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Salivary MMP-8, TIMP-1, and ICTP as markers of advanced periodontitis

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Abstract

Aim: Salivary matrix metalloproteinase (MMP)-8 and -14, tissue inhibitor of matrix metalloproteinase (TIMP)-1, and pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) were analysed aiming to detect potential markers of advanced periodontitis in saliva. In addition, we compared two MMP-8 detection methods, a time-resolved immunofluorometric assay (IFMA) and an enzyme-linked immunoassay (ELISA), to differentiate periodontitis subjects from controls.

Material and Methods: Concentrations of MMP-8, MMP-14, TIMP-1, and ICTP were analysed from salivary specimens of 165 subjects, including 84 subjects having at least 14 teeth with periodontal pocket (pocket depth $\geqslant 4$ mm) and 81 subjects without pocket depth as their controls.

Results: Salivary MMP-8 detection by IFMA differentiated periodontitis subjects from controls more strongly than by ELISA. Salivary MMP-8, TIMP-1, and ICTP concentrations were higher in periodontitis subjects than those in controls. When only smokers were included in the analysis these differences were lost. The MMP-8/TIMP-1 ratio and the combination of MMP-8 and ICTP differentiated periodontitis and control groups even in smoker subjects.

Conclusion: Salivary MMP-8, TIMP-1, ICTP, and especially their ratios and combinations are potential candidates in the detection of advanced periodontitis. Differentiating periodontitis and control subjects with salivary MMP-8 detection is dependent on the selected techniques.

Key words: ICTP; MMP-14; MMP-8; saliva; TIMP-1

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Conflict of interest and sources of funding statement

The authors declare that they have no conflicts of interests.

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In periodontal tissue destruction, the degradation of connective tissue and alveolar bone is mainly induced by activated host cell enzymes (Tatakis & Kumar 2005). Among these enzymes, matrix metalloproteinases (MMPs) form the most important group of proteinases that take part not only in the degradation of matrix proteins during periodontitis but also during normal turnover in health and wound healing. The imbalance between MMPs and tissue inhibitors of matrix metalloproteinases (TIMPs) is considered to trigger the degradation of extracellular matrix, basement membrane, and alveolar bone, and thus to initiate periodontal disease (Sorsa et al. 2004).

MMP-8 in oral fluids, gingival crevicular fluid (GCF), and saliva associates with the initiation and progression of periodontitis and reflects its severity (Sorsa et al. 1988, Uitto et al. 1990, Sorsa et al. 2006). Activation of MMP-8 has been suggested to occur in a cascade of events, where reactive oxygen species and MMP-14 play an important role (Weiss 1989, Holopainen et al. 2003, Sorsa et al. 2006). TIMPs, on the other hand, regulate the activities of MMPs. Although the major function of TIMPs is the inhibition of MMPs, they can also

take part in MMP transportation and stabilization (Sorsa et al. 2006). It has been suggested that periodontal destruction is an outcome of the imbalance between MMPs and their inhibitors (Reynolds 1996). Increase in MMP and decrease in TIMP levels initiate collagen degradation from connective tissue and alveolar bone. Hence, the excessive amount of collagen degradation products, such as pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP), in GCF and/or in saliva is regarded to predict alveolar bone loss in periodontitis (Taba et al. 2005).

In periodontal research, the applicability of salivary diagnostics could be used as specific salivary marker(s) to detect periodontitis. Several host and/or bacteria-originated enzymes, cytokines, and biomarkers of bone turnover have been analysed from saliva of periodontitis patients and compared with those of periodontally healthy controls (Miller et al. 2006, Kinney et al. 2007, Scannapieco et al. 2007, Tobón-Arroyave et al. 2008, Teles et al. 2009). In spite of the several studies mentioned above, having an agreement on MMP-8 as a biomarker of periodontitis is limited mainly due to differences in applied methods. Detection of both latent (precursor) and active forms of MMP-8 (both free and complex) in oral fluids may not necessarily correlate with periodontal disease progression, as the active form is associated with periodontitis, while the latent form is associated with gingivitis (Romanelli et al. 1999, Sorsa et al. 2010, Teles et al. 2010). A time-resolved immunofluorometric assay (IFMA) has been introduced to detect active MMP-8 (Hanemaaijer et al. 1997, Sorsa et al. 2010). The IFMA method differs from the other immunodetection methods by its unique antibody, which detects both PMN- and fibroblast type MMP-8 isotypes, and especially their active forms (Hanemaaijer et al. 1997, Sorsa et al. 2010).

