Matrix Metalloproteinases and Inflammatory Cytokines in Oral Fluid of Patients with Chronic Generalized Periodontitis and Various Construction Materials

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We present the results of comparative enzyme-linked immunosorbent assay of matrix metalloproteinases MMP-2, MMP-8, MMP-9; IL-1β and IL-6; tissue inhibitors of MMP (TIMP-1, TIMP-2), and TNF-α. The object of study was oral fluid of practically healthy subjects with intact periodontium and patients with chronic generalized periodontitis with various structural materials of dental restorations. It was found that MMP-9 in oral fluid could be considered as a marker of chronic generalized periodontitis irrespective of the presence or absence of metal dental restorations. The level of MMP-8 surpassed the normal only in oral fluid of patients with chronic generalized periodontitis with metal restorations. The correlations between the studied parameters in patients attest to relatively similar regulation of MMP, IL and TIMP secretion in oral fluid in practically healthy subjects with intact periodontium. In patients with inflammatory destructive periodontal diseases with and without metal dental restorations, the correlation coefficients indicate initiation of biochemical cascade accompanied by activation of cytokine production in response to etiological factors. Patients with periodontitis and metal orthodontic structures showed more pronounced reaction. Orthodontic structures made from chromium-cobalt, or chromium-nickel alloys in the oral cavity of these patients increased the levels of MMP-2, IL-1β and IL-6 in oral fluid.

Key Words: *periodontitis; matrix metalloproteinase-2; matrix metalloproteinase-8; matrix metalloproteinase-9; inflammatory cytokines; oral fluid; construction materials*

Modern non-invasive techniques based on molecular and biochemical methods for estimating the status of periodontium can be helpful in clinical practice for evaluation of the severity of the disease. This, in turn, is necessary for forming the groups of patients with increased risk of periodontitis and irreversible destructive changes [5,7].

Proteolytic degradation of type I collagen is considered as a key factor of uncontrolled destruction of the extracellular matrix (ECM) in periodontum [2,11]. Matrix metalloproteinases (MMP), the members of multigene family comprising more than 20 zinc-dependent endopeptidases, possess the highest collagenolytic activity. Apart from most ECM components, other proteases, chemotactic molecules, latent forms of growth factors, soluble and membrane-associated proteins binding growth factors, and cytokines may be cleaved by MMP [3,9]. MMP activity in the intercellular space is specifically suppressed by tissue inhibitors (TIMPs), structurally related proteins, three of which (TIMP-1, TIMP-2 and TIMP-4)

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TABLE 1. Groups of Patients according to Nosological Forms and Presence or Absence of Metal Restorations in the Oral Cavity

Groups	Number of observations		Patients with metal restorations		Patients without metal restorations	
	N	%	*N*	%	N	%
Intact periodontium	50	47.62	19	18.10	31	29.52
Mild periodontitis	31	29.52	23	21.90	8	7.62
Moderate periodontitis	12	11.43	7	6.67	5	4.76
Severe periodontitis	12	11.43	9	8.57	3	2.86
Total	105	100.0	58	55.24	47	44.76

are secreted in a soluble form and one (TIMP-3) is ECM-bound.

Among all known MMP, neutrophil collagenase, or MMP-8, has the highest proteolytic activity towards type I collagen. According to experimental and clinical studies, its activity is closely associated with pathological changes in the periodontium [6]. The main substrate of gelatinases MMP-2 and MMP-9 is type IV collagen. These enzymes also contribute significantly to collagen lysis [4]. MMP have a wide substrate specificity including inflammatory cytokines. Therefore they are involved in the processes of periodontal destruction as well as in modulation of inflammatory responses.

According to researchers, oral fluid assay is rather informative and non-invasive test for the status and activity of proteolytic processes in the periodontium [5,13]. It should be noted that not only pathological processes in the periodontium, but also various concomitant diseases such as cardiovascular disease [14] and diabetes mellitus [12], as well as external factors, *e.g.* smoking [10] or structural materials used for dental restoration can induce biochemical changes in the oral cavity [8].

