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Acute myocardial infarction elevates serine protease activity in saliva of patients with periodontitis

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Background and Objective: There are indications that acute myocardial infarction (AMI) may have an effect on the oral environment, which is reflected in the expression of salivary and gingival proteinases. According to our knowledge, no studies have been carried out to investigate the effect of AMI on the activities of two major tissue-destructive serine protease and microbial effectors, elastase and cathepsin G, produced by oral fluid polymorphonuclear granulocytes (PMN). Therefore, we compared the activities of elastase and cathepsin G in saliva from patients with AMI and from systemically healthy subjects (non-AMI) with similar periodontal conditions.

Material and Methods: A total of 92 patients (47 AMI and 28 non-AMI patients with gingivitis or periodontitis, and 17 systemically and periodontally healthy subjects as a control group) were recruited. Clinical periodontal measurements were recorded, and stimulated whole-saliva samples were collected. The patients with AMI were clinically examined within 3–4 d after admission to the coronary care unit. The activities of saliva neutrophil elastase and cathepsin G were measured after collection, at specific time-points during incubation (from baseline to 23 h) by specific synthetic peptide substrate assays.

Results: The saliva of patients with AMI and periodontitis had a significant trend for the highest elastase activities among the study groups. Elastase and cathepsin G activities correlated significantly with each other in the AMI periodontitis group ($r = 0.8$, $p < 0.01$). In a logistic regression analysis, the level of salivary elastase activity associated significantly with periodontitis.

Conclusion: AMI may be reflected in PMN serine protease elastase activity in saliva, despite its strong association with periodontitis.

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Coronary heart disease (CHD) is a major cause of death worldwide. Atherosclerosis, which is the basic cause of CHD and acute myocardial infarction (AMI), is believed to be an inflammatory disorder. Periodontitis is associated with an increased risk of CHD (1). The pathogenic mechanisms involved in CHD and periodontitis have several similarities and have thus been the subject of intensive research. However, the potential links between these diseases have not been completely clarified (2). The link between transient bacteremias caused by periodontal infection and endothelial dysfunction may provide an explanation for the mechanism (3,4). The local inflammation within the artery wall may contribute to the acceleration of atherosclerosis in response to endothelial infection with

oral pathogens, producing alterations in circulating cytokines, acute-phase reactants, white blood cells and responses mediated by the immune system (5). There are also indications that AMI may have an effect on the oral environment, reflected in the expression of salivary and gingival proteinases (6–8).

Enzymes from polymorphonuclear granulocytes (PMNs) are a common factor in both AMI and periodontitis, and they may be the connection between these two diseases (9,10). PMNs are a mediator of tissue destruction because they produce numerous proteolytic and antimicrobial enzymes, such as MMPs, myeloperoxidase, elastase and cathepsin G. All of these enzymes can be measured in the gingival crevicular fluid or in other oral fluids (e.g. saliva and oral rinse samples), with higher levels found in patients with untreated periodontitis than in patients with a periodontally healthy state (11,12). Activation of PMNs is also an early event in AMI and has a potential role in myocardial injury (13). After AMI, an increase occurs in the number of PMNs and they concentrate around damaged myocardial tissue in a triggered state (14). In addition to antimicrobial defense, PMN enzymes have been implicated in thrombotic processes, which are a physiologic tool of the host-defense system to suppress pathogen dissemination (15). Leukocyte adhesion to endothelium and platelets plays an important role in the activation of the coagulation cascade. The major microbicidal enzymes of PMNs – elastase and cathepsin G – also promote blood coagulation and vascular thrombus growth (16), both of which can activate the coagulation cascade and platelets (15). In the absence of pathogen challenge, elastase and cathepsin G can also contribute to thrombosis formation (16). While the role of elastase in thrombotic events seems to be clear, the role of cathepsin G is ambiguous. In patients with CHD, lower levels of cathepsin G have been observed, suggesting that it has an anti-inflammatory role (17); it also has roles in platelet activation and degranulation and in intravascular thrombosis formation (18). The PMN-derived serine proteases, elastase and cathepsin G, can activate pro-MMP-8 and -9, important MMPs associated with the initiation and progression of both periodontitis and CHD (9,10,13,19,20).

To our knowledge, expression of these two major microbicidal effectors of PMNs – elastase and cathepsin G – in oral fluid samples after AMI has not been studied previously. In this study we compared salivary elastase and cathepsin G activities between AMI patients with gingivitis and periodontitis and systemically healthy subjects with gingivitis or periodontitis, and systemically healthy subjects with healthy periodontium as a control group. Our hypothesis was that AMI has an effect on salivary elastase and cathepsin G activities.

