EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Content of Matrix Metalloproteinase-8 and Matrix Metalloproteinase-9 in Oral Fluid of Patients with Chronic Generalized Periodontitis

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The content of MMP-8 and MMP-9 in oral fluid of 145 patients (95 women and 50 men, 18-52 years) was measured by enzyme immunoassay. We examined 63 subjects with the intact periodontium and 82 patients with chronic generalized periodontitis (25 patients with mild disease, 45 patients with moderate disease, and 12 patients with severe disease). All patients were examined during the remission of chronic periodontitis and did not have clinical signs of associated somatic diseases. No significant differences were found in the content of MMP-8 and MMP-9 in oral fluid from periodontitis patients and subjects with the intact periodontium. The content of MMP-8 an MMP-9 in oral fluid of patients with severe periodontium was slightly higher than that in other patients and subjects with the intact periodontium. The depth of the periodontal pocket was shown to be the most reliable clinical sign for the state of periodontal tissues. A strong correlation was revealed between this criterion and MMP-9 content in oral fluid.

Key Words: *periodontitis; matrix metalloproteinase-8; matrix metalloproteinase-9; oral fluid*

Recent morphological, microbiological, and molecular studies showed that some cytokines, growth factors, hormones, and their receptors play a role in inflammatory and atrophic diseases of periodontal tissues [3,5,6,12]. The pathogenesis of these disorders involves microorganisms, general somatic diseases, and metabolic disturbances. The increased production of matrix metalloproteinases (MMP) is of considerable importance for the development and progression and chronic inflammatory processes in periodontal tissues.

MMP belong to a group of enzymes that are produced by various cells and microorganisms in the oral cavity. They are involved in the immune response of the body and cleave the majority of proteins in the extracellular matrix and basal membrane [1,2,4,8]. Under pathological conditions, MMP play a role in degradation and processing of growth factors, cytokines, and other substances that are involved in apoptosis and cell adhesion [4,7]. MMP and other extracellular proteinases can cause irreversible tissue damage [2,3].

Here we EIA analysis of the content of MMP-8 and MMP-9 in oral fluid of patients with chronic generalized periodontitis of different severity and subjects with intact periodontium.

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MATERIALS AND METHODS

We examined 145 patients (50 men (34.48%) and 95 women (65.52%), 18-52 years). The study involved 63 patients (43.45%) with the intact periodontium (21 men (14.48%) and 42 women (28.97%), 25 patients (17.24%) with mild periodontitis (7 men (4.83%) and 18 women (12.41%), 45 patients (31.03%) with moderate periodontitis (17 men (11.72%) and 28 women (19.31%), and 12 patients (8.28%) with severe periodontitis (5 men (3.45%) and 7 women (4.83%).

Clinical examination included an index evaluation of the periodontal state.

The approximal plaque index (API) for dental deposit was determined as described elsewhere [9]. Approximal tooth surfaces were treated with 2% methylene blue to reveal dental deposit. The results of this test were expressed as "yes" or "no". Dental deposit of approximal areas was evaluated (API) on the oral surfaces of the 1st and 3rd quadrants and vestibular surfaces of the 2nd and 4th quadrants. API was calculated as follows: API=(sum of the positive results for dental deposit)/(sum of the evaluations with approximal areas)×100%. API was evaluated as follows: <25%, optimal hygiene of the oral cavity; 25-39%, normal hygiene of the oral cavity; 40-69%, satisfactory hygiene of the oral cavity; and 70-100%, unsatisfactory hygiene of the oral cavity.

The papillary-marginal-alveolar (PMA) index [10,14] was determined as follows: 1, inflammation of the gingival papilla (P); 2, inflammation of the gingival margin (M); and 3, inflammation of the mucous membrane in the jaw alveolar (A) process. The PMA index was calculated as the sum of estimated values for the gingiva of each tooth. The PMA index was calculated as follows:

$$PMA = \frac{\Sigma parameters \times 100}{3 \times number\ of\ teeth}.$$

The periodontal index (PI) [13] illustrates the state of the gingiva and alveolar bone. This index is calculated for each tooth. The calculation was performed with a special scale. Gingival inflammation is characterized by a relatively low index. Resorption of the alveolar bone is characterized by a relatively high index. The indexes for each tooth were summarized. The sum was divided by the number of teeth in the oral cavity. PI of each patient reflects the relative state of periodontal disease irrespective of the type and cause of this disease.

