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REVIEW

Oral fluid matrix metalloproteinase (MMP)-8 as a diagnostic tool in chronic periodontitis

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Abstract: Periodontal diseases that affect the marginal and apical periodontium result from the interaction between bacterial biofilm and the host response. Oral fluid biomarkers might aid clinical diagnosis. Matrix metalloproteinases (MMPs) are a family of 24 proteases that act in physiological and pathological conditions. They can degrade almost all extracellular matrix constituents and regulate inflammatory processes. They are mainly inhibited by tissue inhibitors of metalloproteinases. The aim of this study was to perform a current literature review with a special reference on the diagnostic and clinical utility of oral fluid MMPs, especially MMP-8, and their inhibitors in periodontal and oral diseases. MMP-8 is the main collagenolytic MMP detected in oral fluids, such as saliva, oral mouthrinse, gingival crevicular fluid, and peri-implant fluid. MMP-8, and potentially MMP-9, in oral fluids represent strong biomarker candidates associated especially with periodontal disease diagnosis, severity, progression, and follow-up. Additionally, they show diagnostic potential for systemic conditions, such as pregnancy, myocardial infarction, and smoking. A commercially available mouthrinse, active MMP-8 chair-side/point-of-care lateral flow immunoassay, shows enough sensitivity and specificity to detect clinical signs of periodontitis. The current literature supports that high MMP-8 levels reflect the loss of periodontal supporting tissues rather than inflammation, representing a potentially useful side-diagnostic point-of-care oral disease biomarker, especially in periodontal diseases.

Keywords: gingival crevicular fluid, saliva, biomarkers

Introduction

Periodontal diseases are chronic infections that affect the gingiva (gingivitis) and the supporting tissues of the teeth, namely, the alveolar bone, periodontal ligament, and radicular cementum in periodontitis.[1–4] The later can either affect the marginal periodontium (ie, chronic periodontitis) or the apical periradicular tissues, known as apical periodontitis.[5] They can be considered a global health problem that can lead to tooth loss, widespread oral health dysfunction, and increased susceptibility to other systemic diseases, such as cardiovascular diseases and stroke, diabetes, preterm delivery of low-birth-weight babies, arthritis, and lung inflammation.[6–8]

Periodontitis results from the interaction between bacterial biofilms and the host immune response. Because of its high prevalence and potential systemic health impact, periodontal diseases must be properly detected and treated, in order to preserve, improve, and maintain the remaining teeth, periodontium, or peri-implant tissues in health, comfort, esthetics, and function.[9]

Periodontal diagnosis is presently based on the measurement of a set of clinical parameters that includes probing depth (PD), clinical attachment level (CAL), bleeding

on probing, plaque index, and radiographic recordings.[4,6] However, in large-scale studies, these measurements are laborious, expensive, and time-consuming and require trained dental professionals.[10] Besides, they display information about the past instead of current periodontal tissue breakdown, with an inability to predict future destruction, disease progression, or the patient's response to treatment.[6,11,12] Some limitations of the diagnostic methods are basically derived from the episodic and multifactorial characteristics of periodontal diseases, which are further complicated by the facts that different teeth, and even tooth sites within the same patient, are affected by varying degrees of disease severity and may or may not undergo progression.[13,14] Because the loss of periodontal supporting tissues is cumulative and irreversible, complementary methods aiding early diagnosis and preventing progression are highly desirable.

There is a current need to find a reliable complementary diagnostic tool that ideally aids to screen, differentiate site activity, predict future tissue destruction, and monitor the response to the therapy and medication of periodontal diseases.[6] Oral fluids, such as gingival crevicular fluid (GCF), saliva, mouthrinse, and peri-implant sulcular fluid (PISF) are easily obtained by noninvasive methods and contain various molecular host immune and bacterial-derived components, often referred to as biomarkers. Oral fluid biomarkers can reflect several oral physiological and pathological conditions, including marginal periodontitis, apical periodontitis, peri-implantitis, and orthodontic tooth movement, among others. Qualitative and quantitative changes in oral fluid biomarkers have demonstrated to exert diagnostic and prognostic potential, especially in chronic periodontitis.[5,6,15] Saliva and mouth-rinse represent pooled samples at the patient-level, while GCF and PISF provide site-specific analysis.