Material and methods

A total of 92 subjects: 47 patients with AMI (divided into two subgroups: gingivitis $n = 25$; periodontitis $n = 22$; age range 34–75 years), 28 systemically healthy (non-AMI) subjects (also divided into two subgroups: gingivitis $n = 13$; periodontitis $n = 15$; age range 25–68 years), and 17 systemically and periodontally healthy subjects as a control group (age range 26–44 years) were recruited. The patient population, study protocol and clinical examinations have been discussed in detail in our previous publication (7). The patients in the AMI group were admitted to the Department of Cardiology, University Hospital of Ege because of AMI. AMI was verified by typical changes in the electrocardiogram and elevation of serum enzymes (serum glutamic-oxaloacetic transaminase, creatinine phosphokinase and the creatinine phosphokinase-MB isoenzyme), with or without chest discomfort consistent with AMI. The non-AMI and control groups included dentate patients seeking dental treatment in Ege University School of Dentistry, Department of Periodontology. Complete medical and dental histories were taken. Exclusion criteria were: patients with severe medical disorders, including diabetes mellitus or immunological disorders; patients with any history of any other systemic disease; patients who had received antibiotics or other medicines or periodontal treatment within the past 6 mo; and pregnant women.

In the oral examination, the numbers of teeth present were recorded, excluding third molars. Clinical periodontal data, including probing depth and, bleeding on probing (BOP) (as present or absent), were recorded at six sites per tooth. BOP was expressed as a percentage (number of sites with a positive finding/all sites studied in an individual $\times$ 100%) and probing depth as the mean value of all probing depths. Both AMI and non-AMI groups were further divided into gingivitis and periodontitis subgroups according to the criteria defined in the International Workshop for the Classification of Periodontal Diseases and Conditions (21). Obvious plaque accumulation and accompanying gingival inflammation, as well as a lack of clinical or radiographical signs of attachment loss, were sought for the diagnosis of plaque-induced gingivitis. Chronic periodontitis was assigned when there was at least one pocket in each quadrant with a probing depth of ≥ 5 mm and a clinical attachment level (CAL) of ≥ 6 mm. In addition, the CAL was required to be consistent with the amount of plaque accumulation or local contributing factors.

The study was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2000. The purpose and procedures were explained to patients before participation, and written informed consent was obtained.

Collection of salivary samples

Stimulated whole-saliva samples were obtained from all individuals. In order to stimulate saliva production, the participants chewed a piece of paraffin wax for 7 min. Saliva produced during the first 2 min was discarded and the saliva produced during the following 5 min was collected. The participants chewed the paraffin during the time of saliva collection. The saliva samples were weighed and immediately frozen at −40°C. At the end of the

sample-collection period, the samples were lyophilized and stored at −20°C until required for biochemical analyses.

Analyses of PMN elastase and cathepsin G activities in salivary samples

Saliva samples were analyzed using the chromogenic substrates 1 mM succinyl-alanyl-alanyl-valine-p-nitroanilide (SAAVNA) for PMN elastase activity (22) and 1 mM *N*-succinyl-Ala-Ala-Pro-Phe p-nitroanilide (SAAPPNA) for PMN cathepsin G activity (23). The increase of absorbance (*A*) was detected by spectrophotometry at 405 nm, immediately after sample defrosting (0 h), and at 1, 3, 17 (elastase), 21 (cathepsin G), and 23 h of incubation. Briefly, 10 μL of gingival crevicular fluid sample was incubated with 1 mM SAAVNA or SAAPPNA in 8.8 mM HEPES buffer supplemented with 0.15 M NaCl, 4.2 mM KOH, 2.1 mM $MgCl_2$, 1.7 mM $CaCl_2$ and 0.08% (weight by volume) Brij 35, at 37°C in a flat-bottom 96-well polystyrene ELISA plate (A/S Nunc, Roskilde, Denmark) (24,25). The absorbance at 405 nm was measured with a Labsystems Multiscan PLUS (Labsystems, Helsinki, Finland) and background-corrected. Elastase and cathepsin G values were obtained from calibration curves based on a plot of initial reaction velocities of pure human PMN elastase and cathepsin G standards, respectively (24–26), and the differences in the OD values were used as a measure of elastase and cathepsin G activity (ΔOD_{405}/h).