The index of caries–filling–extracted tooth (CFE) [11] illustrates the degree of caries [11]. This method suggests the individual evaluation of caries by calculating the CFE index. The resistance (predisposition) to caries and further course of the carious process are evaluated prognostically from the severity of existing caries.

Orthopantomography was performed with all patients. Spot-film radiography was conducted when required.

The concentrations of MMP-8 and MMP-9 in unstimulated saliva were measured with Human MMP-8 (total) and Human MMP-9 (total) reagents for direct EIA (QuantikineT, R&D Systems) according to the manufacturer's instructions. Saliva samples were stored at -80ºC, defrosted at room temperature, centrifuged at 10,000 rpm for 2 min, and diluted 100-fold with a standard solution of diluent (composition of the kit). The measurements were performed on an ELX800 automatic universal microplate reader (Bio-Tek Instruments Inc.). The content of MMP-8 and MMP-9 in saliva was expressed in ng/ml.

TABLE 1. Distribution of Study Parameters in Patients

Parameter	Max D	K-S	Lilliefors	W	P
Age	0.118	$p<0.05$	$p<0.01$	0.915	0.000010
Sex	0.420	$p<0.01$	$p<0.01$	0.600	0.000010
PI	0.259	$p<0.01$	$p<0.01$	0.797	0.000010
API	0.151	$p>0.20$	$p<0.01$	0.925	0.004379
PMA	0.121	$p>0.20$	$p<0.01$	0.943	0.002178
Depth of periodontal sockets	0.314	$p<0.01$	$p<0.01$	0.807	0.000010
Number of affected teeth	0.305	$p<0.01$	$p<0.01$	0.816	0.000010
MMP-8	0.187	$p<0.01$	$p<0.01$	0.887	0.000010
MMP-9	0.175	$p<0.01$	$p<0.01$	0.813	0.000010

Note. Max D, maximum difference within the group; K-S, Kormogorov–Smirnov test; Lilliefors, significance level by Lilliefors test; *W*, Shapiro–Wilk test.

The null hypothesis for normal distribution of the results was rejected at $p=0.05$ (Kolmogorov–Smirnov test, Lilliefors test, and Shapiro–Wilk test; Table 1). Therefore, the results were analyzed by nonparametric tests.

RESULTS

No differences were found in the content of MMP-8 and MMP-9 in oral fluid of patients (Table 1). How-

