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Analysis Of MMP-8, MMP-9 Indicators And Their TIMP-1 Inhibitor In Periodontitis Among Patients With Diabetes Mellitus Type II.

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ABSTRACT

This article presents the results of the study of MMP-8, MMP-9 and their TIMP-1 tissue inhibitor among the patients with periodontitis and carbohydrate metabolic disorder (diabetes mellitus type II) and without the latter. 65 patients have been assessed. The reference group has been comprised of 20 volunteers. Blood serum MMP-8 level decrease in both groups of patients in comparison to the reference group and its concentration increase in saliva and blood recovered from the microvascular stream of gum have been verifiably established. Systemic and local increase of MMP-9 concentration in both groups has been observed. When assessing TIMP-1, both local and systemic increase of its concentration in both groups has been established.

Keywords: periodontitis, diabetes mellitus, MMP-8, MMP-9, TIMP-1.

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INTRODUCTION

Periodontal diseases are found in 90-93% of cases of diabetes mellitus, which is dominant among endocrine disorders and tends to rise progressively [3].There is direct correlation between the age and severity of diabetes mellitus and periodontal diseases [6]. Immune perturbations have a significant share in periodontitis progression in the case of diabetes mellitus type II by affecting the dental support complex in one way or another [1,4].

The purpose of our study is to assess the levels of MMP-8, MMP-9 and their tissue inhibitor of TIMP-1 among the patients with periodontitis and carbohydrate metabolic disorder (diabetes mellitus type II) and without the latter.

MATERIALS AND METHODS

65 patients have been assessed, ages of 30 to 60, divided into two groups as follows:

Group I–patients with diabetes mellitus type II and periodontitis of various severity, 30 persons;
Group II–patients with periodontitis of various severity, no comorbidity diagnosed, 35 persons.

The reference group has been comprised of apparently healthy volunteers of the same age (20 persons).

Blood serum, saliva, blood from the microcirculatory stream (MCS) of gum have been used as the materials for study. The levels of MMP-8, MMP-9 and TIMP-1 have been assessed by immune enzyme technique using special reagents by R&D Diagnostics Inc (USA) through sandwich enzyme-linked immunosorbent assay. The results have been measured with the aid of Multi-scan (Finland) enzyme-immunoassay analyzer. The quantity of MMP-8, MMP-9 and TIMP-1 has been registered with a computer-generated calibration curve. The quantity is measured in ng/ml. For statistical deduction the data has been manipulated by SPSSv16 software.

RESULTS OF STUDY

Table 1: Periodontitis determined levels of the MMP-8, MMP-9and TIMP-1 indicators among patients with diabetes mellitus type II and without such

Indicators of M±m ng/ml		Reference group n=20	Group I. Periodontic patients with diabetes mellitus type IIn=30	Group II. Periodontic patients n=35
MMP-8	Serum	15.18±2.2	5.23±0.2***	10.1±0.6*** p1<0.001
	Gingival MCS	56.4±0.5	95.1±0.4***	114.1±1.2***
	Saliva	72.85±11.5	231.6±0.6***	485.1±5*** p1<0.001
MMP-9	Serum	322.03±51.5	422.2±14.6***	364.5±11.7* p1<0.01
	Gingival MCS	4.6±0.06	54.1±0.01***	48.1±0.14***
	Saliva	1.56±0.6	38.1±0.05***	32.5±0.13***
TIMP-1	Serum	88.8±0.5	210.8±3.5***	206.4±1.7***
	Gingival MCS	15.4±0.4	23.2±0.02***	18.7±0.06
	Saliva	3.04±0.01	15.1±0.07***	19.4±0.09***

Note: the statistical significance of differences with the reference group: p<0.05 -*, p<0.01-**, p<0.001-***; p1- the statistical significance of differences between the groups of patients.

Table 1 shows the levels of the assessed indicators of MMP-8, MMP-9 and TIMP-1 in blood serum, saliva and blood from gingival MCS among periodontic patients with comorbid diabetes mellitus type II (Group I) and among periodontic patients with no somatic disorders (Group II).

The analysis of data acquired from the study has established that the level of blood serum MMP-8 is verifiably reduced in both groups of patients compared to the reference group ($p<0.001$), however, Group I shows its more evident decrease ($p1<0.001$). As for the local changes of the MMP-8 indicator, aucontraire, we see increase of its concentrations both in saliva and in blood from gingival MCS, which is particularly evident among the periodontics patients (Group II, $p1<0.001$).

The content of MMP-9 in blood serum exceeds the reference values in both groups, the increase of this indicator being statistically significant among the periodontics patients affected by diabetes mellitus type II. The analysis of the values acquired by examination of saliva and blood from gingival MCS has established their verifiable increase in both groups under study compared to the reference group. The significant difference in MMP-9 local level between the groups of patients has not been established.

The assessment of TIMP-1 has allowed to establish both local and systemic increase of its concentrations in both groups in comparison with the reference group ($p<0.001$). The indicators of TIMP-1 in the assessed groups have shown no significant difference.

The ratio of MMP-8 to TIMP-1 in blood from gingival MCS exceeds the reference values, which is particularly evident among the patients of Group II (6.1±0.09 vs. 4.1±0.08 (Group I), $p<0.001$ and 3.66±0.15 in the reference group). The ratio of MMP-8 to TIMP-1 in saliva is, aucontraire, reduced among the patients of Group I (15.3±0.3 vs. 24±0.5 in the reference group). Patients of Group II have shown no significant difference compared to the reference group.

