

ORIGINAL ARTICLE

EVALUATION OF BONE RESORPTIVE POTENTIAL IN THE TREATMENT OF GENERALIZED PERIODONTITIS

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ABSTRACT

The aim: Is to study dynamics of resorptive potential of bone tissue by indicators of protease and antiprotease systems in the process of treatment generalized periodontitis in patients with age-related osteoporosis and without osteoporotic changes in the skeleton.

Materials and methods: In 102 patients, before the start of treatment of generalized periodontitis and in 2, 4 and 12 weeks, the concentration of pro-resorbing matrix metalloproteinases: stromelysin (MMP-3), collagenase (MMP-8) and gelatinase (MMP-9) were determined in blood plasma and mixed oral fluid. The antiresorbtive potential of bone tissue was evaluated by the concentration of a tissue inhibitor of metalloproteinases (TIMP-1) in plasma. The general antiprotease activity was determined by the activity of α1-antitrypsin (α1-AT) and α2-macroglobulin (α2-MG).

Results: The most significant changes are recorded for the content of MMP-9 in blood and oral fluid, regardless of the presence of systemic disorders of bone metabolism ($P < 0.05$). Concentration of MMP-8 is significantly increased in blood plasma and oral fluid in accordance with the severity of the disease and in the course of treatment ($P < 0.05$). The observed increase in the ratio of MMP-8 to TIMP-1 and MMP-9 to TIMP-1 in patients with generalized periodontitis, complicated by systemic osteoporosis ($P < 0.05$), indicates an imbalance of the protease-antiprotease system.

Conclusions: The resorptive potential of bone tissue in patients with generalized periodontitis allows us to correctly choose treatment tactics and to prevent the development of complications.

KEY WORDS: periodontal diseases, osteopenia, matrix metalloproteinases

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INTRODUCTION

Generalized periodontitis (GP), associated with a significant prevalence among the population, is a multifactorial disease that develops independently or as a result of systemic diseases [1]. The rate of progression of GP is dependent on bone systemic condition, particularly, degree of mineralization and metabolism [2]. The bone resorptive potential is determined mainly by the activity of osteoclasts and osteoblasts, but significant contribution to the destruction of bone matrix is made by periodontal fibroblasts, monocytes, macrophages and others. In the focus of inflammation, immune cells accumulate and secrete cytokines and proteases, thereby causing the release and activation of matrix metalloproteinases (MMPs), that are the key to the resorption of bone matrix [3].

Activity MMPs in physiological conditions is regulated with specific tissue inhibitors (TIMPs). Their concentration characterizes the antiprotease activity of biological fluids. Thus, TIMP-1 and TIMP-2 can inhibit the activity of all known MMPs. TIMP-3 and TIMP-4 are responsible for cell differentiation in extracellular matrix. TIMP-1 is a soluble inducible inhibitor with a wide spectrum of action, preferably produced by cells of the connective tissue matrix; and it is contained in bone tissue in a large quantity. However, the activated proteinases can be free to go out into the bloodstream and other biological fluids, affecting on the homeostasis of the organism. At this level of the regulation of protease-antiprotease balance, hemostatic antiprotease system is connected. Among plasma protease inhibitors, α2- macroglobulin is the most important; it reduces the activity of soluble MMPs [4].

However, it is still not clear what metalloproteinase is the most of all associated with bone resorption in inflammatory destructive processes in periodontal tissues, what value of TIMP-1 and protease inhibitors can be indicators of antiprotease system, in the development of inflammatory and destructive process in periodontal tissues. In our opinion, this kind of research has to have practical output in creating therapeutic and diagnostic methods in the complex treatment of disease; and it prompted us to carry out presented work.

THE AIM

The aim of the study is examination of the dynamics of bone resorptive capacity according the indices of protease and antiprotease systems during the treatment of GP of the II-III degree of severity in patients with age-related osteoporosis and without osteoporotic changes in bone system.