TABLE 2. Significance of Study Parameters in Patients

	Parameter	M	Median	Lower quartile	Upper quartile	Dispersion	δ	M
Intact periodontium	Age (years)	28.38	26.00	22.00	34.00	52.15	7.22	0.86
	PI (score)	0.49	0.20	0.10	0.65	0.38	0.62	0.09
	API (%)	42.69	41.00	35.50	48.00	144.10	12.00	3.00
	PMA (%)	17.98	14.26	7.14	30.00	159.20	12.62	1.90
	CFE (score)	7.22	6.00	4.50	9.00	21.01	4.58	0.56
	MMP-8 (ng/ml)	249	181	92.6	346	46490	216	34.98
	MMP-9 (ng/ml)	541	489	201	747	220161	469	55.7
Mild periodontitis	Age (years)	36.0	37.00	34.00	40.00	90.87	9.53	1.91
	PI (score)	1.15	1.05	0.85	1.45	0.22	0.47	0.23
	API (%)	54.00	52.50	41.00	68.00	207.82	14.42	4.16
	PMA (%)	21.41	20.92	19.42	23.40	8.08	2.84	1.42
	CFE (score)	9.58	8.00	4.00	14.00	51.30	7.16	1.46
	Depth of periodontal sockets (mm)	2.27	2.50	2.00	2.50	0.25	0.50	0.10
	Number of affected teeth	3.52	3.00	0.00	6.00	15.76	3.97	0.79
	MMP-8 (ng/ml)	257	258	94.8	292	49072	222	61.4
	MMP-9 (ng/ml)	439	314	244	594	70646	266	53.2
Moderate periodontitis	Age (years)	40.82	42.00	32.00	50.00	89.42	9.46	1.41
	PI (score)	3.66	4.00	3.20	5.00	1.97	1.40	0.33
	API (%)	49.40	45.00	36.00	65.00	272.42	16.51	3.30
	PMA (%)	43.49	44.65	38.00	49.00	88.69	9.42	2.22
	CFE (score)	10.55	10.00	8.00	13.00	13.23	3.64	0.56
	Depth of periodontal sockets (mm)	4.09	4.00	3.75	4.50	0.20	0.45	0.07
	Number of affected teeth	10.42	10.00	8.00	12.00	11.49	3.39	0.52
	MMP-8 (ng/ml)	350	183	117	519	119725	346	63.2
	MMP-9 (ng/ml)	1018	834	503	1190	736636	858	128
Severe periodontitis	Age (years)	45.75	49.00	44.00	50.00	61.84	7.86	2.27
	PI (score)	5.68	5.80	5.00	6.50	1.17	1.08	0.38
	API (%)	58.67	62.00	39.00	75.00	332.33	18.23	10.53
	PMA (%)	36.51	37.50	30.16	44.00	104.15	10.21	3.61
	CFE (score)	13.83	12.00	11.50	16.50	17.61	4.20	1.21
	Depth of periodontal sockets (mm)	6.08	6.00	5.50	7.00	0.77	0.87	0.25
	Number of affected teeth	13.67	12.00	11.50	16.00	10.61	3.26	0.94
	MMP-8 (ng/ml)	191	170	78.4	326	15677	125	72.3
	MMP-9 (ng/ml)	950	855	376	1038	612157	782	226

ever, MMP-9 concentration in the oral fluid tended to increase in patients with severe periodontitis (as compared to the control group, intact periodontium).

A positive correlation was revealed between the depth of periodontal pockets and MMP-9 concentration in oral fluid (Table 1). The relationships between MMP-8 and MMP-9 were studied in patients of various groups.

No correlations were found between MMP-9 concentration in oral fluid, index of periodontal tissues, and results of X-ray examination in the control group. MMP-8 concentration in patients with the intact periodontium was 181.03 ng/ml (92.60; 345.85), but increased in subjects with mild or severe periodontitis. MMP-8 concentration in oral fluid was shown to decrease in patients with severe periodontitis. However, between-group differences in MMP-8 concentration were not statistically significant ($p > 0.05$; Table 2).

A strong correlation was found between MMP-9 concentration in oral fluid and PMA index in patients with mild periodontitis ($r = 0.73$, $p < 0.00005$, Spearman's test).

A positive correlation was revealed between the depth of the periodontal pocket and API in patients with mild periodontitis ($r = 0.572$, $p = 0.42$, Spearman's test).

The existence of a relationship between API and MPP-9 concentration indicates that the deterioration of hygienic conditions is accompanied by activation of destructive processes in the oral cavity of patients.

No correlation was found between PMA, PI, and MMP-9 concentration in oral fluid. Therefore, the index evaluation of periodontal tissues is not absolutely reliable. New criteria should be developed to characterize the degree of inflammatory and atrophic processes in periodontal tissues.