Decrease of the ratio of MMP-9 to TIMP-1 in blood serum of both groups under study has been established в (2±0.2 (Group I)and 1.76±0.2 (Group II) vs. 3.62±0.13 in the reference group). As for the changes of this indicator in blood from gingival MCS, its slight decrease among the periodontic patients affected by metabolic disorders has been observed. Group II has shown no difference in the ratio. However, the saliva examination has given increase of the indicator in both groups (2.5±0.09 (Group I), 1.7±0.4 (Group II) vs. 0.5±0.05 in the reference group).

Evaluation of results

MMPs play important part in many physiological processes: delivery of immune response, inflammation, physiological and post-wound tissue remodeling, coagulation. But in the presence of pathology, MMPs can cause tissue damage by breaking extracellular matrix. [2] MMPs are regulated at the stage of transcription of their structural genes. Their activity depends on whether they are depressed by endogenous inhibitors, α2-macroglobulin, TIMPs [4].

With reference to studies by D. Pirhamat al (2008), the main role among all MMPs in the development of periodontitis is owned byMMP-8 andMMP-9 [12].

MMP-8 is a marker of neutrophils and their precursors, synthesized by epithelial cells of the sulcus, monocytes, macrophages, fibroblasts of the gingival and parodontal ligaments, differentiated granulocytes in bone marrow. Some authors point to a significant role ofMMP-8 in tissue destruction in periodontitis [9].

As our study has found out, theMMP-8 level in blood serum is decreased in both groups of patients in comparison with the reference group ($p<0.001$), however, Group I shows its more evident decrease ($p1<0.001$). The analysis of local changes of the MMP-8 indicator has revealed the increase of its concentration both in saliva and in blood recovered from MCS, which is especially evident in periodontic patients (Group II,$p1<0.001$), which confirms the similar studies performed by B. C. Raiat al (2004) that established the increase of MMP-8 concentration in gingival tissue extract and saliva in periodontic persons, [13] but does not disprove the results of other scientists in respect to decrease of MMP-8 concentration in gingival fluid in periodontic patients [7].

MMP-9 (gellatinase-B) is identified in neutrophils, macrophages, chondrocytes, fibroblasts and in B-lymphocytes. It is believed that in periodontitis the main source of MMP-9 are neutrophils. [10]The analysis of our data has established thatMMP-9 content in blood serum in Groups I and II exceeded the reference values, in so doing, its level has been higher in patients with carbohydrate metabolic disorder. The assessment of indicators obtained by examination of saliva and blood from gingival MCS, statistically significant increase ofMMP-9 in both groups compared to the reference group has also been found. The result obtained is consistent with studies by O.A. Zorin et al (2011)that established the increase ofMMP-9 in gingival fluid in periodontic patients, wherein its concentration grew as a severity of periodontitis increased. [5] Many researchers deemMMP-9 as the basic gellatinase in inflammatory periodontal tissue diseases, the level of which can be a marker to help assess the severity of periodontitis [8,11].

Regulation of MMPs expression depends on the rate of their synthesis and the level of TIMP endogenous tissue inhibitors. The reason for increase of MMPs' activity is considered to be the imbalance between MMPs and TIMPs towards increase of MMP concentration [2]. TIMP is a family of proteins which inhibit the activity of MMPs in the ratio of 1:1, and which are synthesized by leukocytes and connective tissue cells due to formation of stable bonds with matrix metalloproteinases [14]. Assessment ofTIMP-1 has enabled us to determine a verifiable local and systemic increase of its concentrations in both groups, in comparison with the reference group ($p<0.001$). The values ofTIMP-1 in groups under study did not significantly differ between each other.

MMP-8 to TIMP-1 ratio in blood obtained from the gingival MCS has exceeded the reference value, which is particularly evident in patients suffering from periodontitis (6.1±0.09, Group II, and 4.1±0.08, Group I, vs. 3.66±0.15 in the reference group, $p<0.001$). MMP-8 to TIMP-1 ratio in saliva is, au contraire, reduced in patients from the Group I (15.3±0.3 vs. 24±0.5 in the reference group). Patients from the Group II have shown no significant difference compared to the reference group.

Decrease in MMP-9 to TIMP-1 ratio in blood serum has been detected in both groups under study (2±0.2, Group I, and 1.76±0.2, Group II, vs. 3.62±0.13 in the reference group). As for the changes of this indicator in blood obtained from gingival MCS, we have found its slight decrease in the group of patients suffering from periodontitis associated with metabolic disorders. No difference in the ratio has been detected in Group II. However, decrease in the salivary indicator has been observed in both groups (2.5±0.09, Group I, and 1.7±0.4, Group II, vs. 0.5±0.05 in the reference group).

Thus, the increased local level of MMP-8 in periodontics persons may serve as an additional immunologic marker and have diagnostic significance in localized periodontic inflammatory processes. The verifiable systemic increase in MMP-9 indicator, especially in the group of patients suffering from diabetes mellitus type II, reflects the intensity of inflammatory response which may be used as a marker of periodontitis progression risk in persons with carbohydrate metabolic disorder.

We suppose that the level of MMP-8, MMP-9 and their TIMP-1 tissue inhibitor before and after the performed therapy will be important to evaluate the peculiarities of clinical course and severity of periodontitis in persons suffering from diabetes mellitus type II.

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