Table I. Indicators of protease and antiprotease systems in biological fluids in research groups before treatment (M ± m)

	Indicator		Control	Comparison group (CG)		Basic group (BG)		
				I	II	I	II	III
protease	MMP-3 µg / l	blood plasma	24.65 ± 4.78	29.24 ± 7.43	33.38 ± 9.64 ***	43.56 ± 7.49 ***	42.80 ± 6.61 ***	47.62 ± 7.55 ***
		saliva	3.65 ± 0.57	3.85 ± 0.74	3.93 ± 0.86	4.44 ± 1.18	8.40 ± 1.38 ***	8.45 ± 1.88 ***
	MMP-8 µg / l	blood plasma	3.29 ± 0.59	3.68 ± 0.99	4.24 ± 0.82 ***	2.58 ± 0.76 **	4.31 ± 0.57 ***	5.20 ± 0,99 ***
		saliva	0.63 ± 0.14	1.17 ± 0.25 ***	1.28 ± 0.21 ***	0.75 ± 0.27	1.01 ± 0.18 ***	1.29 ± 0.26 ***
	MMP-9 µg / l	blood plasma	38.05 ± 6.13	50.9 ± 5.79 ***	52.79 ± 5.83 ***	41.49 ± 6.92	57.86 ± 7.05 ***	60.97 ± 8.09 ***
		saliva	13.34 ± 2.26	21.74 ± 4.35 ***	24.46 ± 5.24 ***	15.58 ± 4.93	26.52 ± 4.94 ***	28.80 ± 5,90 ***
	TTA, µM / min / ml	plasma blood	0.51 ± 0.05	0.54 ± 0.07	0.65 ± 0.05 ***	0.50 ± 0.04	0.69 ± 0.08 ***	0.79 ± 0.12 ***
antiproteases	TIMP-1 µg / l	plasma blood	105.60 ± 7.85	58.22 ± 6.51 ***	131.58 ± 11.53***	112.49 ± 13.71	89.70 ± 14.55 ***	81.95 ± 9.34 ***
		saliva	0.79 ± 0.14	1.54 ± 0.30 ***	1.60 ± 0.29 ***	1.05 ± 0.21 ***	1.23 ± 0.18 ***	0.70 ± 0,18
	α1-AT, µM / min / ml	blood plasma	35.89 ± 4.11	58.22 ± 6.51 ***	46.98 ± 3.58 ***	46.73 ± 4.02 ***	50.23 ± 4.67 ***	49.64 ± 6.19 ***
		saliva	1.73 ± 0.32	2.35 ± 0.45 ***	2.57 ± 0.29 ***	1.71 ± 0.50	1.02 ± 0.15 ***	1.21 ± 0.34 ***
	α2-MG, µM / min / ml	blood plasma	6.71 ± 0.70	9.16 ± 0.88 ***	9.98 ± 1.07 ***	7.03 ± 1.30	6.82 ± 1.31	5.09 ± 0.52 ***
		saliva	0.77 ± 0.09	0.88 ± 0.12 ***	0.52 ± 0.08 ***	0.69 ± 0.13 *	0.69 ± 0.14 *	0.55 ± 0.06 ***

Notes. 1. n = 30 for all research groups. 2. * - P < 0.05, ** - P < 0.01, *** - p < 0.001 compared with control group.

MATERIALS AND METHODS

The comprehensive study and treatment of 120 patients with GP, equally men and women, with average age 59.2 **± 5.4 years**, was conducted. The diagnosis was determined by clinical and radiological data [1].

The state of bone tissue was determined by the results of the study of bone mineral density (BMD) by the method of two-energy x-ray absorptiometry using the apparatus Lunar Prodigy. The diagnosis of osteoporotic changes in the skeleton was held by the WHO recommendation by the T-criterion.

Among selected patients with GP, 60 persons had normal bone mineral density, and the remaining 60 ones had age osteoporotic changes in bone tissue. Additionally, the indicators were studied for 30 persons with age-related osteoporosis, whom are not diagnosed GP, and for 30 persons with intact periodontal tissues and without osteoporotic changes.

Criteria for exclusion from the study were receiving drugs with mineral components, the presence of injuries and inflammatory diseases of skeleton and joints. Group of research (basic and comparison) were formed identical in age and gender.

The I basic group (BG) consisted of 30 persons with concomitant age osteoporosis, whom are not diagnosed with inflammatory and destructive changes in periodontal tissues. The II BG included 30 patients with GP of the II degree of severity, chronicity. The III BG included 30 patients with GP of the III degree of severity, chronicity. The criteria for inclusion in the BG was presence of concomitant age osteoporosis provided there is no history of somatic pathology affecting the mineral density of the skeleton.

