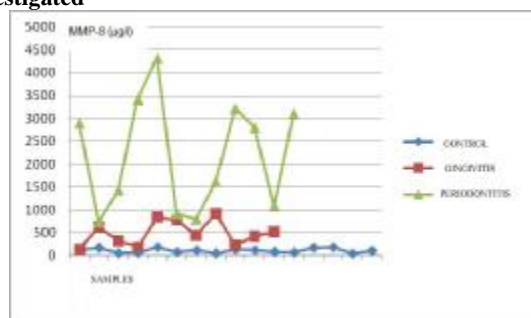
The nonparametric ANOVA Kruskal-Wallis test revealed the value $p < 0.0001$, considered extremely significant value, i.e. the change in the mean values in the investigated groups is significantly higher than expected.

Table no. 1. Descriptive statistics for crevicular MMP-8 (ug/L) by groups

Descriptive statistics for crevicular MMP-8 (ug/L) by groups								
Groups	Mean	Median	Minimum	Maximum	Lower quartile	Upper quartile	Range	StdDev.
Gingivitis (n=15)	481.54	441.00	129	929	255	770	544	211.49
Periodontitis (n=15)	2951.67	2211.00	741	4110	855	1755	2550	1230.75
Control (n=8)	105.55	105.00	30	180	62	150	88	40.55

We used the Dunn multiple comparison statistical test to determine whether there are statistically significant differences between the studied groups pairs. It was noted that there was a statistically significant difference between the concentration levels of MMP-8 in the GCF in patients with chronic periodontitis and the subjects in the control group ($p < 0.001$). Significant difference was also found in concentrations of MMP-8 in the control and gingivitis groups ($p < 0.01$) (figure no. 2).

Figure no. 1. Graphical illustration of the MMP-8 concentration values (µg/L) in the GCF in the three groups investigated



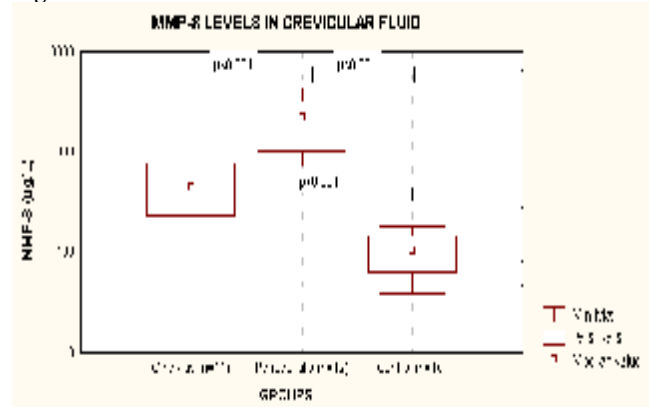
DISCUSSIONS

Matrix metalloproteinases are zinc-dependent endopeptidases derived predominantly from polymorphonuclear leukocytes during acute phases of periodontal disease, and are the key enzyme responsible for the degradation of the extracellular matrix collagen. According to the literature, in the course of periodontal disease high levels of MMP were observed, especially of MMP-8 and MMP-9 in inflamed gingival tissue and GCF.(5)

MMP-8 has the unique ability to degrade collagen type I and III, critical action for periodontal tissue destruction. MMP-8 levels in GCF have been reported to be higher in periodontal disease compared to those found in healthy subjects, observations confirmed by several studies.(6,7,8,9,10)

Establishing a mechanical treatment of scaling and root planning, associated with anti-inflammatory or antibiotic treatment can reduce the level of MMP-8 in the crevicular fluid and can improve the periodontal clinical parameters.(11,12,13,14,15)

Figure no. 2. MMP-8 levels in crevicular fluid



Our results and data in the literature clearly suggest the role of MMP-8 in the progression of periodontal disease, its high level being important in its pathogenesis.

CONCLUSIONS

Immunoassays for the determination of MMP-8 protein in GCF are specific, as opposed to other functional enzyme tests which are currently not typical of individual matrix metalloproteinases.

The concentration of MMP-8 in GCF may be a particularly useful indicator for monitoring the therapeutic response in periodontal disease; thus, for example, patients with gingivitis and positive MMP-8 results of repeated tests can be considered having a high risk of developing a chronic marginal periodontitis, or they may have already suffered minor irreversible damage.

The results obtained by our study allow us to state that the MMP-8 level in GCF differentiates healthy subjects from those with gingivitis and marginal chronic periodontitis.

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