Statistical analyses

Neither PMN elastase nor cathepsin G was normally distributed (Shapiro–Wilk test, $p < 0.05$). Therefore, non-parametric tests were used. Multiple comparisons between the study groups were performed using the Kruskal–Wallis test and pairwise comparisons were performed using the Mann–Whitney *U*-test. The Jonckheere–Terpstra test was used to estimate significance of the trends between the multiple study groups. Crude and adjusted odds ratios (ORs) with their 95% confidence intervals (CIs) and the respective *p*-values were calculated for both elastase and cathepsin G using a logistic regression analysis to find out their association with AMI and with periodontitis. With AMI as a dependent variable, adjustments were made for gender, age, smoking, BOP % and probing depth, and with periodontitis as a dependent variable, adjustments were made for gender, age, smoking, BOP % and systemic diagnosis. In all analyses, $p < 0.05$ was considered statistically significant.

Results

The characteristics of the study groups are shown in Table 1 and represented in our earlier study (7). Salivary PMN elastase activity levels were significantly different between the control and AMI and non-AMI gingivitis and periodontitis groups at 1, 3, 17 and 23 h detection time-points (Table 2). There was a significant trend for the AMI periodontitis group to have the highest salivary elastase activity at respective time-points (1, 3, 7 and 23 h) (Fig. 1, Table 2), although the activities between the AMI and the non-AMI periodontitis groups did not differ statistically significantly at any time-point when tested as a pair (Table 2). The elastase activity in saliva from patients with AMI and periodontitis was significantly higher than the elastase activity in saliva from patients with AMI and gingivitis at 1, 3, 17 and 23 h. No such differences were observed between the non-AMI and gingivitis and non-AMI and periodontitis groups (Table 2). When number of teeth was taken into consideration, the trend for high elastase activities in the AMI and periodontitis group became more obvious and the difference between AMI and periodontitis and non-AMI and periodontitis groups was significant at 17 h and 23 h (Table 3).

Table 1. Demographic and clinical characteristics of the study groups

Parameters	AMI (n = 47)	Non-AMI (n = 28)	p-Value[a]	Control (n = 17)	p-Value[b]
Men, n (%)	38 (80.9)	21 (75.0)	ns[c]	10 (58.8)	ns
Age, mean ± SD	50.7 ± 9.5	45.2 ± 9.5	0.033[d]	35.4 ± 5.1	< 0.001
Smokers, n (%)	15 (31.9)	13 (46.4)	ns[c]	6 (35.3)	ns
Cigarettes per day, median (IQR)	20 (10)	10 (6)	0.001[c]	12.5 (11)	ns
No. of teeth, mean ± SD	21.7 ± 4.9	22.6 ± 3.5	ns[d]	27.4 ± 1.7	< 0.001
Gingivitis/periodontitis, n (%)	25/22 (53.2/46.8)	13/15 (46.4/53.6)	ns[c]		
PPD (mm), median (IQR)	2.4 (1.10)	3.76 (1.47)	< 0.001[c]	2.28 (0.45)	< 0.001
Gingivitis	2.18 (0.66)	3.01 (0.05)	< 0.001[c]		
Periodontitis	3.25 (1.92)	4.37 (0.85)	0.001[c]		
BOP %, median (IQR)	33.3 (40.28)	58 (59.03)	0.040[c]	2.46 (3.14)	< 0.001
Gingivitis	18.45 (21.02)	23.13 (26.16)	ns[c]		
Periodontitis	57.98 (12.8–94.8)	82.5 (20.36)	ns (0.075)[c]		

[a] Comparisons between acute myocardial infarction patients (AMI) and systemically healthy subjects with similar periodontal conditions (non-AMI).
[b] Comparisons between control group, AMI and non-AMI; Kruskal–Wallis test.
[c] Mann–Whitney *U*-test.
[d] *t*-test.
BOP %, percentage of sites with bleeding on probing; IQR, interquartile range; ns, nonsignificant; PPD, periodontal probing depth; SD, standard deviation.