The present study is a continuation of our salivary diagnostic studies, aiming to detect infectious and pro-inflammatory markers of periodontitis (Könönen et al. 2007, Gursoy et al. 2009, Paju et al. 2009). The aims of the present study were (i) to compare the applicability of two MMP-8 immunodetection methods, IFMA and enzyme-linked immunoassay (ELISA), in saliva for differentiating periodontitis subjects from controls and (ii) to identify the potential of MMP-8, MMP-14 (an activator of MMP-8), TIMP-1 (an inhibitor of MMP-8), and a bone degradation marker, ICTP, as salivary markers of periodontitis.

Material and Methods

Study population and specimen collection

The present population of 165 subjects is a subsample of the nationally representative population of the ''Health 2000 Health Examination Survey''. The survey was conducted in five districts of Finland by the National Institute for Health and Welfare (formerly National Public Health Institute) during the years 2000–2001. A total of 8028 subjects, aged $\geqslant 30$ years and steadily living in Finland, participated in the health examination, including data on their health behaviour and smoking habits collected by interviews, and data on their general and oral health examined in the field. Periodontal measurements included pocket depths and bleeding on probing, measured from four sites of each tooth. The teeth were recorded as: tooth with pocket depth ≥ 4 mm or tooth with pocket depth $\geqslant 6$ mm. Bleeding on probing data were recorded at the sextant level. Periodontal status was examined by specially trained dentists, as described in the report of the National Public Health Institute (http://www.terveys2000.fi/julkaisut/oral_health.pdf). All protocols had been approved by the institutional ethics committees.

In the southern Finland district, paraffin-stimulated whole saliva samples were collected from 1294 of the Health 2000 subjects, and frozen at -70°C until further use. The present study includes 165 saliva samples; 84 samples from subjects with advanced periodontitis, each subject having at least 14 teeth with a probing pocket depth (PPD) $\geqslant 4$ mm (the periodontitis group), and 81 samples from subjects with no teeth with PPD $\geqslant 4$ mm (the control group). All 165 subjects had at least 20 teeth. More detailed information on saliva collection procedures and oral examinations can be found elsewhere (Könönen et al. 2007, Gursoy et al. 2009).

Salivary analysis of MMP-8, MMP-14, TIMP-1, and ICTP

Frozen saliva samples were thawed and centrifuged at 9,300 *g* for 3 min. and the supernatants were aliquoted for the assays of salivary markers. MMP-8 was analysed using two different methods, IFMA and a commercial ELISA kit (Amersham, GE Healthcare, Buckingamshire, UK).

The IFMA method has been described in detail previously by Hanemaaijer et al. (1997). Briefly, the monoclonal MMP-8-specific antibodies 8708 and 8706 (Medix Biochemica, Kauniainen, Finland) were used as catching and tracer antibodies, respectively. The tracer antibody was labelled using europium-chelate. The assay buffer contained 20 mM Tris-HCl (pH 7.5), 0.5 M NaCl, 5 mM $CaCl_2$, 50 μM $ZnCl_2$, 0.5% bovine serum albumin, 0.05% sodium azide, and 20 mg/l diethylenetriaminepentaacetic acid. Salivary samples were diluted in the assay buffer and incubated with the catching antibody for 1 h, followed by incubation for 1 h with the tracer antibody. Enhancement solution was added and after 5 min. fluorescence was measured using a 1234 Delfia Research Fluorometer (Wallac, Turku, Finland).

The ELISA analyses for MMP-8, TIMP-1, and MMP-14 were performed with commercial test systems (Amersham, GE Healthcare, Buckinghamshire, UK) according to the manufacturer's instructions. The concentration of MMP-14 was analysed both in the presence and absence of 1.5 mM 4-aminophenylmercuric acetate (APMA, Sigma-Aldrich, St. Louis, MO, USA). As an optimal organomercural pro-MMP activator, APMA was included to measure the total activity of MMP-14.

The ICTP concentrations were measured using an enzyme immunoassay (Orion Diagnostica Oy, Espoo, Finland).