In turn, the I comparison group (CG) consisted of 30 patients with GP of the II degree of severity, chronicity. The II CG consisted of 30 patients with III degree of severity of disease. All patients, included CG, did not have osteoporotic changes in skeleton bone. The control group included 30 relatively healthy persons without both inflammatory and destructive changes in periodontal tissues and osteoporotic changes in the skeleton.

Patients with GP received combined treatment according to standard protocol [1]. The material for biochemical studies was plasma (serum) of blood and saliva before treatment of GP, and in 2, 4 and 12 weeks after its beginning.

For the comprehensive assessment of the activity of proteolytic processes in the bone tissue, it was studied the concentration of MMPs of three main subclasses, participating in the resorption of bone matrix, namely stromelizyn (MMP-3), collagenase (MMP-8) and gelatinase (MMP-9) in blood plasma using R&D Diagnostics Inc. kit (USA). Also, trypsin-like total activity (TTA) of plasma, which is based on the these enzymes' decomposition colorless synthetic substrate N-benzoyl-DL-arginine-4-paranitroanilinohydrochloride with the formation of p-nitroaniline with yellow colour, were determined. The degree of color were recorded at a wavelength of 410 nm using a photometer 2000 Human (Human, Germany). Quantitative evaluation TTA in plasma was performed using the calibration graph, where the various concentrations of aniline, the final cleavage product of trypsin-like enzymes, were used as standard solutions. Specific trypsin-like activity in blood plasma was determined in µmol / minute of fermented substrate in terms of 1 liter of serum (µM / minute / l).

The anti-resorptive potential of bone tissue was evaluated by the concentration of MMPs tissue inhibitor TIMP-1 in blood plasma using the R&D Diagnostics Inc. kit (USA).

Based on the fact that the concentration of MMPs does not fully reflect the activity of degradation of the intercellular matrix, which is mainly due to the resistance to proteinase specific inhibitors, we additionally determined the total anti-proteolytic activity of biological fluids according

