CLINICAL PRACTICE

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Salivary matrix metalloproteinases activity in periodontal disease

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Abstract: *Introduction: Matrix metalloproteinases (MMPs) are key enzymes involved in degradation of extracellular colagen matrix during periodontal disease progression. The aim of our work was to study the levels of MMP-1, MMP-2, MMP-7, MMP-9 and MMP-10 in saliva of patients with chronic periodontitis.*

Materials and methods: The study included 22 patients with chronic periodontal disease and 15 controls. We collect unstimulated whole saliva from all volunteers included in the study. Salivary levels of mentioned MMPs were analyzed using magnetic bead-based multiplex assays and Luminex technology.

Results: MMP-1, MMP-7, MMP-9 and MMP-10 present statistically increased salivary levels at patients with periodontal disease versus the control group ($p<0.05$). Salivary level of MMP-2 at patients with chronic periodontitis was increased but statistically insignificant compared with healthy subjects ($p=0.1$).

Conclusions. The increased level of these enzymes in patients diagnosed with periodontal disease reflects their implication in periodontal tissue degradation. Saliva is an excellent fluid useful in monitoring the severity of this oral disease that affects about half of the adult population around the world.

Keywords: *saliva, inflammation, proteases, periodontal tissue*

INTRODUCTION

Periodontal disease is one of the most common oral disease and affects the adult population around the world. Periodontal disease is a chronic inflammatory disorder that affects the supporting tissues of the tooth, leading to the progressive destruction of conjunctival tissue and alveolar bone.

The role of microorganisms in the etiology of periodontitis has been very well studied, from 600 types of bacteria that are able to colonize oral cavity about 10% plays an important role in the initiation of periodontitis. Porphyromonas gingivalis, Tanerella forsythensis and Troponema denticola, are directly associated with chronic periodontitis. Actinobacillus actinomycetemcomitans, was observed in early stages of periodontal disease and aggressive periodontitis. These

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microorganisms cause periodontal tissue damage either by their degradation products (toxins, enzymes, and lipopolysaccharide) or by activating of the host´s immune system, which leads to the activation of inflammatory cells, cytokine generation, proteolytic enzyme release, and osteoclast activation [1-4].

This inflammatory process is characterized by the increased levels of IL-1α, IL-1β, IL-6, and TNF-α and release of enzymes such as MMPs. MMPs-1, 2, 3, 8 and 9 are key proteases found in biopsy specimens of human inflammatory periodontal tissue [5].

The many advantages of the saliva, namely the rapid, inconvenient collection, anywhere and anytime, the lack of contamination has led many researchers to use it as a new diagnostic fluid for both systemic and oral disorders. Saliva is secreted by major and minor salivary glands, contains 99.5% water, 0.2% inorganic substances and 0.3% organic substances. Salivary proteins include proteins synthesized by salivary glands (mucoproteins and enzymes) and proteins of blood origin, with over 20 protein fractions. Plasma proteins may exceed 20% of salivary proteins and include: albumin, globulin (IgA, IgM, and IgG), α- and β-globulins. The total concentration of γ-globulins is less than 0.5% of the plasma concentration and is generally not affected by the salivary flow. Saliva also contains and non-protein organic compounds such as glucose, lipids, hormones, uric acid, vitamins [6-8].

The main aim of the present research is to detect salivary levels of MMP-1, 2, 7, 9 and 10 in unstimulated whole saliva of patients diagnosed with chronic marginal periodontitis using Luminex technology and Magnetic Bead-Based Multiplex assays.

MATERIAL AND METHODS

Patient selection: We included in our study 22 patients with chronic periodontitis and 15 healthy subjects who agreed to participate voluntary and signed and informed consent. The present research was approved by the Ethics Committee of Science Research from „Carol Davila University of Medicine and Pharmacy". Twenty-two subjects with chronic periodontal disease were recruited from a dental private office in Bucharest. We excluded from our study subjects under 18 years old or with systemic diseases, such as: diabetes mellitus, osteoporosis, chronic hepatitis, liver cirrhosis, HIV/AIDS, autoimmune diseases. All volunteers were diagnosed by the same experienced medical professional.

Clinical data: Demographic data, including age, sex, medical history, medication used and smoking habits (smoker/nonsmoker), were collected. The periodontal parameters performed were: for bleeding index (BI), probing depth (PD), plague index (PI). Chronic periodontitis was diagnosed according to the clinical case definitions proposed by the Center for Disease Control and Prevention.