Here we measured by ELISA and compared the levels of MMP-2, MMP-8, MMP-9, IL-1β and IL-6, tissue inhibitors of MMP (TIMP-1, TIMP-2), and TNF-α in oral fluid of patients suffering from chronic generalized periodontitis with dental restorations made of various materials.

MATERIALS AND METHODS

A total of 105 individuals (18-52 years) were included in the study: 40 (38.10%) males and 65 (61.90%) females. The group of patients with fixed metal dental restorations included 58 individuals (55.24%), 24 males (22.86%) and 34 females (32.38%). Twenty-three of these patients developed mild periodontitis; 7, moderate periodontitis; 9, severe periodontitis. Eighteen subjects had healthy periodontium. The group of patients without metal dental restorations in oral cavity comprised 47 subjects (44.76%), 16 (15.24%) males, and 31 (29.52%) females. Eight of these patients developed mild periodontitis, 5 had moderate periodontitis, and 3 had severe periodontitis. Thirty one-person had healthy periodontium. Cobalt-chromium and chromium-nickel alloys were the main structural materials of metal and metal-ceramic restorations.

Clinical examination included an index evaluation of the periodontal state as described elsewhere [1]. The following parameters were evaluated: plaque index (API) by D. E. Lange *et al*. (simplified); papillary-marginal-alveolar index (PMA) by M. Massler M. *et al.* and H. C. Sandler; periodontal index (PI) by A. L. Russell *et al.*; carious, filled and extracted teeth index (CFE) by G. Nikiforuk *et al*. Orthopantomography was performed on all patients, spot-film radiography, according to indications. Division of examined patients into groups by the severity of periodontitis is presented in Table 1.

MMP-2, MMP-8, and MMP-9 levels in oral fluid were measured by direct ELISA using Human/Mouse/Rat MMP-2, Human MMP-8, and Human MMP-9 total kits; TIMP-1 and TIMP-2, using Human TIMP-1 and Human TIMP-2 (Quantikine^{T}, R&D Systems); IL-1β and TNF-α, using Human IL-1β Platinum and Human TNF-α Platinum ELISA kits (Bender MedSystems); IL-6, using Human IL-6 ELISA kit (Biosource International) according to manufacturer's instructions. Oral fluid samples stored for 2 weeks at -80°C were thawed at room temperature and centrifuged at 10,000 rpm for 2 min. Absorbance was measured on an automated Bio-Tek Instruments ELX800 universal microplate reader.

Kolmogorov–Smirnov test, Lilliefors test, and Shapiro–Wilk test demonstrated that the data did not conform the normal distribution and the null hypothesis was rejected at p=0.05. Therefore, the results were analyzed by nonparametric tests.

RESULTS

The levels of MMP-9 and MMP-8 significantly differed in oral fluid of patients with intact periodontium and patients with chronic generalized periodontitis with metal restorations in the oral cavity (p=0.0168 and 0.0363, respectively; Table 2).

In patients without metal restorations, MMP-9, TIMP-2 and MMP-2 differed significantly (p=0.0069; p=0.045; p=0.003). No significant differences were found between the other parameters. Statistical differences between MMP-9 and MMP-8 levels in examined subjects with healthy periodontium and chronic generalized periodontitis confirm the important role of MMP-9 in the development of inflammatory destructive periodontal diseases.

Nonparametric analysis of variance yielded statistically significant differences between the levels of MMP-2 in oral fluid in four groups of patients (X^2=9.98; p=0.0188; Fig. 1). The maximum MMP-2 levels were observed in patients with periodontitis and without metal restorations (4.43±1.37 ng/ml); minimal values of MMP-2 (0.25±0.13 ng/ml) were found in subjects with intact periodontium and without metal restorations. The same pattern was observed in patients with periodontitis and metal restorations: 1.86±0.93 ng/ml in patients with periodontitis and 0.95±0.75 ng/