Table 2. Elastase activities (ΔA_{405}/h) at 0, 1, 3, 17 and 23 h detection time-points according to systemic and periodontal diagnosis

		AMI		Non-AMI			Control			
Incubation	Group	Median (IQR)	*p*-Value[a]	Median (IQR)	*p*-Value[a]	*p*-Value[b]	Median (IQR)	*p*-Value[c]	*p*-Value[d]	*p*-Value[e]
0 h	All	0.124 (0.108)	0.475	0.163 (0.097)	0.717	0.163	0.122 (0.073)	0.054	0.184	0.543
	G	0.122 (0.125)		0.171 (0.148)		0.259				
	P	0.133 (0.088)		0.154 (0.082)		0.435				
1 h	All	0.302 (0.428)	0.005	0.333 (0.771)	0.928	0.381	0.167 (0.168)	0.009	0.001	0.017
	G	0.218 (0.189)		0.345 (0.575)		0.052				
	P	0.508 (0.697)		0.320 (1.023)		0.593				
3 h	All	0.581 (0.975)	0.001	0.458 (1.422)	0.964	0.573	0.190 (0.275)	0.014	0.001	0.007
	G	0.282 (0.440)		0.432 (1.047)		0.097				
	P	1.056 (0.886)		0.551 (1.157)		0.319				
17 h	All	1.119 (1.059)	0.001	0.954 (0.990)	0.992	0.839	0.417 (0.956)	0.027	0.001	0.005
	G	0.514 (0.938)		0.927 (1.035)		0.168				
	P	1.381 (0.266)		1.227 (0.997)		0.181				
23 h	All	1.201 (1.039)	0.001	1.089 (0.959)	0.650	0.930	0.450 (0.989)	0.031	< 0.001	0.003
	G	0.611 (0.989)		1.078 (0.999)		0.150				
	P	1.402 (0.300)		1.275 (1.007)		0.098				

[a] Comparisons between acute myocardial infarction patients (AMI) and systemically healthy subjects (non-AMI) with periodontitis and gingivitis, Mann–Whitney *U*-test.
[b] Comparisons between AMI and non-AMI groups, Mann–Whitney *U*-test.
[c] Comparisons among AMI, non-AMI and control groups, Kruskall–Wallis test.
[d] Comparisons among AMI gingivitis, AMI periodontitis, non-AMI gingivitis, non-AMI periodontitis and control groups, Kruskall–Wallis test.
[e] Comparisons for trend among AMI gingivitis, AMI periodontitis, non-AMI gingivitis, non-AMI periodontitis and control groups, Jonckheere–Terpstra test.
G, gingivitis; IQR, interquartile range; P, periodontitis.

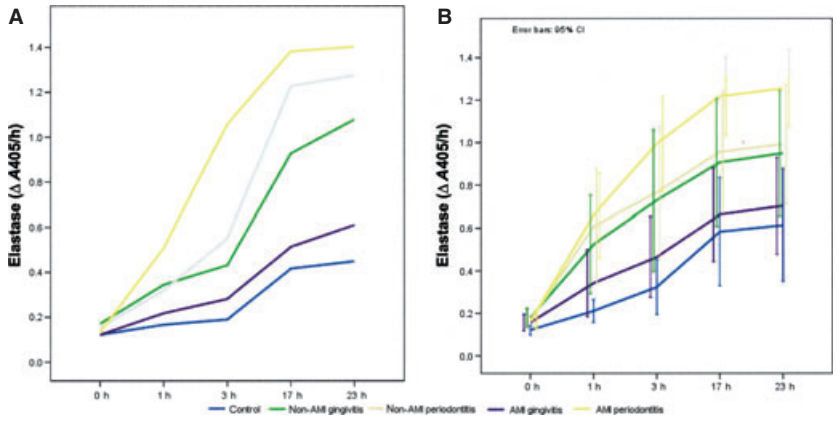

Fig. 1. Median (A) and mean (95% CI) (B) elastase activities at 0, 1, 3, 17 and 23 h (ΔA_{405}/h) detection time-points for the five study groups. AMI, acute myocardial infarction; Non-AMI, systemically healthy subjects with similar periodontal conditions.

Cathepsin G activity was similar in control, AMI and non-AMI gingivitis and periodontitis groups at all time-points (Table 4). When the number of teeth was taken into consideration, the control group had a lower cathepsin G activity than AMI and non-AMI gingivitis and periodontitis groups at all time-points (Table 5).

Smoking had no effect on elastase activity in the AMI group, neither on the total activity (Fig. 2A) nor on the activity when the number of teeth was considered. Instead, the total elastase activity of saliva from nonsmoking, non-AMI subjects was higher than the total elastase activity of saliva from non-AMI smoker subjects (the differences were significant at 1- and 3-h time-points) (Fig. 2B). The difference was less obvious when the number of teeth was considered (0 h, $p = 0.717$; 1 h, $p = 0.029$; 3 h, $p = 0.467$; 17 h, $p = 0.098$; and 23 h, $p = 0.156$). Smoking had no effect on cathepsin G activity in any study group, when determined either by total measures or by considering the number of teeth.