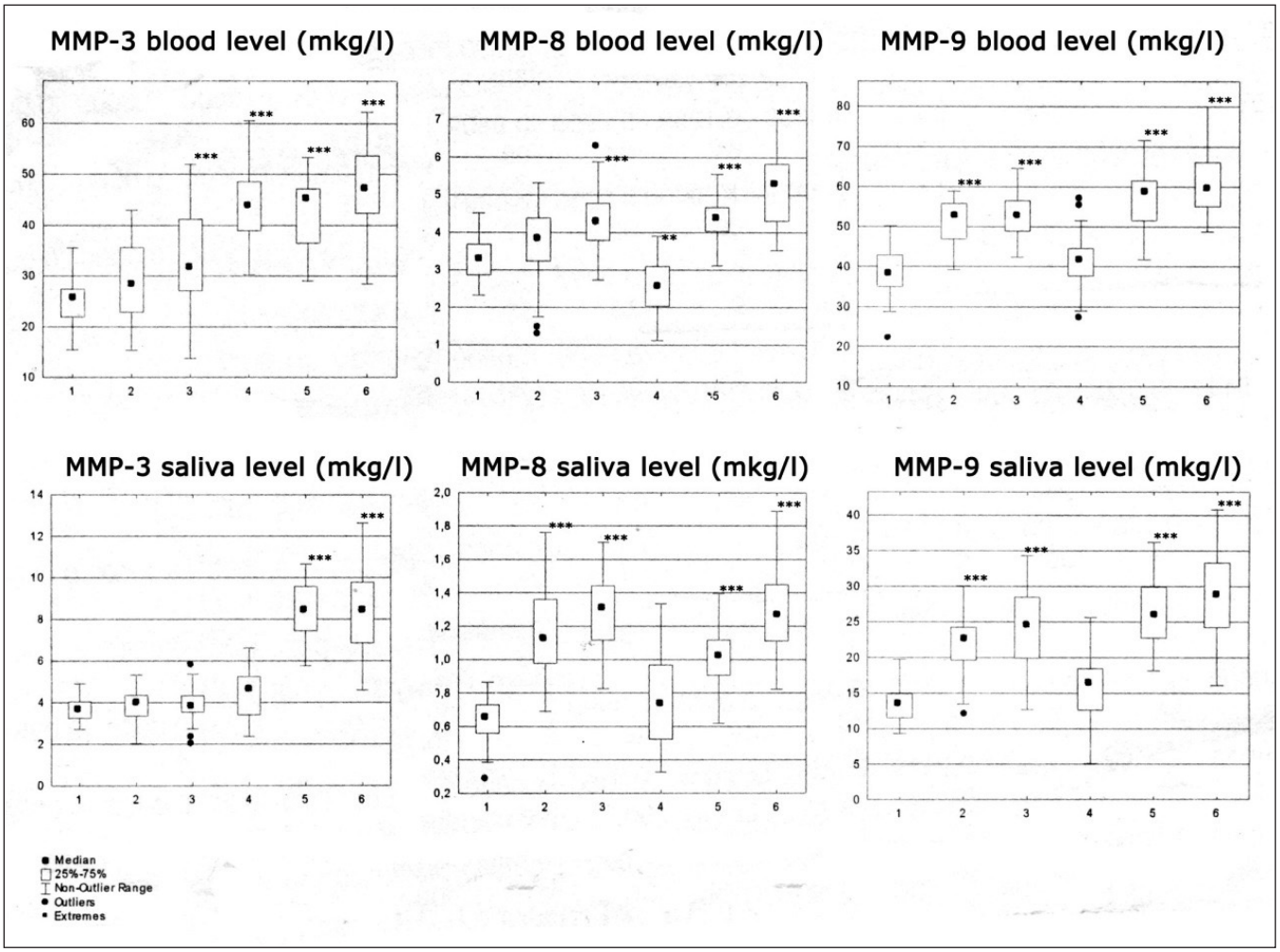

Fig.1. Concentration of MMPs in biological fluids (1 - control group; 2 – the I CG; 3 – the II CG; 4 – the I BG, 5 – the II BG, 6 – the III BG) before treatment (M ± m)

the activity of α1-antitrypsin (α1-AT) and α2-macroglobulin (α2-MG) by the Nartikova & Pashina's method [5]. It should also be noted that prior to the studies, samples were normalized by the total protein concentration determined by the method [6].

Statistical analysis was performed using the package Statistica 8.0 (Statsoft Inc., USA).

RESULTS

Systematic content of MMPs in blood plasma and saliva varied depending, firstly, on the stage of GP and, secondly, on the presence of concomitant osteoporosis, increasing according to the severity of the pathological process in periodontal tissues and its complications under systemic disorders of bone metabolism (Table I).

In patients of the BG, nonsignificant increase in the level of MMP-3 in plasma was observed, while the local concentration of the MMPs in saliva varied significantly ($P < 0.001$). At the same time, in patients of BG, significant differences of MMP-3 concentration in plasma and saliva, compared to each other, were not observed ($P > 0.05$).

In patients with GP, the MMP-8 level significantly increased in plasma ($P < 0.05$), most notably against background of systemic osteoporosis and the third degree of the destructive and inflammatory process in periodontal tissues ($P < 0.001$). Regarding the MMP-8 content in saliva, this index showed the same, but more pronounced, dynamics, that in plasma. Its differences with values of the control group proved to be reliable in patients with GP in all observation groups (Figure 1).

According to the results, the MMP-9 concentration also increased in the blood and saliva of patients with GP, but the differences of the indices for the saliva in GP of the II degree of severity were less pronounced ($P < 0.001$).

Thus, the analysis of the study of MMPs proved representativeness of content MMP-8 and MMP-9 in both blood plasma and saliva, regarding the description of the activity of the destructive process in the periodontium. However, as expected, the most significant changes were found in MMP-8 concentrations in biological fluids studied in the patients of the basic and comparison groups in GP of the III degree of severity ($P < 0.001$). Changes in the index of saliva, unlike MMP-9, were registered in all variants of GP. The level of MMP-3 is more related to osteoporotic bone lesions and was not significant in assessing the severity of GP.

Table II. Indicators of protease and antiprotease systems in plasma in research groups during treatment (M ± m)

