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Salivary matrix metalloproteinase-8 in patients with and without coronary heart disease may indicate an increased susceptibility to periodontal disease

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Background and Objective: Tissue destruction caused by periodontitis may increase the number of cytokines implicated in the pathogenesis of cardiovascular diseases. We measured the concentration of the leukocyte-derived proteolytic enzyme, salivary neutrophil collagenase-2 [matrix metalloproteinase-8 (MMP-8)], as a marker of periodontal disease and assessed its relationship to coronary heart disease (CHD). Our aim was to study whether salivary MMP-8 levels were different among patients with and without CHD. The hypothesis was that patients with heart disease might present higher salivary MMP-8 levels than cardiologically healthy controls.

Material and Methods: Saliva samples were taken from 256 patients with CHD and from 250 matched controls with known oral and general health status. The MMP-8 levels in saliva were analyzed by immunofluorometric assay, salivary albumin was assessed by enzyme-linked immunosorbent assay (ELISA) and total protein was determined using the colorimetric method. We further investigated the molecular forms and isoform distribution of salivary MMP-8 by western immunoblotting. The MMP-8 results were adjusted for the number of teeth and salivary protein concentrations.

Results: The adjusted logarithmic MMP-8 values were 0.145 ± 0.245 μg/l in patients with CHD and 0.088 ± 0.115 μg/l in controls ($p < 0.01$). The respective MMP-8 : total protein and MMP-8 : albumin ratios were also significantly higher in CHD patients than in non-CHD subjects.

Conclusion: Elevated salivary MMP-8 levels seemed to associate with CHD, suggesting more tissue breakdown as a result of periodontitis among the patients with heart disease.

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Increased serum inflammatory markers, in particular elevated C-reactive protein (CRP), have been accepted by the American Heart Association as predictors of heart disease risk (1). Periodontitis has been associated with cardiovascular disease (CVD) and it appears to increase serum inflammatory markers, including CRP (2). The evidence is not unequivocal, however, owing to imprecise assessment of periodontitis in earlier studies. Parameters such as clinical periodontal pocket depth or attachment loss, used in earlier studies, reflect past disease experience and may dilute the effects of current disease activity, at least at the biochemical level.

Elevated levels of matrix metalloproteinases (MMPs), especially neutrophil collagenase-2 (MMP-8), are markers for tissue destruction in inflammation (3–7). The severity and course of periodontitis-associated tissue destruction can be quantified by measurement of MMP-8 concentrations in oral fluid (4,5,8,9). MMP-8 (or collagenase-2) has repeatedly been shown to be the major collagenase or MMP present in inflamed periodontitis-affected human gingival crevicular fluid (GCF), saliva and mouth rinse samples (4–9). Association between increased GCF, saliva and mouth rinse MMP-8, and progressive loss of connective tissue attachment, has been demonstrated and longitudinal studies have shown a significant decrease in MMP-8 in GCF, saliva and mouth rinse samples following successful periodontal treatment (4–9). However, recently MMP-8 has been shown, in addition to its surrogate catalytic action (3,7), also to exert anti-inflammatory or defensive characteristics (10).

We examined salivary MMP-8, total protein and albumin, as well as periodontal disease status, of 256 patients with coronary heart disease (CHD) of at least New York Heart Association class II, verified by angiography. For comparison, 250 non-CHD subjects were investigated. The study hypothesis was that CHD patients might present a greater activity of periodontal disease than controls, assessed by measuring the salivary MMP-8 levels.

Material and methods

Patients and controls

The study material, with exclusion and inclusion criteria, has been presented in detail previously (2,11). In brief, 256 patients (mean age 60 ± 9 yrs), referred for open-heart surgery to the Kuopio University Hospital, Kuopio, Finland, were included in the study. The study protocol was approved by the Ethical Committee of the University Hospital, and the principles of the Helsinki Declaration were followed throughout the study. The patients' heart disease was of the New York Heart Association grades II–IV. For comparison, 250 age- and gender-matched non-CHD patients (mean age 61 ± 10 yrs) were also included. The sample size (total: 506 subjects) was based on practical reasons without any power calculation. Of the subjects, 64% were men. All patients were examined in the hospital and their full medical and dental records were available. Saliva samples were taken at the dental examination by giving the subject a 1-g piece of paraffin-wax to chew. The collection time was 5 min. The samples were then centrifuged and deep frozen (−75°C) until analyzed.

Methods

Salivary MMP-8 levels were analyzed by immunofluorometric assay (IFMA) (10,12). MMP-8 concentrations in saliva samples were determined by a time-resolved IFMA, and the concentrations of monoclonal antibodies 8708 and 8706 for MMP-8 were 1.5 μg and 0.5 μg per assay, respectively.