Samples collection

For all participants in the study, saliva samples were collected after clinical examination. The collection method followed a well-established protocol and took place as follows: 2-4 mL of unstimulated whole saliva was given by each participant in the morning around 9 AM. The participants were asked not eating, drinking or smoking prior to saliva collection. All subjects rinsed with 5 mL of distilled water before collection. Immediately after the collection, the samples were kept on ice and then centrifuged at 5000 rpm for 10 minutes. Samples were aliquoted and stored at -80 ºC until further determinations.

Matrix metalloproteinases detection

All MMPs were detected using Luminex technology and Magnetic Bead-Based Multiplex assays. This method enables simultaneous detection of multiple salivary human biomarkers. Thus salivary levels of MMP-1, -2, -7, -9, -10 were assessed using MILLIPLEX MAP Human MMP Magnetic Bead Panel 2 – Immunology Multiplex Assay. All samples and controls were processed following the manufacturer's specifications. The assay required 25 µL of saliva diluted at 1:20; each MMP had its own detection interval as follows: MMP-1: 27 – 20,000 pg/mL; MMP-2: 68 -50,000pg/mL; MMP-7: 548 – 400,000pg/mL; MMP-9: 14 – 10,000pg/mL; MMP-10: 27 – 20,000pg/mL. The assay involved a 2 hour multistep procedure involving preparation of reagents, samples and controls, preparation of the 96 well-plate and

analysis using a Luminex® 200™ and the xPONENT® software for data acquisition and analysis.

Statistical analysis: The statistical analysis was performed using SPSS software. The characteristics were expressed as median (quartile 1; 3). Statistically significant correlations were found using the Mann-Whitney test (a two-sided p-value less than 0.05 was noted as statistically significant).

Results:

Clinical data: Results for demographic data and clinical examination are present in Table 1. No significant differences were detected for gender and age between the periodontal patients and the control group. Table 2 present the MMPs values obtained in the two groups of patients included in the study.

Table 1. Clinical date of patients included in the study

Variables	Patients	Controls	p-values
Age (*years*)	44.88±12.81	20.67±2	-
Gender (*female/male*)	10/12	10/5	-
Smoking status	0	0	-
BI (*%*)	45±0.11	17±0.20	<0.05
GI (*%*)	59.7±0.24	12.36±0.12	<0.05
PD (*%*)	4.80±0.23	0	<0.05

Table 2. Salivary levels for MMP-1,-2,-7,-9 and -10

Variables	Patients	Controls	p-values
MMP-1 (*pg/mL*)	348.375±144.6	190.125±144.6	<0.05
MMP-2 (*pg/mL*)	116.37±230.12	9.56±230.12	=0.1
MMP-7 (*pg/mL*)	886.68±79.81	527.59±79.81	<0.05
MMP-9 (*pg/mL*)	6885.75±2800.08	5224.911±2800.08	<0.05
MMP-10 (*pg/mL*)	1569.56±168.04	598±168.044	<0.05

DISCUSSIONS

Salivary biomarkers are used in the diagnosis and monitoring of autoimmune disorders (cystic fibrosis, Sjrogen's syndrome), cardiovascular disease, diabetes, HIV, oral cancer, oral lichen planus and periodontal disease [9-13].

MMPs are metal-dependent proteolytic enzymes involved in both physiological (apoptosis, bone remodeling, angiogenesis) and pathological processes such as cardiovascular disease, cancer and periodontal disease [14].

In macrophages and other types of cells from periodontal tissue, lipopolysaccharide from Porphyromonas gingivalis, binds to Toll-like receptor 4 (TLR4) and activates an inflammatory cascade with the release of inflammatory cytokines, chemokines and prostaglandins. This inflammatory response actives inflammatory cells to release MMPs mainly from polymorphonucleate leukocytes (PMN) which will degrade extracellular collagen matrix [5, 15].

Because the extracellular periodontal matrix contains especially type 1 collagen, special attention has been given to MMPs capable of degrading this type of collagen such as MMP-2 MMP-8, MMP-9, MMP-13 [16, 17].

MMPs-1, -2, -8, -9 and -13, had been detected so far in patients with periodontal diseases, mostly from gingival crevicular fluid (GCF) and less from unstimulated whole saliva. The results of the studies reflects increased levels of all mentioned MMPs and their implication in periodontal tissue degradation [18, 19].