Group			Indicator						
			MMP-3 µg / l	MMP-8 µg / l	MMP-9 µg / l	TTA, µM/min/ml	TIMP-1 µg / l	α1-AT, µM/min/ml	α2-MG, µM / min / ml
Control			24.65 ± 4.78	3.29 ± 0.59	38.05 ± 6.13	0.51 ± 0.05	105.60 ± 7.85	35.89 ± 4.11	6.71 ± 0.70
Comparison (CG)	I	before treatment	29.24 ± 7.43	3.68 ±0.99	50.90 ± 5.79	0.54 ± 0.07	135.91 ± 10.08	58.22 ± 6.51	9.16 ± 0.88
		2 weeks	27.82 ± 7.07	3.55 ± .96	46.83 ± 5.33	0.53 ± 0.07	139.57 ± 10.35	50.51 ± 5.65 ***	7.22 ± 0.69
		4 weeks	27.23 ± 6.92	3.22 ±0.87	45.51 ± 5.18	0.52 ± 0.07	127.05 ± 9.42	37.64 ± 4.21 ***	8.06 ± 0.77 ***
		12 weeks	28.02 ± 7.12	3.79 ±1.02	46.10 ± 5.24	0.55 ± 0.07	118.25 ± 8.77 ***	38.89 ± 4.35 ***	7.34 ± 0.70 **
	II	before treatment	33.38 ± 9.64	4.24 ±0.82	52.79 ± 5.83	0.65 ± 0.05	131.58 ± 11.53	46.98 ± 3.58	9.98 ± 1.07 ***
		2 weeks	28.43 ± 8.21	3.90 ±0.76	50.06 ± 5.53	0.58 ± 0.05 *	130.59 ± 11.44	37.17 ± 2.83 ***	9.76 ± 1.04
		4 weeks	26.29 ± 7.59 *	3.42 ± 0.66 **	49.73 ± 5.50	0.59 ± 0.05	128.26 ± 11.24	38.68 ± 2.94 ***	8.89 ± 0.95 **
		12 weeks	27.43 ± 7.92	3.63 ± 0.70	43.57 ± 4.82 ***	0.55 ± 0.05 ***	115.88 ± 10.15 ***	38.59 ± 2.94 ***	8.02 ± 0.86 ***
Basic (BG)	I	before treatment	43.56 ± 7.49	2.58 ± 0.76	41.49 ± 6.92	0.50 ± 0.04	112.49 ± 13.71	46.73 ± 4.02	7.03 ± 1.30
	II	before treatment	42.80 ± 6.61	4.31 ± 0.57	57.86 ± 7.05	0.69 ± 0.08	89.7 ± 14.55	50.23 ± 4.67	6.82 ± 1.31
		2 weeks	40.15 ± 6.20	4.03 ± 0.53	52.28 ± 6.37 *	0.61 ± 0.07 **	92.26 ± 14.97	45.64 ± 4.24 *	6.97 ± 1.34
		4 weeks	41.32 ± 6.38	3.75 ± 0.50	49.39 ± 6.02 ***	0.63 ± 0.07	107.34 ± 17.41 ***	45.71 ± 4.25 *	7.12 ± 1.37
		12 weeks	38.77 ± 5.99	3.49 ± 0.46 **	47.46 ± 5.78 ***	0.60 ± 0.07 ***	103.43 ± 16.78 **	39.1 ± 3.64 ***	7.40 ± 1.42
	III	before treatment	47.62 ± 7.55	5.20 ± 0.99	60.97 ± 8.09	0.79 ± 0.12	81.95 ± 9.34	49.64 ± 6.19	5.09 ± 0.52
		2 weeks	45.73 ± 7.25	5.11 ± 0.97	55.98 ± 7.43	0.77 ± 0.11	96.5 ± 11.00 ***	48.70 ± 6.07	5.29 ± 0.54
		4 weeks	43.60 ± 6.91	4.34 ± 0.82 **	47.68 ± 6.33 ***	0.73 ± 0.11	108.21 ± 12.34 ***	49.68 ± 6.19	5.47 ± 0.56
		12 weeks	41.47 ± 6.57	3.62 ± 0.69 ***	45.72 ± 6.07 ***	0.71 ± 0.11 **	114.53 ± 13.06 ***	48.59 ± 6.06	5.89 ± 0.60

Notes. 1. n = 30 for all research groups. 2. * - P < 0.05, ** - P < 0.01, *** - p < 0.001 compared with control group.

Data on changes in the concentration of MMPs in plasma and saliva during treatment are given in the Tables II and III. The level of MMP-3 remained unchanged in both biological fluids. The results obtained after treatment exceeded the control values almost twice (P < 0.01). The concentration of MMP-9, as a result of the treatment, decreased rather rapidly, especially in the blood plasma, more dynamically in the groups with GP of the III degree of severity (P < 0.05 compared to the concentration before treatment), but did not reach plasma parameters in the control group at the end of treatment. On the contrary, MMP-8 concentration was normalized in both saliva and blood plasma over the treatment period, and the final results were almost consistent with the control values (P < 0.05).