The molecular forms of MMP-8 were analyzed by a western immunoblot method, with specific polyclonal antibody for MMP-8 used at 2 μg/ml final concentrations. Saliva samples for western immunoblot analysis from the CHD patients and controls were selected by random choice. Each sample contained 21 μg of protein. Human polymorphonuclear leukocytes (PMN) and rheumatoid synovial culture media were used as positive controls for neutrophil- and mesenchymal-type MMP-8, respectively (12,13). Salivary total protein was analyzed by the colorimetric Lowry method, and albumin was analyzed by enzyme-linked immunosorbent assay (ELISA) (11).

Statistical analyses

SPSS for Windows, version 13, was used for statistical analyses. The differences of background variables between CHD patients and non-CHD subjects were analyzed with the chi-square and Mann–Whitney tests, when applicable. To remove skewness in the MMP-8 values, they were first re-expressed as base 10 logs. The analysis of covariance (ANCOVA) was used for the MMP-8 analyses, with confounding parameters of general health (i.e. diabetes, age and smoking) and the number of teeth as covariates. p-Values of < 0.05 were considered statistically significant.

Results

Table 1 gives the dental status characteristics of the patients and controls. Of the dentate CHD patients, 64.4% had periodontitis compared with 63.9% of the non-CHD subjects. However, higher salivary MMP-8 levels were found in CHD patients than in non-CHD subjects after controlling for the number of teeth (0.145 ± 0.245 μg/l in CHD patients, 0.088 ± 0.115 μg/l in controls; $p = 0.006$, Fig. 1A). Furthermore, the MMP-8 : total protein and MMP-8 : albumin ratios were higher in CHD patients than in non-CHD subjects ($p < 0.001$, Fig. 1B,C). The mean salivary total protein concentrations (± standard deviation) were 1.3 ± 0.6 mg/ml in CHD patients, and 1.5 ± 0.6 mg/ml in non-CHD patients ($p < 0.001$). Respectively, the mean salivary albumin concentrations were 188 ± 208 mg/l in CHD patients and 204 ± 157 mg/l in non-CHD patients ($p < 0.05$).

ANCOVA showed that the estimated log MMP-8 means were 1.219 (standard error 0.059) in the CHD group and 0.999 (standard error 0.057) in the non-CHD group. Regarding the molecular forms of salivary MMP-8 in CHD patients, western immunoblotting revealed both 65–75 kDa neutrophil-type isoforms and 45–55 kDa mesen-

Table 1 . Dental status of patients with or without coronary heart disease (CHD)

	CHD patients ($n = 256$)	Non-CHD patients ($n = 250$)	Significance
Edentulousness (n, %)	89 (34.8%)	37 (14.8%)	***
Maxilla	157 (61.3%)	77 (30.0%)	***
Mandible	90 (35.2%)	41 (16.4%)	***
Mean no. of teeth (± SD)	8.8 ± 9.1	17 ± 10.5	***
Mean DS index (± SD)	0.09 ± 0.7	0.08 ± 0.3	***
Mean no. of endodontally treated teeth (± SD)	0.4 ± 1.1	1.4 ± 1.7	***
Mean no. of teeth with gingival pockets > 6 mm (± SD)	0.3 ± 0.9	0.4 ± 0.9	
Mean no. of teeth with calculus or overhangs	2.1	1.3	***
Mean no. of surfaces per tooth with plaque	0.4	0.2	*

DS index, decayed surfaces index; SD, standard deviation.
*$p < 0.05$; ***$p < 0.001$.

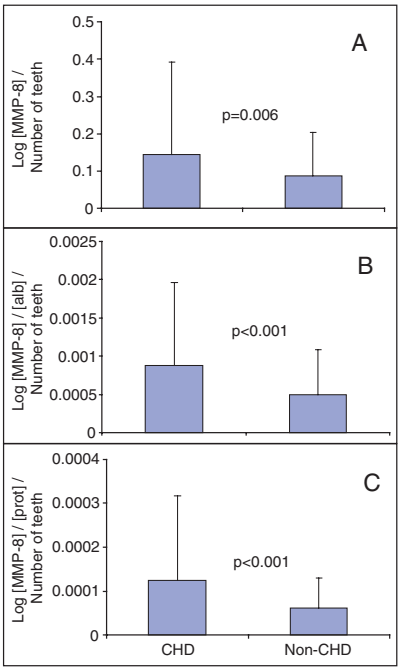

Fig. 1. The logarithmically transformed mean salivary matrix metalloproteinase-8 (MMP-8) (A) levels and MMP-8/salivary albumin (alb) (B) and MMP-8/salivary total protein (prot) (C) ratios in patients with coronary heart disease (CHD, $n = 256$) and in non-CHD subjects ($n = 250$). The bars show the means and the whiskers give the standard deviations.