MMP-1 or collagenase-1 is the main collagenase found at patients with aggressive periodontitis. GCF and salivary levels of MMP-1 were increased during

periodontal disease and decreased levels were observed after periodontal treatment [20-22].

MMP-1 present increased levels in inflammation, autoimmune and cardiovascular diseases [14, 23].

Our results revealed statistically increased salivary levels of MMP-1 in patients with chronic periodontitis compared with controls.

MMP-2 and MMP-9 are gelatinases that can be released by various type of cells from oral cavity such as gingival fibroblast and gingival keratinocytes [24].

In the present study, we obtained increased salivary levels of MMP-2 but statistically insignificant in patients with chronic periodontal disease versus the healthy group.

The salivary level of MMP-9 was statistically increased in our study at patients diagnosed with periodontal disease versus the healthy group.

Increased levels for MMP-2 and MMP-9 were observed in GCF at patients with periodontitis, versus patients' gingivitis and healthy subjects [25].

MMP-9 in involved in cell apoptosis, tissue remodeling, being associated with several systemic diseases such as systemic sclerosis and osteoarthritis. Meanwhile MMP-2 is involved in angiogenesis and pathological processes such as cardiovascular disease [14, 23].

MMP-7 or matrilysin-1 is produced in non-injured exocrine and mucosal epithelia. Levels of MMP-7 in GCF of patients with periodontal disease are the same with healthy subjects [26].

MMP-7 is associated with inflammation, cardiovascular and lung diseases and to physiological cell apoptosis [14, 23]. Salivary levels of MMP-7 at chronic periodontitis patients involved in our study reflect increased statistically levels compared with controls.

MMP-10 or stromelysin -2 acts on types III, IV and V of collagen and has been linked to cell apoptosis, liver and lung diseases, tissue remodeling [14, 19, 23].

Still now, MMP-10 has not been detected in saliva or GCF in patients with periodontal disease. Our study revealed increased statistically levels at periodontitis patients versus healthy volunteers.

CONCLUSIONS

In conclusion, increased levels of MMP-1, -2, -7, 9, and 10 are directly associated with the severity and progression of periodontal disease, especially through involvement in alveolar bone resorption and periodontal tissue breakdown. Salivary detection of this MMPs is a modern alternative to monitoring the activity of these enzymes.

References:

1.Kinney J, Ramseier C, Giannobile WV. Oral fluid- based biomarkers of alveolar bone loss in periodontitis. Ann N Y Acad Sci, 2007, 1098:230-251.

2.Miller CS, Foley JD, Bailey AL, Campell CL, Humphries R L, Floriano NCP, Simmons G. Curent developments in salivary diagnostics. Biomarkers Med, 2010, 4(1): 1-19.

3.Miricescu D, Totan A, Calenic B, Mocanu B, Didilescu A, Mohora M, Spinu T, Greabu M. Salivary biomarkers: Relationship between oxidative stress and alveolar bone loss in chronic periodontitis. Acta Odontol Scand, 2014, 72(1):42-7.

4.Bascones-Martínez A, Muñoz-Corcuera M, Noronha S, Mota P, Bascones-Ilundain C, Campo-Trapero J. Host defence mechanisms against bacterial aggression in periodontal disease: Basic mechanisms. Med Oral Patol Oral Cir Bucal, 2009,14:680-5.

5.Rai B, Kaur J, Jain R, Anand SC. Levels of gingival crevicular metalloproteinases-8 and 9 in periodontitis. Saudi Dental J, 2010, 22: 129-131.

6.Javaid MA, Ahmed AS, Durand Robert, Tran SD, Saliva as a diagnostic fluid for oral and systemic diseases. J Oral Biol Craniofac Res, 2016, 6(1):66-75.

7. Miricescu D, Greabu M, Totan A, Mohora M, Didilescu A, Mitrea N, Arsene A, Spinu T, Totan C, Rădulescu R. Oxidative Stress – A possible link between systemic and oral diseases. Farmacia, 2011;3: 329-337.

8. Battino M, Mohora M, Totan A, Didilescu A, Spinu T, Totan C, Miricescu D, Radulescu R. Saliva-a diagnostic window to the body, both in health and in disease. J Med Life, 2009, 2 (2):124-132.

9.Goswami Y, Mishra R, Agrawal AP, Agrawal LA. Salivary Biomarkers-a review of powerful diagnostic tool. J Dent Med

Sci, 2015, 14, 3: 80-87.