Statistical analysis

The SPSS statistical program (Version 15.0; SPSS Inc., Chicago, IL, USA) was used to analyse the data. Logarithmic conversion did not bring the values to normality; therefore, non-parametric tests were applied. The Mann–Whitney *U*-test and Kruskal–Wallis *H*-test were used for the comparisons of the MMP-8, MMP-14, TIMP-1, and ICTP concentrations between the periodontitis and control groups. The receiver operating characteristics (ROC) curve analysis and corresponding area under the curve (AUC) analyses were performed for predicting the accuracy of the multiple salivary markers to differentiate perio-

dontitis subjects from controls. The AUC was calculated for the discriminative efficacy of each marker alone and for the ratios of MMP-8 (by IFMA)/TIMP-1 and MMP-8 (by ELISA)/TIMP-1. A logistic regression was performed to combine MMP-8 (by IFMA) and ICTP, and MMP-8 (by ELISA) and ICTP. ROC analysis was then performed using the saved probabilities from the model. Statistical significance was defined as $p < 0.05$.

Results

The characterization of the study population by age, gender, and periodontal status is presented in Table 1. The mean age and the proportions of men and smokers were higher in the periodontitis group than in the control group.

MMP-8, MMP-14, TIMP-1, and ICTP were detected in all saliva samples. Of the tested salivary markers, MMP-8, determined by both IFMA ($p < 0.001$) and ELISA ($p = 0.044$), and ICTP ($p = 0.039$) were present in higher concentrations but TIMP-1 ($p = 0.001$) was present in a lower concentration in the periodontitis group than in the control group (Table 2). While differentiating the periodontitis subjects from the controls, the IFMA technique was determined to be more effective (the mean ± SD MMP-8 concentration in the periodontitis group was 1001.0 ± 717.7 pg/ml and in the control group was 451.0 ± 420.4 pg/ml) than by the ELISA method (the corresponding concentrations were 101.6 ± 72.2 and 80.3 ± 46.6 pg/ml, respectively).

When the subjects were subgrouped according to their smoking habits, significantly higher MMP-8 concentrations (by IFMA $p < 0.001$ and by ELISA $p = 0.014$) but lower TIMP-1 concentrations ($p = 0.001$) and slightly higher ICTP concentrations ($p = 0.056$) were found in the non-smoking periodontitis subjects than in the non-smoking control subjects. In smokers, only the TIMP-1 concentration tended to differ ($p = 0.055$) between the periodontitis and control subjects.

When the markers were analysed as ratios and combinations, the MMP-8 (by IFMA)/TIMP-1 ratio ($p = 0.027$) was the only parameter with a statistically significant difference between the perio-

Table 1. Study population by age, gender, smoking habits, and periodontal status

	Periodontitis ($n = 84$)		Control ($n = 81$)		Periodontitis *versus* control *p*
	smoker (52.3%)	non-smoker (47.6%)	smoker (17.2%)	non-smoker (81.5%)	
Age in years (mean ± SD)*	48.6 ± 5.3 *	50.7 ± 4.9	44.4 ± 4.3 *	48.6 ± 5.7	0.025*
% of men†	52.3	67.5	42.9	33.3	0.003†
No. of teeth (mean ± SD)*	26.7 ± 2.2	27.3 ± 2.4	27.4 ± 2.2	27.5 ± 2.4	0.634*
No. of teeth with PPD ⩾ 4 mm (mean ± SD)*	20.3 ± 4.0 *	18.2 ± 3.4	0	0	
No. of teeth with PPD ⩾ 6 mm (mean ± SD)*	4.8 ± 6.0 *	3.6 ± 3.8	0	0	
No. of bleeding sextants (mean ± SD)*	5.0 ± 2.0	5.5 ± 1.1	2.3 ± 1.9 *	1.2 ± 1.5	< 0.001*

*p-value < 0.05.
†Kruskal–Wallis and χ^2-tests were applied in comparisons between ''smoker and non-smoker'' and ''periodontitis and control'' groups.
PPD, probing pocket depth.