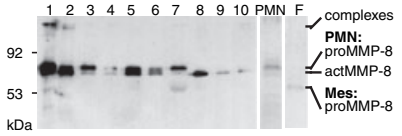

Fig. 2. Representative collagenase-2 [matrix metalloproteinase-8 (MMP-8)] western immunoblot of salivary samples from patients with coronary heart disease (CHD) and from non-CHD subjects. Samples were selected by random choice. The amount of protein in each sample was 21 µg. Lanes 1–5, saliva from patients with CHD; lanes 6–10, saliva from non-CHD subjects; lane PMN, human neutrophil culture media; lane F, rheumatoid synovial fibroblast culture media. Complexes indicate > 100 kDa high-molecular-weight MMP-8 species; PMN proMMP-8 indicates neutrophil-type MMP-8; PMN actMMP-8 indicates neutrophil-type active MMP-8, and Mes proMMP-8 indicates mesenchymal-type proMMP-8. The positions of molecular weight markers are indicated.

chymal cell-type isoforms (12,13) (Fig. 2). Especially, the neutrophil-type MMP-8 was partially converted to the active form. Furthermore, slight immunoreactivities to high-molecular-weight isoforms (> 100 kDa) could also be observed (Fig. 2). This might eventually represent shed forms of membrane-associated MMP-8 species or MMP-8 bound to inhibitors (8,13).

Discussion

Our results confirmed and further extended the study hypothesis indicating that periodontitis might be more active in CHD patients than in non-CHD subjects, as reflected by the MMP-8 findings. As regards the salivary protein concentrations analyzed, the values in both groups were within the range observed in elderly patients of the same population base used in the present investigation (14).

Periodontitis has been associated with an increased risk of CHD and its complications in longitudinal studies (15). It may indeed be a risk factor or a risk indicator for cardiovascular pathology, and these disease entities may share common pathogenic pathways (2). However, clinical studies examining the link between cardiovascular diseases and periodontitis have been criticized for not using validated measures for periodontal disease. In several such studies, the prevalence of periodontitis has been assessed by different clinical parameters and self-reported questionnaires (2). MMPs, especially MMP-8 or collagenase-2, are the major pivotal mediators of tissue destruction in periodontitis (4–7,9,10). Neutrophil MMP-8 has been shown to enter saliva from GCF, and patients suffering from periodontitis consequently have elevated GCF levels and salivary MMP-8 concentrations (4–7,9,10). Thus, an elevated salivary MMP-8 concentration may reflect the severity of periodontal disease and can be considered as a biomarker for the disease (4–9). MMP-8 is the major collagenase present in inflamed gingival tissue, GCF and saliva of periodontitis patients. Association between elevated MMP-8 concentrations, activity and activation with progressive loss of periodontal connective tissue attachment has been shown, and a significant decrease in GCF MMP-8 activity following successful treatment has been demonstrated (4–9). With this background we suggest that an oral fluid MMP-8 test could be used in future studies when examining the association between periodontitis and CHD.

The active form of MMP-8 may also be partly responsible for degradation of the collagen cap of atherosclerotic plaque and thus contribute to the pathogenesis of vascular diseases (16). Previous studies have established the thinning and weakening of fibrous cap, which is the major mechanism render-

ing an atheroma susceptible to rupture. The stability of the fibrous cap depends primarily on the content of the intact type I collagen, which is the major load-bearing molecule. In this regard, the atheroma plaques with histological signs of vulnerability exhibit enhanced collagenolytic activity, evidently as a result of the action of collagenolytic MMPs (17). Among the interstitial collagenases MMP-1, -8 and -13, MMP-8 exhibits three-fold greater catalytic activity against type I collagen, which eventually makes it the most efficient type I collagenolytic enzyme in humans (18). Additionally, MMP-8 can process pro- and anti-inflammatory chemokines, cytokines and apoptosis factors, and thereby process immune responses (10). The inflammatory burden enhanced by periodontitis and periodontopathogenic bacteria can induce macrophages, endothelial cells and smooth muscle cells to express increased concentrations of MMP-8. The mechanisms behind the periodontal disease–atherosclerosis paradigm have been recently reviewed by Meurman and coworkers (19).

The present study was the first to test a host-derived oral fluid surrogate biomarker of periodontitis (MMP-8) in analyzing the susceptibility of CHD patients to periodontal disease. It was an interesting finding that CHD patients might indeed have an increased susceptibility to periodontal tissue destruction. Alternatively, depending on the characteristics of MMP-8, the present findings may also reflect, at least in part, anti-inflammatory or defensive reactions (8). Finally, we have recently discussed the effect of oral infections, other than periodontal disease, in the chronic infection–atherosclerosis perspective (2). The present observations support the hypothesis that local infections, such as periodontitis, may indeed play a role in the pathogenesis of cardiovascular diseases by triggering inflammatory mediators and causing the up-regulation of enzymes, such as MMP-8 investigated in the present study.

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