The elastase activity and the cathepsin G activity correlated significantly with each other in the saliva of subjects with AMI and periodontitis (Spearman's $\rho = 0.846$, $p < 0.01$; Pearson correlation coefficient = 0.804, $p < 0.01$) (Fig. 3). Although the correlation coefficients between elastase and cathepsin G in other study groups were relatively high (*p*-value at level 0.01), the correlations could not be confirmed with graphically.

Logistic regression analyses found the saliva elastase activity to be associated with periodontitis, both in unadjusted analyses at 1, 3, 17 and 23 h and in multivariate analyses adjusted by gender, age, smoking, BOP % and systemic diagnosis at 3, 17 and 23 h time-points (Table 6). Cathepsin G activity was not associated with periodontitis. Neither elastase nor cathepsin G activities were associated with AMI either in

Table 3. Elastase activities when number of teeth was taken into consideration (ΔA_{405}/h) at 0, 1, 3, 17 and 23 h detection time-points according to systemic and periodontal diagnosis

		AMI		Non-AMI			Control			
Incubation	Group	Median (IQR)	*p*-Value[a]	Median (IQR)	*p*-Value[a]	*p*-Value[b]	Median (IQR)	*p*-Value[c]	*p*-Value[d]	*p*-Value[e]
0 h	All	0.007 (0.01)	0.321	0.007 (0.01)	0.586	0.580	0.004 (0.0)	0.001	0.681	0.018
	G	0.006 (0.01)		0.008 (0.01)		0.314				
	P	0.007 (0.01)		0.007 (0.0)		0.795				
1 h	All	0.015 (0.03)	0.002	0.016 (0.03)	0.683	0.518	0.008 (0.01)	< 0.001	0.010	0.001
	G	0.011 (0.01)		0.016 (0.02)		0.032				
	P	0.025 (0.04)		0.013 (0.03)		0.290				
3 h	All	0.026 (0.05)	0.250	0.021 (0.04)	0.339	0.844	0.009 (0.01)	0.052	0.466	0.021
	G	0.012 (0.02)		0.020 (0.04)		0.879				
	P	0.051 (0.04)		0.022 (0.04)		0.658				
17 h	All	0.045 (0.05)	< 0.001	0.044 (0.04)	1.0	0.948	0.015 (0.03)	0.003	0.001	< 0.001
	G	0.029 (0.04)		0.044 (0.04)		0.079				
	P	0.062 (0.03)		0.044 (0.04)		0.033				
23 h	All	0.045 (0.05)	< 0.001	0.047 (0.04)	0.964	0.904	0.016 (0.04)	0.003	0.001	< 0.001
	G	0.031 (0.07)		0.048 (0.04)		0.085				
	P	0.062 (0.03)		0.046 (0.04)		0.030				

[a] Comparisons between acute myocardial infarction patients (AMI) and systemically healthy subjects (non-AMI) with periodontitis and gingivitis, Mann–Whitney *U*-test.
[b] Comparisons between AMI and non-AMI groups, Mann–Whitney *U*-test.
[c] Comparisons among AMI, non-AMI and control groups, Kruskall–Wallis test.
[d] Comparisons among AMI gingivitis, AMI periodontitis, non-AMI gingivitis, non-AMI periodontitis and control groups, Kruskall–Wallis test.
[e] Comparisons for trend among AMI gingivitis, AMI periodontitis, non-AMI gingivitis, non-AMI periodontitis and control groups, Jonckheere–Terpstra test.
G, gingivitis; IQR, interquartile range; P, periodontitis.

Table 4. Cathepsin G activities (ΔA_{405}/h) at 0, 1, 3, 21 and 23 h detection time-points according to systemic and periodontal diagnosis

		AMI		Non-AMI			Control			
Incubation	Group	Median (IQR)	*p*-Value[a]	Median (IQR)	*p*-Value[a]	*p*-Value[b]	Median (IQR)	*p*-Value[c]	*p*-Value[d]	*p*-Value[e]
0 h	All	0.106 (0.120)	0.147	0.124 (0.071)	0.294	0.681	0.061 (0.097)	0.201	0.174	0.119
	G	0.080 (0.098)		0.118 (0.069)		0.649				
	P	0.120 (0.120)		0.126 (0.080)		0.891				
1 h	All	0.474 (0.528)	0.201	0.612 (0.472)	0.201	0.677	0.295 (0.529)	0.190	0.163	0.119
	G	0.434 (0.605)		0.352 (0.648)		0.976				
	P	0.622 (0.459)		0.684 (0.412)		0.614				
3 h	All	1.003 (0.807)	0.394	0.994 (0.915)	0.316	0.706	0.700 (0.916)	0.265	0.309	0.198
	G	0.035 (0.849)		0.647 (1.147)		0.903				
	P	0.980 (0.950)		1.253 (0.722)		0.572				
21 h	All	2.416 (1.302)	0.515	2.243 (1.005)	0.217	0.956	2.146 (1.545)	0.271	0.417	0.140
	G	2.390 (1.403)		2.185 (1.219)		0.808				
	P	2.444 (1.141)		2.467 (0.838)		0.772				
23 h	All	2.447 (1.284)	0.558	2.313 (0.971)	0.201	0.755	2.209 (1.451)	0.253	0.364	0.191
	G	2.447 (1.345)		2.294 (1.187)		0.903				
	P	2.450 (1.144)		2.491 (0.795)		0.511				