Among host-derived biomarker candidates, matrix metalloproteinases (MMPs) represent a large family of calcium-dependent, zinc-containing proteases that act in physiological and pathological conditions.[16–18] Currently, there are 24 genetically distinct, but structurally related, human MMPs types, which are divided into subgroups, such as collagenases (MMP-1, -8, and -13), gelatinases (MMP-9 and -2), membrane-type MMPs (MMP-14, -15, -16, -17, -24, and -25), and others.[19] Together, they can degrade almost all extracellular matrix and basement membrane constituents. They can also regulate cellular and inflammatory processes, through limited and decisive proteolysis of nonmatrix bioactive substrates (enzymes, chemokines, cytokines, growth factors, complement components, receptors, and serum components, among others).[4,20–22] Downregulation of MMP-8

to physiological or normal levels appears to contribute to the resolution of inflammation through the processing of anti-inflammatory chemokines and cytokines and suggest, at least in part, also a defensive role for MMP-8 in periodontitis.[23,24]

The activities of MMPs can be regulated by delicate inductive cascades through their gene expression, the conversion of zymogen to the active enzyme, and the presence of specific inhibitors.[25] Tissue inhibitors of metalloproteinases (TIMPs)-1, -2, -3, and -4 represent the main MMP physiological inhibitors,[19] and both, inhibitors and activating factors (MMPs and reactive oxygen species as other host and microbial proteases) determine the activities of MMPs in periodontitis. Interactions between their inhibitors and activating factors will thus determine MMP activity, which finally accounts for the rate of matrix turnover or destruction,[6,26] as well as immune bioactive substrate processings. Total MMP levels can be measured by enzyme-linked immunosorbent and immunofluoromentric assays (ELISA and IFMA, respectively). The variety of MMP isoforms, proforms, active forms, fragmented species, and others, can be analyzed by Western immunoblotting, while the intrinsic and total MMP activities can be assessed by functional MMP activity assays, such as zymography or commercially available catalytic and immune assays.

Collagen I represents the bulk component of the periodontal extracellular matrix. Accordingly, collagenase or collagenolytic MMPs (MMP-1, -8, -13, and -14) and gelatinase MMPs (MMP-2 and -9) play a pivotal role in the loss of periodontal support on the basis of their collagen-degrading properties.[27] Collagenases process native collagen I and III, and gelatinases further degrade the resultant denatured collagens.[28] Among them, the main MMPs involved in periodontal tissue destruction are MMP-8, -13, and -9.[6,29]

MMP-8 constitutes the main collagenolytic MMP detected in gingival tissue and oral fluids (80% of collagenases in GCF) and has emerged as a promising periodontal biomarker.[30] The major cellular source of MMP-8 are polimorphonuclear neutrophils (PMN), but it can also be produced by various non-PMN-lineage cells such as gingival fibroblasts, endothelial cells, epithelial cells, plasma cells, macrophages, and bone cells.[31] MMP-8 has been related to periodontal disease diagnosis, severity of periodontal inflammation, progression, and treatment follow-up.[32]

MMP-13 has been observed in gingival sulcular epithelium, fibroblasts, macrophages, plasma cells, and osteoblasts; and MMP-13, together with MMP-9, has been associated with bone resorption.[33–37] MMP-9 is also present in PMN granules,

as a variety of other periodontal cell types, and it represents the main gelatinase in oral fluids. MMP-9 levels and active forms have been directly associated with periodontal inflammation and breakdown.[38] Other MMPs that are also detected in oral fluids (GCF, PISF, saliva, and mouthrinse) in minor quantities are MMP-1, -2, -3, -7, -12, -14, -25, and -26 and TIMP-1 and -2.[6,27] The aim of this study is to perform a current literature review with a special reference on the diagnostic and clinical utility of oral fluid MMPs, especially MMP-8, and their inhibitors in periodontal and oral diseases.