10. Miricescu, Maria Greabu, Alexandra Totan, Andreea Didilescu, R. Rădulescu. The antioxidant potential of saliva: clinical significance in oral diseases. Therapeutics, Pharmacology and Clinical Toxicology, XV,2011, 2:139-143.

11. Greabu M, Miricescu D, Totan A, Calenic B. Salivary and serum enzymes as diagnostic biomarkers in patients with periodontal disease. Stoma. Edu J,2014, 1(1):22-26.

12. Totan A, Miricescu D, I Parlatescu, M Mohora, M Greabu. New possible salivary and serum biomarkers in oral lichen planus. Biotechnic&Histochemistry, 2015, 19:1-7.

13. Nunes LAS, Mussavira S, Bindhu OS. Clinical and diagnostic utility of salivaas a non-invasive diagnostic fluid: a systemic review. Biochemia Medica, 2015, 25(2):177-92.

14. Cavalla F, Hernandez-Rios P, Timo S, Biguetti C, Hernandez M. Matrix metalloproteinases as regulators of periodontal inflammation. Int. J. Mol. Sci, 2017, 18, 440.

15. Ara T, Kurata K, Hirai K, Uchihashi T, Uematsu T, Imamura Y, Furusawa K, Kurihara S, Wang P. Human gingival fibroblasts are critical in sustaining inflammation in periodontal disease. J. Periodontal Res, 2009, 44:21–27.

16. Hernández MVR., Mäntyla P, Tervahartiala T, Sorsa T, Gamonal J. Chronic Periodontitis: The Role of Immuno-Inflammatory Response in the Pathogenesis of Chronic Periodontitis and Development of Chair-Side Point of Care Diagnostics in Periodontitis and Related Systemic Inflammation. InTech; Rijeka, 2012, 33–54.

17. Sorsa T, Gursoy UK., Nwhator S, Hernandez M, Tervahartiala T, Leppilahti J, Gursoy M, Könönen E, Emingil G, Pussinen PJ, et al.. Analysis of matrix metalloproteinases, especially MMP-8, in gingival creviclular fluid, mouthrinse and saliva for monitoring periodontal diseases. Periodontol. 2000, 2016,70:142–163.

18. Parmar NK, Nisha KJ, Guru S, Padmanabhan. Role of matrixmetalloproteinases in periodontal disease-a review. Biomed.J.Sci, 2018, 2 (1):1-6.

19. Maciejczyk M, Pietrzykowska A, Zalewska A, Knas M, Daniszewska. The significance of matrix metalloproteinases in oral diseases. Adv.Clin. Exp. Med, 2016, 25(2):383-390.

20. Tüter G, Kurtiş B, Serdar M. Effects of phase I periodontal treatment on gingival crevicular fluid levels of matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1. J Periodontol, 2002;73:487-93.

21. Marcaccini AM, Novaes AB Jr, Meschiari CA, Souza SL, Palioto DB, Sorgi CA, et al. Circulating matrix metalloproteinase-8 (MMP-8) and MMP-9 are increased in chronic periodontal disease and decrease after non-surgical periodontal therapy. Clin Chim Acta 2009,409:117-22.

22. Marcaccini AM, Meschiari CA, Zuardi LR, de Sousa TS, Taba M Jr, Teofilo JM, et al. Gingival crevicular fluid levels of MMP-8, MMP-9, TIMP-2, and MPO decrease after periodontal therapy. J Clin Periodontol, 2010,37:180-90.

23. Kim WU, Min SY, Cho ML, et al. Elevated matrix metalloproteinase-9 in patients with systemic sclerosis. Arthritis Res Ther, 2005,7(1):71-9.

24. Makela M, Salo T, Uitto V-J, Larjava H. Matrix Metalloproteinases (MMP-2 and MMP-9) of the Oral Cavity: Cellular Origin and Relationship to Periodontal Status. J. Dent. Res, 1994, 73(8):1397–1406.

25. Maeso G, Bravo M, Bascones A. Levels of metalloproteinase-2 and -9 and tissue inhibitor of matrix metalloproteinase-1 in gingival crevicular fluid of patients with periodontitis, gingivitis and healthy gingiva. Quitessence Int, 2007, 38: 247-252.

26. Emingil G, Tervahartiala T, Mãntylã P, Määttä M, Sorsa T, Atilla G, Gingival crevicular fluid matrix metalloproteinase (MMP)-7, extracellular MMP inducer, and tissue inhibitor of MMP-1 levels in periodontal disease. J Periodontol, 2006, 77(12):2040–2050