[a] Comparisons between acute myocardial infarction patients (AMI) and systemically healthy subjects (non-AMI) with periodontitis and gingivitis, Mann–Whitney *U*-test.
[b] Comparisons between AMI and non-AMI, Mann–Whitney *U*-test.
[c] Comparisons among AMI, non-AMI and control group, Kruskall–Wallis test.
[d] Comparisons among AMI gingivitis, AMI periodontitis, non-AMI gingivitis, non-AMI periodontitis and control group, Kruskall–Wallis *U*-test.
[e] Comparisons for trend among AMI gingivitis, AMI periodontitis, non-AMI gingivitis, non-AMI periodontitis and control group, Jonckheere–Terpstra test.
G, gingivitis; IQR, interquartile range; P, periodontitis.

Table 5. Cathepsin G activities when number of teeth was taken into consideration (ΔA_{405}/h) at 0, 1, 3, 21 and 23 h detection time-points according to systemic and periodontal diagnosis

		AMI		Non-AMI			Control			
Incubation	Group	Median (IQR)	*p*-Value[a]	Median (IQR)	*p*-Value[a]	*p*-Value[b]	Median (IQR)	*p*-Value[c]	*p*-Value[d]	*p*-Value[e]
0 h	All	0.005 (0.01)	0.067	0.005 (0.0)	0.440	0.844	0.002 (0.0)	0.017	0.202	0.005
	G	0.003 (0.0)		0.005 (0.0)		0.693				
	P	0.006 (0.01)		0.006 (0.0)		0.350				
1 h	All	0.021 (0.03)	0.186	0.025 (0.02)	0.440	0.887	0.011 (0.02)	0.033	0.388	0.015
	G	0.019 (0.03)		0.020 (0.02)		0.927				
	P	0.033 (0.04)		0.027 (0.02)		0.472				
3 h	All	0.042 (0.05)	0.250	0.041 (0.04)	0.339	0.844	0.026 (0.03)	0.052	0.466	0.021
	G	0.040 (0.05)		0.035 (0.04)		0.879				
	P	0.054 (0.05)		0.052 (0.04)		0.658				
21 h	All	0.101 (0.05)	0.201	0.097 (0.04)	0.650	0.547	0.077 (0.06)	0.013	0.474	0.004
	G	0.101 (0.06)		0.097 (0.04)		0.879				
	P	0.115 (0.08)		0.052 (0.04)		0.435				
23 h	All	0.104 (0.06)		0.102 (0.04)		0.697	0.080 (0.06)	0.008	0.593	0.005
	G	0.101 (0.06)	0.233	0.1 (0.04)	0.717	0.976				
	P	0.114 (0.08)		0.104 (0.04)		0.572				

[a] Comparisons between acute myocardial infarction patients (AMI) and systemically healthy subjects (non-AMI) with periodontitis and gingivitis, Mann–Whitney *U*-test.
[b] Comparisons between AMI and non-AMI groups, Mann–Whitney *U*-test.
[c] Comparisons among AMI, non-AMI and control groups, Kruskall–Wallis test.
[d] Comparisons among AMI gingivitis, AMI periodontitis, non-AMI gingivitis, non-AMI periodontitis and control groups, Kruskall–Wallis test.
[e] Comparisons for trend among AMI gingivitis, AMI periodontitis, non-AMI gingivitis, non-AMI periodontitis and control groups, Jonckheere–Terpstra test.
G, gingivitis; IQR, interquartile range; P, periodontitis.