TABLE 3. Correlation Analysis of Data

	Parameter	Spearman R	p	Gamma	p	Kendall Tau	P
Mild periodontitis	MMP-9 & age	0.032	0.835	0.060	0.568	0.059	0.568
	MMP-9 & PI	0.347	0.158	0.288	0.122	0.267	0.122
	MMP-9 & API	0.020	0.923	0.010	0.943	0.010	0.943
	MMP-9 & PMA	0.298	0.229	0.231	0.196	0.223	0.196
	MMP-9 & depth of periodontal sockets	0.127	0.404	0.112	0.356	0.095	0.356
	MMP-9 & number of affected teeth	0.323*	0.033*	0.292*	0.011*	0.265*	0.011*
	API & MMP-8	0.597*	0.040*	0.484*	0.034*	0.469*	0.034*
	Dental defect & MMP-8	-0.782*	0.008*	-0.714*	0.011*	-0.630*	0.011*
	MMP-9 & MMP-8	0.895*	0.000*	0.714*	0.001*	0.714*	0.001*
	API & depth of periodontal sockets	0.577*	0.050*	0.474*	0.047*	0.439*	0.047*
Moderate periodontitis	MMP-9 & age	0.179	0.154	0.138	0.111	0.135	0.111
	MMP-9 & PI	0.340	0.168	0.266	0.141	0.254	0.141
	MMP-9 & API	0.117	0.492	0.075	0.526	0.073	0.526
	MMP-9 & PMA	0.351	0.154	0.252	0.153	0.247	0.153
	MMP-9 & depth of periodontal sockets	0.330*	0.006*	0.253*	0.005*	0.232*	0.005*
	MMP-9 & number of affected teeth	0.413*	0.0001*	0.332*	0.0001*	0.309*	0.0001*
Severe periodontitis	MMP-9 & age	-0.032	0.920	-0.055	0.822	-0.050	0.822
	MMP-9 & PI	0.360	0.381	0.364	0.268	0.320	0.268
	MMP-9 & API	1.000	0.117	1.000	0.117	1.000	0.117
	MMP-9 & PMA	-0.074	0.862	-0.040	0.894	-0.038	0.894
	MMP-9 & age	-0.252	0.430	-0.236	0.330	-0.215	0.330
	MMP-9 & number of affected teeth	0.022	0.947	0.018	0.940	0.017	0.940
	Depth of periodontal sockets & PI	0.760*	0.048*	0.733*	0.047*	0.629*	0.047*
	Depth of periodontal sockets & MMP-9	0.918*	0.000*	0.931*	0.002*	0.836*	0.002*

Note. *$p < 0.05$.

A strong correlation was revealed between the depth of periodontal pockets and MMP-9 concentration in patients with moderate periodontitis (r=0.33, p<0.0056, Spearman's test). The correlation coefficients by Gamma test and Kendall Tau test were r=0.25 and r=0.23, respectively (p=0.0047). A strong positive correlation was found between MMP-9 concentration in oral fluid and number of affected teeth (r=0.41, p<0.0005, Spearman's test). The correlation coefficients were also evaluated by Gamma test (r=0.33, p=0.0002) and Kendall Tau test (r=0.31, p=0.0002).

Some correlations were revealed between the age of patients, indexes of the periodontal state, and clinical signs of periodontal tissues in subjects with moderate periodontitis. The Spearman correlation coefficient for the age of patients and API index was r=0.55 at p=0.0041. The correlation coefficient for the age of patients and PMA index was r=0.57 at p=0.0144. A positive correlation was found between the age of patients and number of affected teeth (r=0.28, p=0.03).

The existence of these relationships indicates that age-related changes in the human body play a role in the pathogenesis of inflammatory and atrophic processes in the periodontium. Further studies are required to evaluate the pathogenetic mechanisms of inflammatory and atrophic diseases of the periodontium and to characterize the role of cytokines, growth factors, and molecular-and-biological markers in these disorders.

A strong correlation was found between MMP-9 concentration and depth of periodontal pockets in patients with severe periodontitis (r=0.92, p<0.00047, Spearman's test). The correlation coefficients by Gamma test and Kendall Tau test were r=0.93 and r=0.84, respectively (p=0.0017).

A correlation was revealed between PI and MMP-9 concentration in oral fluid (r=0.76, p<0.0475, Spearman's test). The correlation coefficients by Gamma test and Kendall Tau test were r=0.73 and r=0.63, respectively (p=0.047).

Our results indicate that oral cavity hygiene (API index) and relationship between this factor and MMP-8 concentration in oral fluid have an important role in the early stage of inflammatory and atrophic diseases of the periodontium. The progression of these disorders is associated with age-related changes in the human body. This factor has an important role in the pathogenesis of periodontitis. These data suggest that inflammatory and atrophic processes in the periodontium occur in stages. Each stage is characterized by the appearance of new pathogenetic factors or potentiation of their negative effects.

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