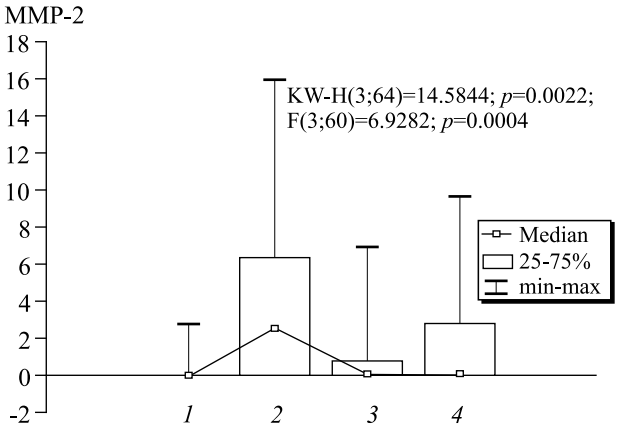

Fig. 1. Comparison of MMP-2 concentrations in oral fluid of examined groups. *1*) patients with intact periodontium and without metal restorations; *2*) with periodontitis and without metal restorations; *3*) with intact periodontium and metal restorations; *4*) with periodontitis and metal restorations.

TABLE 2. Biochemical Parameters of Oral Fluid in Examined Patients

Group	Parameter	Patients with intact periodontium				Patients with periodontitis				*P*
		M	Median	σ	*m*	*M*	Median	σ	*m*	
Patients with metal restorations in oral cavity	MMP-9	537.65*	299.80*	631.57*	144.89*	863.24*	579.30*	691.02*	116.80*	0.0168*
	MMP-8	223.23*	102.43*	295.05*	93.30*	329.51*	221.70*	251.12*	53.54*	0.0363*
	IL-6	10.54	0.00	28.81	9.11	8.56	7.56	18.49	3.86	0.4107
	IL-1β	278.25	180.34	269.81	74.83	401.91	203.56	425.30	78.98	0.6243
	TNF-α	175.23	65.30	191.86	53.21	199.98	152.32	201.91	37.49	0.7236
	TIMP-1	157.10	146.65	44.37	22.18	189.13	172.50	61.85	35.71	0.2888
	TIMP-2	19.67	16.30	9.20	4.60	35.90	36.28	23.42	13.52	0.4795
	IL-2	0.29	0.04	0.85	0.27	1.02	0.54	3.32	0.68	0.9397
	MMP-2	0.95	0.04	2.24	0.75	1.86	0.04	3.10	0.93	0.7612
Patients without metal restorations in oral cavity	MMP-9	574.58*	489.40*	500.67*	96.35*	1478.38*	955.20*	1057.44*	293.28*	0.006935*
	MMP-8	283.58	247.10	201.91	46.32	235.24	241.75	80.39	40.19	0.81
	IL-6	1.41	0.00	3.43	0.79	4.54	4.44	11.10	2.34	0.52
	IL-1β	286.63	240.50	190.55	38.11	312.04	226.04	208.49	93.24	0.89
	TNF-α	232.75	134.46	231.91	46.38	170.27	156.16	79.53	35.57	0.72
	TIMP-1	200.98	178.80	126.87	56.74	268.73	304.05	174.72	71.33	0.58
	TIMP-2	21.31*	14.65*	15.77*	7.05*	44.22*	46.96*	17.56*	7.17*	0.044611*
	IL-2	0.22	0.04	0.48	0.17	1.19	0.74	3.56	0.61	0.90
	MMP-2	0.25*	0.01*	0.72*	0.13*	4.43*	2.49*	5.12*	1.37*	0.003069*

Note. * Statistically significant differences in the studied parameters determined by Mann-Whitney test.