MMPs as diagnostic biomarkers of periodontal diseases in saliva and mouthrinse

Saliva is a clear, slightly acidic oral secretion, mainly produced by the major (parotid, submandibular, and sublingual) and minor salivary glands.[39] It is composed of organic and inorganic compounds, with bacterial and host proteins, as well as dietary, serum, and blood components, leaked to the mouth by GCF or oral wounds.[29,40]

Saliva as a diagnostic fluid has a long history and constitutes a noninvasive, readily available, easily collected sample or matrix.[6,41] Saliva collection methods through drooling, the utilization of cotton swabs, or paraffin gum chewing show a similar protein composition, with small differences in saliva volume.[10,29] It can reflect the aspects of oral and systemic health status, aiding in diagnostics of cancers, cardiovascular diseases, immunologic syndromes, and hereditary deficiencies.[29,42,43] Additionally, other environmental and systemic factors, such as smoking, diabetes, and medications, may eventually also influence biomarker concentrations.[44–46] In periodontal disease, saliva constitutes an overall view of the disease status, with a pooled sample constituted by all periodontal sites and saliva secretion.[47]

MMP-8 has been described as one of the strongest salivary biomarkers for detecting alveolar bone destruction, associated with different clinical and radiological parameters, such as deepened periodontal pockets, progression of attachment loss, alveolar bone loss, and bleeding on probing (BOP).[10] MMP-8 (mainly detected by IFMA), TIMP-1, MMP-8/TIMP-1 molar ratio, and *carboxy-terminal* telopeptide of type I collagen (ICTP) are able to distinguish between periodontitis and control groups, defined by bone resorption or the presence of periodontal pockets and ongoing periodontal attachment loss.[46,48]

MMP-8 has been observed at higher concentrations in generalized severe periodontal bone destruction than localized alveolar resorption, together with the increase of the MMP-8/TIMP-1 molar ratio and interleukin (IL)-1β, a proinflammatory cytokine.[46] MMP-9 and MMP-13 have been linked to MMP-8 and higher concentrations associated with generalized periodontitis, even when the highest levels of MMP-9 and -13 are found in localized periodontitis patients.[49] Indeed, elevated levels of MMP-8 and -9 are found in advanced periodontitis patients in comparison with a healthy/gingivitis group of subjects, and these biomarkers, in combination with red complex periodontal pathogens (such as *Porphyromonas gingivalis* and *Treponema denticola*), can more accurately predict the different disease categories.[50] MMP-14 measurements in other studies, do not show any difference between generalized periodontitis, localized periodontitis, and control group.[48] Salivary MMP-8 has also been associated with the response to therapy and medications.[26,51,52] MMP-8 and IL-1β decrease significantly in relation to scaling and root planning, and MMP-8 shows significant correlations with the changes in posttreatment clinical parameters.[52]

It should be considered that the diagnostic value of MMPs in saliva can be affected by systemic and environmental conditions, such as smoking. Smoking impairs the salivary levels of cytokines and enzymes and results in lower MMP-8 and MMP-8/TIMP-1 molar ratio levels in comparison with nonsmoker subjects.[31,46] A lower MMP-8 proteolytic activity has also been found in smokers in relation to ex-smoker subjects.[53] These findings are similar to those found in other systemic conditions, such as pregnancy, where MMP-8 shows smaller postpartum than prepartum levels, which inversely changes in relation to pregnancy gingivitis.[54] Elevated salivary MMP-8 activation has also been associated with acute myocardial infarction.[55]