Table 2. Study population by salivary biomarkers

	Periodontitis ($n = 84$)		Control ($n = 81$)		Periodontitis *versus* control *p*
	smoker (52.3%)	non-smoker (47.6%)	smoker (17.2%)	non-smoker (81.5%)	
MMP-8 (by IFMA) (ng/ml)	703.1 (338.6–1646.7)	1075.5 (345.2–1715.9)	392.6 (230.9–758.1) *	296.8 (209.7–402.2)	< 0.001
MMP-8 (by ELISA) (ng/ml)	83.6 (52.9–114.5)	96.7 (61.8–144.7) *	72.8 (48.3–151.9)	75.6 (43.2–95.6)	0.044
TIMP-1 (ng/ml)	73.0 (42.0–164.0) α	45.5 (23.3–112.3) *	121.5 (88.3–282.5)	108.5 (62.8–158.3)	0.001
ICTP (ng/ml)	0.75 (0.60–0.94)	0.74 (0.56–0.96) *	0.78 (0.56–0.94)	0.66 (0.54–0.79)	0.039
MMP-14 (with APMA) (ng/ml)	176.2 (112.7–288.3)	229.3 (138.1–360.3)	210.6 (109.1–297.0)	196.6 (142.4–336.6)	0.518
MMP-14 (without APMA) (ng/ml)	9.31 (4.3–12.8)	8.48 (2.21–11.88)	10.34 (8.38–12.09)	7.97 (4.51–10.41)	0.835
MMP-8 (by IFMA)/TIMP-1 ratio	8.26 ± 11.05 *	15.44 ± 21.48	3.03 ± 6.63 *	2.97 ± 5.34	< 0.001
MMP-8 (by ELISA)/TIMP-1 ratio	0.69 ± 0.72	1.30 ± 1.27 *	0.47 ± 0.75	0.47 ± 0.57	< 0.001
MMP-8 (by IFMA) and ICTP combination	0.58 ± 0.23 α	0.62 ± 0.23 *	0.45 ± 0.16 *	0.39 ± 0.14	< 0.001
MMP-8 (by ELISA) and ICTP combination	0.52 ± 0.12	0.55 ± 0.12 *	0.51 ± 0.11 *	0.47 ± 0.07	0.001

MMP-8, TIMP-1, ICTP, and MMP-14 values are given as medians and interquartile ranges (in parentheses).
*p-value < 0.05 and α indicates borderline significance ($p = 0.05$).
MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix metalloproteinase; ICTP, pyridinoline cross-linked carboxyterminal telopeptide of type I collagen.

dontitis and control groups in smokers (Table 2). Also, when using the combination of MMP-8 (by IFMA) and ICTP, there was a trend to distinguish between these groups ($p = 0.052$).

Figure 1 presents the ROC curves for discriminating the periodontitis and control groups. The MMP-8, TIMP-1, and ICTP concentrations, and the MMP-8 (by IFMA)/TIMP-1 and MMP-8 (by ELISA)/TIMP-1 ratios were able to distinguish between the subjects with and without periodontitis and provided AUCs larger than 0.5 (Fig. 1a). In whole population, the AUCs of the MMP-8/TIMP-1 ratio and the combination of MMP-8 and ICTP were slightly higher than those obtained by MMP-8 alone (Fig. 1b). When the smoking subjects were selected for further statistical analyses, the MMP-8 (by IFMA)/TIMP-1 ratio and the combination of MMP-8 (by IFMA) and ICTP increased the AUCs (Table 3).