The values of the protease system (TTA) of the blood plasma also changed according to the degree of destruction of periodontal bone under the influence of local inflammatory-destructive process and systemic osteoporotic changes. Its highest values were obtained for patients with GP of the II-III degrees of severity on the background of age-old systemic osteoporosis (P < 0.001 when compared with control values), the lowest – for the II degree without systemic changes in the skeleton (P > 0.05 when compared with control values). However, this index was not indicative in the dynamics of treatment, the final results significantly exceeded the control values (P < 0.01).

The levels of α1-AT and α2-MG in the blood plasma, as indicators of the state of the antiproteinase system, are slightly increased in the CG (P < 0.05), and in the main group these indicators tend to decrease and do not differ significantly from the control group (P > 0.05). However, with GP of the III degree of severity in both groups, the activity of α2-MG in saliva decreased, which indicates a decrease in the local antiproteinase protection of periodontal tissues. Changes in these parameters during treatment occured, but without significant dynamics in patients with osteoporosis (P > 0.05), which limits their diagnostic value.

In addition, the release of MMPs in the presence of destructive phenomena in the bone system of general or only local origin, as discussed above, was offset by an increase in the concentration of TIMP-1, both in blood plasma and saliva (Table I), which was most noticeable in patients with GP of the II-III severity without osteoporotic changes. Regarding studied dynamics of TIMP-1 indices in the biological fluids, it was proved to be quite indicative of the inhibition of pathological processes in periodontium due to the treatment.

The ratio of MMPs concentration to the TIMP-1 in blood plasma and saliva was changing in different directions (Table IV). The most revealing was increased MMPs to TIMP-1 in saliva in patients with GP, complicated system osteoporosis (P < 0.001). This demonstrated a local imbalance of protease-antiprotease system. These indices grown most notably in GP of the III degree, in both the basic and the comparison group. The ratio MMP-3 to TIMP-1 in saliva increased only in BG, i.e.in patients with GP, occurring against the background of systemic osteoporosis. In blood plasma the ratio of MMP-3 to TIMP-1 was increased only in the BG and the ratio of MMP-8 and MMP-9 to TIMP-1 increased more than twice in groups with GP on the background of osteoporosis (P < 0.001).

Table III. Indicators protease and antiprotease systems in saliva in research groups during treatment (M ± m)

Group			Indicator					
			MMP-3 µg / l	MMP-8 µg / l	MMP-9 µg / l	TIMP-1 µg / l	α1-AT, µM/min/ml	α2-MG, µM/min/ml
Control			3.65 ± 0.57	0.63 ± 0.14	13.34 ± 2.26	0.79 ± 0.14	1.73 ± 0.32	0.77 ± 0.09
Comparison (CG)	I	before treatment	3.85 ± 0.74	1.17 ± 0.25	21.74 ± 4.35	1.54 ± 0.30	2.35 ± 0.45	0.88 ± 0.12
		2 weeks	3.80 ± 0.73	0.78 ± 0.17 ***	20.33 ± 4.06	1.20 ± 0.23 ***	2.16 ± 0.42	0.86 ± 0.12
		4 weeks	3.90 ± 0.75	0.71 ± 0.16 ***	19.47 ± 3.89	1.13 ± 0.22 ***	2.21 ± 0.43	0.81 ± 0.11
		12 weeks	3.66 ± 0.70	0.68 ± 0.15 ***	17.12 ± 3.42	1.05 ± 0.21 ***	2.04 ± 0.39 *	0.79 ± 0.11
	II	before treatment	3.93 ± 0.86	1.28 ± 0.21	24.46 ± 5.24	1.60 ± 0.29	2.57 ± 0.29	0.52 ± 0.08
		2 weeks	5.36 ± 1.17 **	0.88 ± 0.14 ***	20.47 ± 4.39	1.22 ± 0.22 ***	2.50 ± 0.29	0.51 ± 0.08
		4 weeks	4.46 ± 0.97	0.79 ± 0.13 ***	19.61 ± 4.20 *	1.19 ± 0.21 ***	2.08 ± 0.24 ***	0.59 ± 0.09
		12 weeks	4.07 ± 0.89	0.68 ± 0.11 ***	19.09 ± 4.09 ***	1.03 ± 0.19 ***	1.98 ± 0.23 ***	0.64±0.10**
Basic (BG)	I	before treatment	4.44 ± 1.18	0.75 ± 0.27	15.58 ± 4.93	1.05 ± 0.21	1.71 ± 0.50	0.69 ± 0.13
	II	before treatment	8.4 ± 1.38	1.01 ± 0.18	26.52 ± 4.94	1.23 ± 0.18	1.02 ± 0.15	0.69 ± 0.13
		2 weeks	7.93 ± 1.30	0.95 ± 0.17	22.51 ± 4.20	1.12 ± 0.16	0.98 ± 0.15	0.71 ± 0.13
		4 weeks	8.49 ± 1.40	0.92 ± 0.16	18.91 ± 3.52 ***	1.03 ± 0.15 *	1.17 ± 0.17	0.71 ± 0.13
		12 weeks	8.40 ± 1.38	0.84 ± 0.15	17.65 ± 3.29 ***	1.02 ± 0.15 *	1.30 ± 0.19	0.74 ± 0.14
	III	before treatment	8.45 ± 1.88	1.29 ± 0.26	28.80 ± 5.90	0.70 ± 0.18	1.21 ± 0.34	0.55 ± 0.06
		2 weeks	8.52 ± 1.90	1.23 ± 0.25	28.13 ± 5.77	0.69 ± 0.17	1.25 ± 0.35	0.56 ± 0.06
		4 weeks	8.56 ± 1.91	1.08 ± 0.22 **	27.62 ± 5.66	0.83 ± 0.21	1.33 ± 0.38	0.58 ± 0.06
		12 weeks	7.14 ± 1.59 **	0.97 ± 0.19 ***	23.28 ± 4.77 ***	0.86 ± 0.21	1.40 ± 0.40	0.62 ± 0.07