TABLE 3. Correlation Analysis of the Obtained Data in Examined Groups

Groups	Parameters	Spearman rank correlation coefficient (*R*)	*P*
Without metal restorations			
intact periodontium	MMP-9 & MMP-8	0.84	0.0001*
	MMP-9 & TIMP-1	0.90	0.0374*
	MMP-8 & IL-1β	0.48	0.0364*
	IL-6 & TNF-α	-0.47	0.0437*
	IL-1β & PI	-0.39	0.0542
	TNF-α & MMP-2	-0.39	0.0569
	TIMP-2 & MMP-2	0.97	0.0048*
periodontitis	MMP-9 & MMP-8	0.57	0.0415*
	MMP-8 & PI	-0.65	0.0389*
	MMP-8 & PMA	-0.68	0.0217*
	MMP-2 & age	0.60	0.0236*
	MMP-2 & PI	0.61	0.0216*
	MMP-2 & PMA	0.74	0.0024*
With metal restorations			
intact periodontium	MMP-9 & MMP-8	0.87	0.0012*
	MMP-9 & IL-6	0.62	0.0544*
	MMP-8 & IL-6	0.70	0.0240*
	MMP-8 & IL-1β	0.81	0.0049*
periodontium	MMP-9 & MMP-8	0.74	0.0001*
	MMP-9 & IL-6	0.47	0.0225*
	MMP-9 & IL-1β	0.6	0.0009*
	MMP-9 & TNF-α	0.43	0.025*
	MMP-8 & API	0.46	0.0455*
	MMP-8 & periodontal pocket depth	-0.52	0.0139*
	MMP-8 & IL-1β	0.69	0.0004*
	IL-6 & periodontal pocket depth	-0.61	0.0021*
	IL-6 & IL-1β	0.57	0.0061*
	IL-1β & PMA	0.86	0.0137*
	IL-1β & TNF-α	0.5	0.0057*
	MMP-2 & PI	0.78	0.0218*

Note. * Statistically significant correlation coefficients ($p<0.05$).

ml subjects with healthy periodontium. Patients with periodontitis in both groups demonstrated the highest MMP-2 levels. However, in the group with metal restorations the difference in MMP-2 levels between patients with healthy periodontium and periodontitis was lower, which was apparently due to suppressive effect of metal alloy components on MMP-2 production.

Correlation analysis of the data obtained on examined subjects with healthy periodontium without metal restorations in the oral cavity (Table 3) revealed a positive correlation between MMP-9 and MMP-8 levels in the oral fluid. A positive correlation was also found between MMP-9 and TIMP-1 values and between MMP-2 and TIMP-2. Negative correla-

tions were found between IL-6 and TNF-α levels and between IL-1β and PI.

Patients with periodontitis and without metal restorations (Table 3) also showed significant positive relationship between MMP-9 and MMP-8 levels. MMP-2 was positively associated with the age of patients and periodontal condition indices (PI, PMA). MMP-8 levels were found to correlate negatively with periodontal condition indices (PI, PMA). The strength of correlations between MMP-9 and MMP-8 was lower than in subjects with healthy periodontium. Significant correlations between MMP-2 and age, PI, and PMA indicate close association between MMP-2 oral fluid levels in patients with periodontitis and periodontal condition indices and confirms the previously published data on the impact of age-related changes on frequency of inflammatory destructive periodontal diseases.

The group of examined subjects with intact periodontium and with metal restorations revealed positive correlations between the oral fluid concentrations of MMP-9 and MMP-8 (Table 3). MMP-9 levels correlated also with IL-6 values. MMP-8 levels in this group had a strong positive correlation with parameters of IL-6 and IL-1β.

In periodontitis patients with metal restorations in oral cavity, positive relationships were found between oral fluid values of MMP-9, and MMP-8, IL-6, IL-1β as well as TNF-α. MMP-8 values in oral fluid had a positive relationship with IL-1β levels and API index. A negative correlation was revealed between MMP-8 values in oral fluid and periodontal pocket depth. Positive correlation was found between IL-6 and IL-1β oral fluid levels and negative one, between IL-6 and periodontal pocket depth parameter. There were positive correlations between IL-1β and TNF-α as well as between IL-1β and PMA levels. MMP-2 in this group positively correlated with PI index.

The data obtained suggest that regulatory mechanisms underlying MMP, IL, and TIMP production in the oral cavity of controls with healthy periodontium are relatively similar. In patients suffering from chronic generalized periodontitis with and without metal dental restorations obtained correlation coefficients indicate triggered biochemical cascade accompanied by the activation of cytokine production in response to the etiologic factors. The group of patients with periodontitis and metal restorations demonstrated more marked reaction. Correlations identified in this group indicate that enhanced MMP-2, MMP-8, and MMP-9 production increased the levels of IL-1β and IL-6.

Thus, MMP-9 in oral fluid can be considered as a marker of chronic generalized periodontitis. At the same time, orthodontic structures made from chromium-cobalt or chromium-nickel alloys increase the oral fluid levels of MMP-2, IL-1β , and IL-6 in these patients.

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