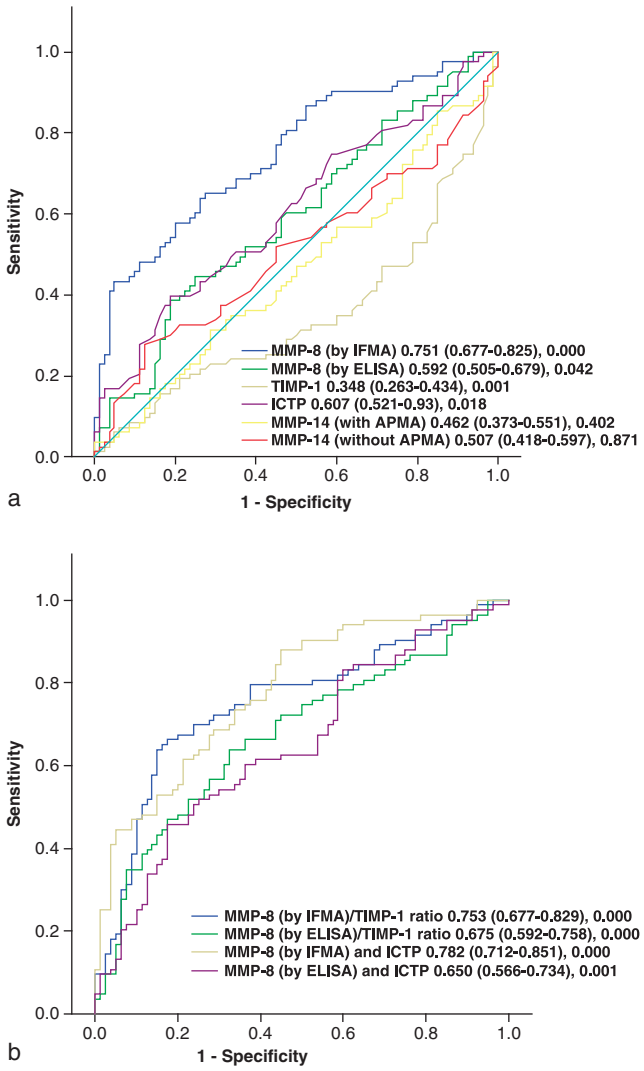

Fig. 1. Receiver operating characteristics analysis of each salivary biomarker tested (a) alone and (b) in combination or in proportion. The numbers next to each marker refer to the area under the curve (95% confidence intervals) and *p*-values.

Discussion

Periodontal health requires a balance between tissue destruction enzymes and their inhibitors. MMP-8, also called neutrophil collagenase-2, is one of the major collagenases that has a major part in the destruction of connective tissue and alveolar bone in periodontitis. MMP-8 can be activated by other MMPs (namely MMP-2, MMP-13, and MMP-14), reactive oxygen species, and inhibited by TIMP-1 and TIMP-2 (Sorsa et al. 2006). The main finding of the present study is that the concentrations of MMP-8, TIMP-1, and ICTP in saliva are associated with advanced periodontitis, while smoking, however, attenuated this association. In addition, the proportional or combined use of the salivary markers further increased the accuracy of periodontal diagnosis in these smoking subjects. Moreover, the differentiation of periodontitis subjects from controls by the IFMA technique proved to be stronger than the widely used ELISA method, based on salivary MMP-8 detection.

As a study specimen, saliva has advantages as being easily and non-invasively collected. On the other hand, it fails to detect the exact site of active disease. Also, variations in the salivary flow rate, use of antimicrobial medications, and smoking habits may have an impact on salivary analysis. Indeed, a good periodontitis marker should function at a population level, regardless of the subjects' smoking behaviours or of other modifiers. Our preliminary results need to be verified in a larger population with different stages of periodontal diseases.

In the present study, the detection of salivary MMP-8 by the IFMA technique distinguished the periodontitis and control groups with a higher accuracy than the traditional ELISA method. One explanation can be the difference in specificities between the IFMA and ELISA antibodies (Tuomainen et al. 2007, Sorsa et al. 2010). The antibody used in IFMA identifies neutrophilic and fibroblast-type MMP-8 isotypes and in particular their active forms (Hanemaaijer et al. 1997, Sorsa et al. 2010). Unlike IFMA, the ELISA method detects all forms of MMP-8, as the antibodies have been produced against full-size MMP-8 (Knäuper et al. 1990). With regard to our latest study (Sorsa et al. 2010) on the detection of MMP-8 in GCF with IFMA antibody, it was shown that the major bands in Western blots were 55 kDa MMP-8 species in the periodontitis group. Also, other MMP-8 species (complexes and fragments), but in clearly lesser amounts, can be detected in periodontitis and gingivitis groups, as well as in a positive control. It has been shown that the active form of MMP-8 is mainly found in sites with active periodontitis, while a latent MMP-8 associates with gingivitis (Lee et al. 1995, Mancini et al. 1999, Romanelli et al. 1999, Kiili et al. 2002, Sorsa et al. 2010, Teles et al. 2010). Hanemaaijer et al.

Table 3. AUCs obtained by the ROC analysis for identifying periodontitis in smokers and non-smokers