However, some inconsistencies in the literature have been found between single salivary biomarkers, partially explained on the basis of different study populations, analytical methods, systemic illnesses, and the large variety of biomarkers involved in periodontal disease.[29,56–58] To overcome those difficulties, the cumulative risk score index considers a set of biomarkers that include *P. gingivalis* representing infection, IL-1β representing inflammation, and MMP-8 reflecting tissue degradation. The calculation of subscores, considering the tertile values of each biomarker, classifies the subjects into three levels of risk (I, II, and III), from the lowest to the highest one.[29,58] According to recent studies, the cumulative risk score is more accurate than any of the biomarkers alone and is able to differentiate periodontitis subjects from their controls, regardless of the coronary artery disease of the patients.[10,58] Recent studies have repeatedly demonstrated that commercially available

mouthrinse aMMP-8 chair-side/point-of-care (PoC) lateral flow immunoassay (PerioSafe® and Oral Risk Indicator®, Dentognostics GmHb, Jena, Germany) detects at least two sites with pathological deepened periodontal pockets and BOP but exerts low sensitivity for single deepened pockets and BOP (Figure 1).[29,59] This mouthrinse aMMP-8 PoC/chair-side assay is sensitive and specific enough to detect initial or early signs of periodontitis in adolescents (Figure 1).[60] The cut off value of aMMP-8 lateral flow mouthrinse immune assay is 25 ng/mL.[60] The PoC aMMP-8 immunoassay PerioSafe showed >70% sensitivity and 96% specificity.[29,60]

MMPs as diagnostic biomarkers of periodontal/peri-implant diseases in GCF and PISF

GCF and PISF are serum transudates that bathe the gingival sulcus in physiologic conditions. In the presence of inflammation, it becomes an exudate that carries molecules from both interstitial periodontal/peri-implant tissues and general circulation.[61] Thus, GCF constituents as candidate biomarkers might reflect local periodontal and systemic inflammation, respectively.[62] Higher GCF and PISF collagenolytic MMP levels, particularly of MMP-8, have been widely reported in periodontitis compared with healthy sites overcoming the protective shield provided by TIMP-1. It also associates with the levels of type I collagen degradation products, whereas the nonsurgical periodontal treatment improves clinical parameters along with the reductions of MMP-8, along with MMP-9, and -13 levels and activities.[29,30,34,63–66]

Currently, the challenge in biomarker site-specific GCF and PISF assessments point out a need to identify a diagnostic

Figure 1 Mouthrinse aMMP-8 point-of-care/chair-side assay.
Notes: Constituents (purified rinse water, cup, and syringe with filter) and lateral flow test cassette of aMMP-8 chair-side/point-of-care oral fluid immunoassay. Two lines (indicated by arrows) indicate increased, >25 mg/mL, MMP-8 in mouthrinse and elevated risk for periodontitis; even a thin second line indicate the risk (1, 2). One line indicates no risk (3) for periodontitis, the patient is under control, patient's treatment and medication have been successful and/or is in good maintenance.
Abbreviations: MMP, matrix metalloproteinase; aMMP, active matrix metalloproteinase.

array of biomarkers to account for a disease's biological profile and disclose current or future periodontal and peri-implant loss.[6,12,67] Until now, few studies have evaluated biomarkers' site-specific diagnostic potential to differentiate periodontitis sites from inflamed sites with no attachment loss, as well as their predictive/prognostic potential to identify sites at risk of progression and to evaluate treatment response.[29]

Regarding the diagnostic potential, elevated MMP-8 levels in GCF and PISF measured by two different quantitative methods (IFMA and commercial ELISA) along with myeloperoxidase (MPO), discriminate chronic periodontitis from gingivitis and healthy sites.[68] MPO is an antimicrobial neutrophil-derived enzyme that catalyzes the synthesis of hypochlorous acid, reported to oxidatively activate latent proMMP-8 and -9 in vitro.[69,70] In this context, gingivitis/peri-implant mucositis represents a control for inflamed sites with no periodontal/peri-implant attachment loss.[29] Another previous study incorporated gingivitis controls, testing a quantitative MMP-8 assay (IFMA) and a qualitative test-stick with similar results.[66] Interestingly, there is good agreement between the different tests for GCF MMP-8 assessments, but thresholds/cut-off values for diagnosis depend on the utilized specific monoclonal/polyclonal antibodies and detection method.[14,66] Nevertheless, the findings of Mäntylä et al,[66] utilizing monoclonal anti-MMP-8 antibodies, have been confirmed by Akbari et al,[71] utilizing polyclonal anti-MMP-8 antibodies. Mäntylä et al[66] and Akbari et al[71] have independently demonstrated, thus confirming each other, that the specificity and sensitivity obtained with monoclonal and polyclonal anti-MMP-8 antibodies are practically same, validating the dip-stick strip MMP-8 chair-side GCF test kit.[71] Furthermore, MMP-8 and myeloperoxidase (MPO) show a high potential for site-specific diagnosis of periodontitis determined by receiver operating characteristic curves (diagnostic accuracy 0.90–0.98). Both the markers (MMP-8 and MPO) and MMP-8 quantitative methods (IFMA and ELISA) showed very high diagnostic sensitivity and specificity discriminating from healthy and gingivitis sites, supporting their potential usefulness for PoC diagnostics. These results emphasize that high levels of MMP-8, along with MPO, reflect the loss of periodontal supporting tissues rather than solely inflammation.[68]