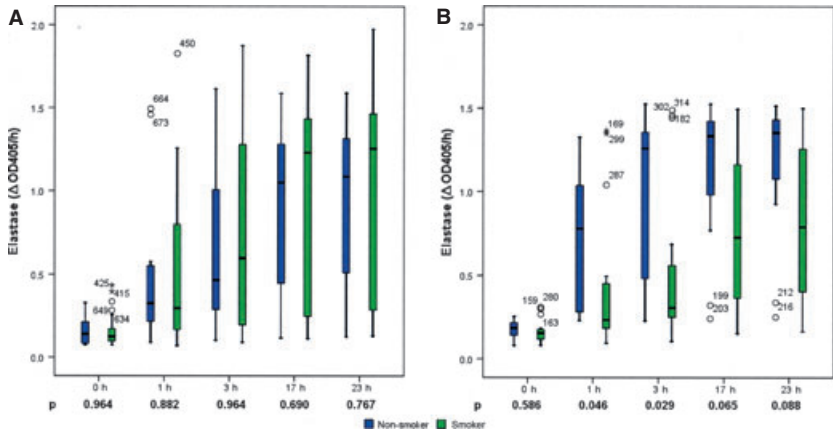

Fig. 2. Box plots with medians, quartiles and statistical outlier values (symbols and numbers above or below the box-plot bars) showing the effect of smoking on salivary elastase activities in systemically healthy subjects (non-AMI) (A) and acute myocardial infarction (AMI) (B) (periodontitis and gingivitis patients together). The *p*-values indicated in the figure at all detection time-points (0–23 h) were calculated using the Mann–Whitney *U*-test for pairwise comparisons between AMI and non-AMI.

unadjusted analysis or when adjusted with gender, age, smoking, periodontal probing depth and BOP % at any detection time-point.

Discussion

In the present study, the activities of salivary PMN elastase and cathepsin G were analyzed in a time-dependent manner over a 23-h period and the results were compared between patients who had recently suffered an AMI and systemically healthy subjects. Both systemic diagnosis groups contained patients with gingivitis and periodontitis, and systemically and periodontally healthy subjects formed a control group. Our main finding was that as a consequence of AMI, salivary elastase activity increased in subjects with periodontitis, and the elevated elastase levels correlated significantly with the cathepsin G activity. Thus, the effect of AMI seems to be reflected in the oral environment. To our knowledge, there are no earlier studies on the effect of AMI on the activities of PMN serine proteases elastase and cathepsin G in oral fluid.

The clinical features of our study groups have been discussed previously (7): the gingival/periodontal condition of the non-AMI subjects was more pronounced than that of the AMI patients when measured as pocket probing depth, as was the inflammatory status of the periodontium when measured as BOP %. Regardless, the salivary elastase activity of AMI patients with periodontitis was higher than in non-AMI subjects during the 23-h detection time-period. Number of teeth had only a minor effect on the elastase activities, indicating that elastase in saliva obviously originates also from sources other than teeth-supporting structures. Saliva is a highly

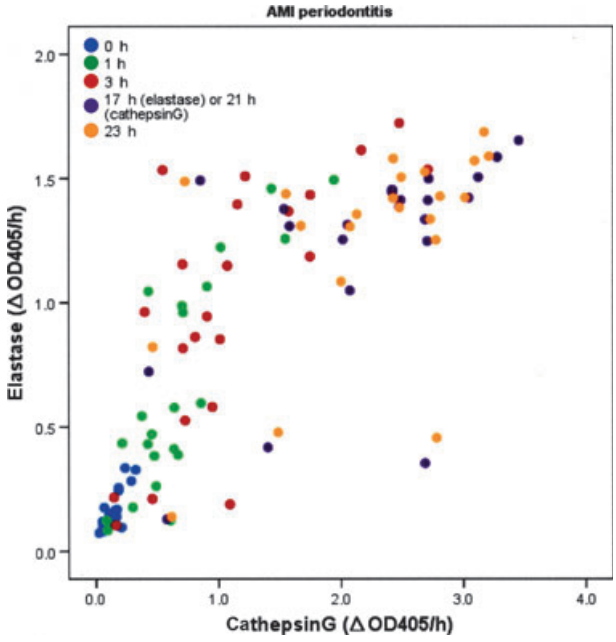

Fig. 3. Scatter plot showing the correlation between elastase and cathepsin G activities of patients with acute myocardial infarction (AMI). All detection time-points (0–23 h) are considered. Spearman's rho 0.846, $p < 0.01$; Pearson correlation coefficient 0.804, $p < 0.01$.