	AUC (95% confidence intervals), *p*	
	Smokers ($n = 58$)	Non-smokers ($n = 106$)
MMP-8 (by IFMA) (ng/ml)	0.633 (0.475–0.791), 0.138	0.806 (0.721–0.890), <0.001
MMP-8 (by ELISA) (ng/ml)	0.506 (0.315–0.697), 0.948	0.642 (0.531–754), 0.014
TIMP-1 (ng/ml)	0.328 (0.172–0.484), 0.055	0.298 (0.189–0.408), 0.001
ICTP (ng/ml)	0.531 (0.351–0.711), 0.732	0.611 (0.496–0.727), 0.056
MMP-14 (with APMA) (ng/ml)	0.428 (0.243–0.612), 0.420	0.516 (0.398–0.634), 0.787
MMP-14 (without APMA) (ng/ml)	0.381 (0.224–0.538), 0185	0.518 (0.395–0.640), 0.762
MMP-8 (by IFMA)/TIMP-1 Ratio	0.698 (0.548–0.847), 0.027	0.817 (0.734–0.900), <0.001
MMP-8 (by ELISA)/TIMP-1 Ratio	0.613 (0.446–0.780), 0.270	0.766 (0.669–0.862), <0.001
MMP-8 (by IFMA) and ICTP combination	0.674 (0.519–0.830), 0.052	0.819 (0.738–0.900), <0.001
MMP-8 (by ELISA) and ICTP combination	0.520 (0.329–0.711), 0.824	0.707 (0.602–0.812), <0.001

MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix metalloproteinase; ICTP, pyridinoline cross-linked carboxyterminal telopeptide of type I collagen; ELISA, enzyme-linked immunoassay; AUC, area under the curve; ROC, receiver operating characteristics.

(1997) demonstrated that, in addition to PMN-type MMP-8, the IFMA antibody can detect autoactivated fibroblast-type MMP-8. Therefore, we propose that the ability of IFMA to detect both PMN-type and fibroblast-type MMP-8, and especially in their active forms, explains its strength in differentiating periodontitis and healthy subjects.

An important aspect is that gingival inflammation regardless of subjects' pocket depths can influence MMP-8 and TIMP-1 secretions, and consequently, their concentrations in saliva. Unfortunately, due to the data collection protocols of the present study, it was not possible to form a gingivitis group from the same health survey population to analyse the effects of gingival inflammation itself on salivary MMP-8 and TIMP-1 concentrations. Therefore, in order to evaluate the impact of gingival inflammation, we later analysed the salivary MMP-8 and TIMP-1 concentrations in a relatively small group ($n = 18$) of gingivitis patients, and we found that the medians and interquartile ranges for the salivary MMP-8 (by IFMA) and TIMP-1 concentrations in this gingivitis group were 341.3 (147.32–762.8) and 95.61 (79.8–141.1), respectively (own unpublished data). When these values were compared with those of the present periodontitis and control groups, no differences in MMP-8 and TIMP-1 concentrations were observed between the control and gingivitis groups, whereas both of them were statistically different from the values of the periodontitis group. Hence, although gingival inflammation may affect salivary MMP-8 and TIMP-1 concentrations, significantly increased MMP-8 and decreased TIMP-1 concentrations in saliva are related to periodontal attachment loss.

MMP-14 concentrations, on the other hand, did not differ between the study groups. We made the MMP-14 analyses both in the presence and in the absence of APMA, an activator of latent enzymes (Syggelos et al. 2001), and the MMP-14 concentrations proved to be similar regardless the presence of APMA. To our knowledge, no studies exist on salivary MMP-14 levels in periodontitis subjects. In an experimental gingivitis model, it was shown that, after the placement of ligature, MMP-14 expression reduces within a week (Lorencini et al. 2009). As MMP-14 is known to activate several other MMPs, it may be expressed at higher levels at an early phase of disease, e.g., in gingivitis. Its weak expression in periodontitis could be through a regulatory mechanism for controlling the periodontal disease progression (Lorencini et al. 2009). Nevertheless, soluble forms of MMP-14 have been detected in periodontitis-affected GCF, bronchoalveolar lavage, tear fluid, and diabetic urine (Sorsa et al. 2006). Although MMP-14 has been shown to exhibit collagenolytic activity, this proteinase is unlikely to contribute significantly to pathologically elevated collagen degradation during periodontitis, as shown in the present study.

We found TIMP-1, a regulator of MMP-8 activity, at lower levels in the periodontitis group than in the control group. TIMP-1 is released by various cells of the periodontium (fibroblasts, keratinocytes, and endothelium) and by inflammatory cells (monocytes/macrophages) as a regulator of normal connective tissue remodelling (Reynolds 1996). Our results confirm the earlier findings on lower salivary TIMP-1 concentrations in periodontitis subjects in comparison to periodontally healthy controls (Hayakawa et al. 1994). After periodontal treatment, levels of TIMP-1 appear to increase in GCF (Pozo et al. 2005); however, some studies have failed to show such a change in the saliva (Birkedal-Hansen 1993, Ingman et al. 1996). Within the limits of the present study, it is not possible to explain these controversies.