Significant positive correlations between collagenases (MMP-8, -13, and -14) are hinted in gingivitis and are clearly evidenced in periodontitis sites.[68,72] Noteworthy, total MMP-8 levels and its active isoforms repeatedly correlate with MPO levels in periodontitis sites, suggesting that pro-MMP-8 activation by MPO-derived Hypochlorous acid represents a key activating mechanism for triggered neutrophils.

A similar correlation is also described for MPO and MMP-9.[65] Altogether, clinical evidence suggests that inflammation contributes to the loss of periodontal homeostasis through both proteolytic and oxidative mechanisms activating key MMPs in periodontal tissues.[69,72]

In a recent study, the site-specific diagnostic accuracy of MMP-8 and MPO in GCF was further confirmed in chronic periodontitis and novel biomarker candidates emerged, including MMP-9 and soluble tartrate-resistant acid phosphatase (TRAP)-5; the latter is regarded as a direct marker of osteoclastic activity and bone resorption.[73] Moreover, the potential of GCF side-diagnosis in apical periodontitis was explored. The performance of MMP-8, MPO, MMP-9, and TRAP-5 was not limited to their high diagnostic accuracy in chronic periodontitis (0.91–1.0), but they also reflected disease severity, correlating to PD, CAL, and/or radiographic bone level. Moreover, data from our group (Hernández-Ríos P and Hernández M) demonstrate a positive correlation between bone resorption, reflected by TRAP, MMP-8, and MMP-9 activities (correlation coefficient=0.81 and 0.74, respectively, $P<0.05$). Although GCF volume and total protein concentration are limited (<1 µL and <1 mg/mL, respectively),[62] these enzymes, as opposed to proinflammatory and osteolytic cytokines, such as IL-1β, IL-6, tumor necrosis factor-α, receptor activator of nuclear factor κB ligand, and osteoprotegerin, can be easily detected and quantified in GCF, representing high diagnostic accuracy biomarkers for site-specific diagnosis of chronic periodontitis. Moreover, proteolytic metalloproteinases can regulate osteolytic cytokines, such as IL-6 and receptor activator of nuclear factor κB ligand.[74–76]

The changes in GCF composition do not seem to be limited to chronic periodontitis. MMP-8 and -9 can also differentiate between healthy and asymptomatic apical periodontitis (AAP); at higher levels, they discriminate chronic periodontitis from AAP and show diagnostic potential for the later, though lower compared with chronic periodontitis (accuracy of 0.90–0.93 for active MMP-9 and -8 ELISA, respectively).[5] The hallmark of AAP is the destruction of periradicular periodontal tissues leading to the formation of an osteolytic apical lesion in the absence of clinical symptoms.[2] Few earlier studies including GCF analysis have been conducted in AAP, but the results are consistent with these findings. Higher levels of pro-MMP-9 were reported for the first time in teeth with apical lesions and healthy contra lateral controls in a split mouth design.[77] Accordingly, higher gelatinolytic MMP-9 activity in human apical lesions was later confirmed in comparison with healthy periodontal ligament controls.[8] In addition to MMP-9, MMP-8 has also been immunolocalized to apical lesions and inflamed pulp tissue

and its levels decreased with statistical significance after intracanal calcium hydroxide medication;[78] nevertheless, no previous studies have been conducted in GCF.