Table 6. Association of salivary elastase activity with periodontitis by means of logistic regression analysis at separate detection time-points

Detection time-point	Unadjusted		Adjusted*	
	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value
0 h	7.906 (0.032–1956.8)	0.462	5.874 (0.0–464571)	0.758
1 h	6.285 (1.973–20.02)	0.002	8.615 (0.713–104.16)	0.090
3 h	5.063 (2.078–12.34)	< 0.001	13.26 (1.007-174.59)	0.049
17 h	4.922 (2.018–12.01)	< 0.001	26.988 (1.764–413.01)	0.018
23 h	4.818 (1.974–11.76)	0.001	21.31 (1.643–276.34)	0.019

*Adjusted for gender, age, smoking, percentage of sites with bleeding on probing (BOP %), and systemic diagnosis.
95% CI, 95% confidence interval: OR, odds ratio.

nonspecific fluid that contains cellular fragments and biochemical components from several sources and it is affected by the composition of gingival crevicular fluid. Elevated levels of proteolytic enzymes of PMN origin have been detected in blood serum or plasma after AMI (7,27–33), among them PMN elastase (13). Therefore, systemic status may have an effect on saliva consistency and its proteolytic enzyme levels.

There are contradictory findings concerning the effect of smoking on salivary elastase. Lower salivary elastase levels have been found in smoking periodontitis patients compared with nonsmoking patients (34), and smoking can also reduce elastase activity in adolescents' saliva (35). However, significantly higher mean levels of neutrophil elastase activity have been detected in the gingival crevicular fluid of smokers compared with nonsmokers (36). In the current study, non-AMI smoker subjects had lower elastase activities than non-AMI nonsmoker subjects, but smoking had no effect on salivary elastase activities in AMI patients, although AMI patients smoked a significantly higher number of cigarettes per day than non-AMI smoker subjects. This may be a further indication of the origin of oral fluid elastase other than solely the oral environment (i.e. as a reflection of increased blood serum elastase as a consequence of AMI and further influx through oral tissues into the oral cavity).

In common with other biological markers, elastase activities in saliva samples showed a wide distribution in all study groups. The distribution of elastase activity showed a wide overlap in saliva from patients with AMI and periodontitis and in saliva from non-AMI patients with periodontitis, and in pairwise comparisons, no significant differences were detected between these groups; however, the median levels of elastase in patients with AMI and periodontitis tended to be higher at 1–23 h detection time-points and the median activation curve was steeper (Fig. 1A). This indicates that the higher salivary elastase activity of AMI patients with periodontitis may be caused, in part, by the recent myocardial infarction. Moreover, our findings that salivary elastase and cathepsin G levels have a strong correlation in the saliva from patients with AMI and periodontitis support the hypothesis that AMI, together with a strong oral inflammatory burden as a result of periodontitis, increases the neutrophilic serine protease activities in the oral cavity and in oral fluid. In our previous study we detected higher MMP8 activation levels in the saliva of AMI subjects, irrespective of periodontal diagnosis (7). The current study suggests that AMI, together with periodontitis, may have an especially detrimental effect on oral health through increasing MMP8 activation and elastase activity. Serine proteases from PMNs may act as pro-MMP activation-cascade inducers in both periodontitis and AMI (9,20).

Although the activities of cathepsin G and elastase correlated significantly in the saliva of patients with AMI and periodontitis, the cathepsin G activity did not differ significantly between the study groups. Only when the number of teeth was considered were the cathepsin G activities of both AMI and non-AMI gingivitis and periodontitis patients higher than the cathepsin G

activity of the control subjects. This may be an indication that salivary cathepsin G originates mainly from the periodontium and reflects the gingival condition rather than the condition of deeper periodontal structures (i.e. deep gingival pockets). Salivary elastase activity associated significantly with periodontitis in logistic regression analysis. This can be regarded as a logical finding because both AMI patients with periodontitis and non-AMI patients with periodontitis expressed higher elastase activities than gingivitis subjects.

In conclusion, the current study suggests that AMI may have an effect on the oral environment and this seems to be reflected especially in those AMI patients who have the highest oral inflammatory burden caused by periodontal infection/inflammation. The increase of oral neutrophilic serine protease levels and activities, as well as MMP8 activation levels, is an indication of strengthened antimicrobial attack from the host, leading to an intensified host response reaction with a possible two-way effect in AMI patients (7). In AMI patients, in addition to the possible worsening of periodontal disease status, also as a consequence of a local periodontal intensified host response, is a further increase of the systemic inflammatory mediator burden possible. This bidirectional effect needs further studies but it also provides a good reason for dental clinicians to take seriously the need to treat all oral inflammatory conditions.

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