Notes. 1. n = 30 for all research groups. 2. * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$ compared with control group.

According to the analysis of results for the basic and comparison groups for the purpose of evaluation of the total antiproteinase activity of blood plasma, in patients with GP of the II-III degrees of severity without osteoporotic changes, the antiproteinase activity was increased. On the contrary, under GP of the II-III degrees of severity with osteoporotic changes, the antiproteinase system was overloaded due to the significant activation of the proteinases, so the TTA in the BG tended to decrease.

DISCUSSION

The study of MMP-3, MMP-8, MMP-9 and TIMP-1 content in patients with GP of the II-III degree of severity, including cases, complicated systemic osteoporosis, revealed their multiple changes depending on the severity of the disease and its treatment. In our opinion, that indicates the key role of disorders of the balance of protease and antiprotease systems of bone matrix in the development of periodontal pathology.

MMP-8 plays leading role in the destruction of periodontal tissues: it is a major destructive factor in the progression of GP, unburdened by disorders of bone metabolism.

In patients with GP in both research groups, the concentration of MMP-9 was significantly higher than normal. We consider that this is due to the high content of the cellular elements of the periodontal enzyme in the inactive form; the MMP-9 is activated by both cytokines and other proteinases. On the one hand, this indicates the greatest contribution to the destruction of periodontal tissues MMP-9, on the other hand – this MMP is a nonspecific indicator of inflammatory periodontal diseases and their complications.

At least, MMP-3 characterizes the destruction of periodontal tissues, in particular its bone component. Its activity is the greatest under the GP on the background of osteoporosis. While, if the process becomes more active, other proteinases are activated, which have a greater contribution to the destruction of the periodontal tissues. This is due to the lower content of MMP-3 in bone tissue and the activation of this MMP under the action of hormones and internal regulatory factors. However, it should be noted that the determination of MMP-3 is valuable in the diagnosis of systemic osteoporosis and the assessment of systemic activity of its course, as confirmed by the results of studies of precursors [7].

Despite the level of MMP-3, which is within the normal range of control and comparison individuals, the concentration of this MMP in the I BG is higher than normal, and in the II and III BG s it is significantly higher than normal ($P < 0.01$). This is indirectly indicative of the background course of osteoporotic changes in bone tissue and coincides with data from previous studies [8-9], which show the high value of MMP-3 in the processes of non-inflammatory destruction of the bone matrix. In this regard, the level of MMP-3 remains high enough in the II and III BG, despite the comprehensive treatment and elimination of the inflammatory component of osteodestruction. Also, these results indicate a latent course of osteodestruction and the achievement of temporary remission in the treatment of GP on the background of osteoporosis.