The mechanisms explaining how GCF composition might be modified during AAP remain unknown. Bacteria, their products, and/or inflammatory mediators may diffuse from the apical lesions and/or infected endodontic canals either through periodontal circulation or periodontal ligament through root fractures, microcracks, exposed dentinal tubules, and lateral canals. Inflammatory mediators might also extravasate from systemic circulation. Accordingly, recent studies support that AP might associate with low-grade systemic inflammation.[79] Overall, destructive chronic inflammatory processes of the periodontium at both marginal and periapical levels can modify GCF composition with the potential for periodontal-side diagnostics and eventually to assess systemic involvement.[80]

Emerging evidence supports that host-derived MMPs can also be applied to evaluate the risk and course of periodontitis progression. MMP-8 levels and MMP-13 activity were higher in nontreated progressing versus nonprogressing periodontitis sites, categorized by the tolerance method, although they are statistically significant only for MMP-13.[72,81] After nonsurgical periodontal treatment, MMP-8 along with MPO levels were reduced, but the association of MPO and MMP-8 persisted in progressive sites.[72] In another study, stable sites that maintained their CAL and PD improvements after periodontal treatment up to the end of the maintenance phase (1 year follow-up) showed a sustained reduction of MMP-8 levels measured by IFMA and ELISA; whereas unstable sites, that showed further CAL and PD positive changes, showed marked MMP-8 picks during the maintenance phase.[14] In line with these findings, elevated concomitant levels of IL-1β and MMP-8 after nonsurgical periodontal treatment (1 year) associated with increased risk of subsequent periodontal attachment loss in postmenopausal women (2 years), identifying sites more prone to progress. Adjunctive subantimicrobial dose doxycycline medication was effective to drop the risk of periodontitis progression along with MMP-8 levels.[82]

The ability of a panel including host-derived and bacterial biomarkers in GCF saliva, serum, and dental plaque is assessed in progressing and stable chronic periodontitis patients. Progression is defined as any site undergoing CAL >2 mm from the baseline measurement, followed by 6 months untreated (disease monitoring phase) and 6 months after nonsurgical treatment (recovery phase). In GCF, mild and moderate periodontitis groups showed significantly reduced MMP-8 levels during recovery. Statistically significant higher baseline levels are found for IL-1β, osteoprotegerin, MMP-8,

and -9 in progressive versus stable sites. The authors state that singular analysis of GCF biomarkers is useful to predict periodontal disease progression, but when combined with clinical and other biological measures, the predictability rises. The greatest degree of sensitivity for progression is found for saliva biomarkers and the greatest specificity for GCF. Serum, on the other hand, shows no prognostic role to identify future periodontal progression.[13]

It is noteworthy that tobacco smoking can influence MMP-8 levels in GCF from periodontitis subjects. Tobacco seems to downregulate MMP-8 levels; however, the levels remain high after nonsurgical treatment in progressive sites of both non-smokers and smokers.[32] Moreover, high MMP-8 levels at baseline significantly predict a weak treatment response in smokers, defined by the patterns of CAL reductions. According to their findings, the authors recommend monitoring of MMP-8 levels during the maintenance period.[83] For this purpose, a PoC/chair-side oral fluid MMP-8 assay (PerioSafe) is a very practical, inexpensive, and fast diagnostic tool to be performed by dental and medical professionals, and even by the patients themselves (Figure 1). Overall, testing of MMP-8 in GCF, PISF, and/or oral fluids (saliva and mouthrinse) represents a promising chair-side/PoC diagnostic tool to identify patients' and sites at risk for periodontal/peri-implant disease progression and predict treatment response.

Acknowledgments

Disclosure

Professor Timo Sorsa is an inventor of US patents 5652227, 5736341, 5866432, and 6143476. The authors report no other conflicts of interest in this work.

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