ICTP, a 12–20 kDa fragment of type I collagen of bone, is released after bone resorption or collagen matrix degradation. Increased ICTP levels in GCF are considered to reflect alveolar bone degradation and periodontal disease activity (Giannobile 1997). There are few studies in the literature correlating its presence in saliva with an active periodontal disease. Recent studies by Ng et al. (2007) and Frodge et al. (2008) failed to detect ICTP in the saliva of most subjects with periodontal disease. In contrast, we found significantly higher salivary ICTP concentrations in the periodontitis group than in the control group.

When biomarkers of host and/or microbe origin are combined, the detection of periodontitis may be significantly improved (Hyvärinen et al. 2009, Ramseier et al. 2009). The MMP-8/TIMP-1 ratio and the MMP-8 and ICTP combination slightly increased the AUC, in comparison with an AUC by each marker alone, as defined by an ROC analysis; when MMP-8 was analysed by IFMA, the AUC increased from 0.751 (MMP-8) to 0.753 (the MMP-8/TIMP-1 ratio) and to 0.782 (the MMP-8 and ICTP combination). The increase was more prominent when the ELISA method was used to detect MMP-8; the corresponding AUC results were 0.592, 0.675, and 0.650, respectively. After

combining MMP-8 and ICTP, increases in AUC were especially stronger in smokers. As the increase in MMP and decrease in TIMP levels initiate collagen degradation from the connective tissue and alveolar bone, multiple uses of these markers, for example ratios of degradation enzymes and their inhibitors, or degradation enzyme and degradation product combinations, may increase the capability of these markers to detect periodontitis, in comparison with their single use.

Smoking, an important risk factor of periodontitis, may impair the salivary levels of cytokines and enzymes, explaining the controversial reports in the literature. In a study by Kibayashi et al. (2007), smokers had lower levels of prostaglandin E_2 and higher levels of lactoferrin, albumin, aspartate aminotransferase, lactate dehydrogenase, and alkaline phosphatase, whereas MMP-8, MMP-9, and IL-1β levels were similar between smokers and non-smokers. Raitio et al. (2005), on the other hand, reported that total MMP-9 levels were lower in smokers' saliva, whereas MMP-8 levels did not differ between smokers and non-smokers. According to the present study, smoking strongly affects the detection of periodontal disease markers. This notifiable impact of smoking on periodontal disease markers in saliva may be explained by its direct effect on periodontal and inflammatory cells, through their presence and activity in periodontal disease pathogenesis. Another explanation for the low MMP-8/TIMP-1 ratio in the smoking subjects could be that MMPs are less effective in mediating tissue degradation in smokers than in non-smokers. It is possible that other host-derived proteinases, particularly elastase, are more active in periodontal tissue breakdown in smokers (Palcanis et al. 1992). Also, another endogenous proteinase inhibitor, α1-antitrypsin, which is inactivated by smoking (Söder et al. 2002), could play a role in the ratio of active proteinase/endogenous inhibitor.

Distinguishing periodontitis subjects from controls by the detection of salivary MMP-8 is dependent on selected antibodies and detection techniques. We conclude that MMP-8, TIMP-1, and ICTP are valuable marker candidates in the detection of periodontitis in saliva, and problems caused by smoking can be overcome by their proportional (MMP-8 (by IFMA)/TIMP-1) or combined [MMP-8 (by IFMA) and ICTP] calculations.

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Clinical Relevance

Scientific rationale for the study: In periodontics, the applicability of salivary diagnostics could be used for specific salivary marker(s) in saliva to determine the activity of periodontitis or the outcome of periodontal treatment, or to detect periodontitis in field studies.

Principal findings: Distinguishing periodontitis subjects from controls by the detection of salivary MMP-8 was found to be dependent on detection techniques. The MMP-8, TIMP-1, and ICTP concentrations and the MMP-8 (by IFMA)/TIMP-1 and MMP-8 (by ELISA)/TIMP-1 ratios were able to separate the subjects with and without periodontitis.

Practical implications: Single or multiple uses of salivary MMP-8, TIMP-1, and ICTP can act as valuable marker candidates in the detection of periodontitis in saliva, especially in smoker subjects; however, in order to clarify the effect of smoking on salivary markers, studies with larger populations are needed.