Table IV. Ratio of matrix metalloproteinases concentration to the TIMP-1 in biological fluids (M ± m)

Biological fluids	Indicator	Control	Comparison group (CG)		Basic group (BG)		
			I	II	I	II	III
blood plasma	MMP-3 / TIMP-1	0.24 ± 0.05	0.22 ± 0.06	0.25 ± 0.07	0.39 ± 0.09	0.49 ± 0.11	0.59 ± 0.12
	MMP-8 / TIMP-1	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
	MMP-9 / TIMP-1	0.36 ± 0.07	0.38 ± 0.04	0.40 ± 0.06	0.37 ± 0.08	0.66 ± 0.10	0.75 ± 0.13
saliva	MMP-3 / TIMP-1	4.83 ± 1.30	2.60 ± 0.75	2.55 ± 0.76	4.43 ± 1.52	6.96 ± 1.60	12.77 ± 4.15 *
	MMP-8 / TIMP-1	0.82 ± 0.21	0.78 ± 0.23	0.83 ± 0.22	0.74 ± 0.30	0.84 ± 0.19	1.92 ± 0.31 *
	MMP-9 / TIMP-1	17.57 ± 4.61	14.67 ± 4.39	15.71 ± 4.40	15.49 ± 6.76	22.05 ± 5.67 *	43.2 ± 12.66 *

Notes. 1. n = 30 for all research groups. 2. * – $P < 0.001$ compared with control group.

Describing the role of expression and activation of MMPs by signs that have been determined, it should be emphasized that normally the tissues do not contain active MMPs and the concentration of their precursors is at a minimal level. Thus, both stages of regulation are necessary for the accumulation in the bone tissue of the active form of the matrix.

TIMP-1 inhibits the activity of MMP-3, MMP-8, and MMP-9 in a 1:1 ratio, directly interacting with the active center of MMPs [10]. In the comparison group, the ratio of MMP-8 to TIMP-1 in the blood plasma increased in comparison with the control in 2.3 and 2.6 times, depending on the severity of the disease ($P < 0.01$). This was confirmed by the fact that with the activation of inflammation there is an increase in the concentration of MMP-8, "responsible" for the destruction of tissues of the periodontal complex, and the compensatory increase in the level of TIMP-1 was insufficient, which led to a violation of the balance of proteolytic activity. In addition, TIMP-1 can be inactivated by proteolytic enzymes – trypsin, chemotrypsin and neutrophil elastase – thus significantly increasing the activity of MMPs.

According to our observations, the ratios of MMPs to TIMP-1 in the blood plasma of the subjects were uninformative. In the BG, the ratio of MMPs to TIMP-1 in saliva increased significantly, indicating a pronounced local imbalance of the protease and antiprotease system, which causes the severity of periodontitis in patients with age-related osteoporosis and confirms the role of MMPs in the pathogenesis of disorders of the extracellular matrix.

Indicators of the antiproteinase system state were not indicative of the course as inflammatory-destructive phenomena in periodontal tissues. Although, we observed some changes that caused the activation of pathological phenomena. While the study of qualitative and quantitative characteristics of MMPs and their inhibitors represents a promising area of basic research that will allow the development of new approaches to the diagnosis and treatment of inflammatory-destructive periodontal diseases.

CONCLUSIONS

In patients with GP, indicators of MMP-9 content in blood and saliva characterize the severity of inflammatory-destructive processes in bone tissue during generalization of the process. They are not indicative for evaluation of treatment effectiveness. The concentration of MMP-8 in patients with GP increases in plasma and saliva according to the severity of the disease; it changes also during the treatment. The increase in the ratio of MMPs to TIMP-1 in saliva in patients with GP, complicated by systemic osteoporosis, indicates a local imbalance of the protease-antiprotease system. The concentration of MMP-3 in saliva and blood plasma characterizes general changes in bone tissue, but is not representative of the activity of GP. In patients with GP, in saliva the level of α1-AT and α2-MG decreases, which indicates a decrease in local antiproteinase protection of periodontal tissue. In patients with GP, assessment of bone resorptive potential is to identify the leading pathogenetic mechanism of bone resorption at the current stage of disease and allows choosing the tactics of treatment: to prescribe the correct pharmacotherapy and to prevent the development of complications. Wherein, MMP-8 and TIMP-1 are the most significant markers of the inflammatory-destructive process in the periodontal tissues.

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