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SHORT COMMUNICATION

Linking oral microbial proteolysis to aMMP-8 PoC diagnostics along with the stage and grade of periodontitis: A cross-sectional study

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Through this brief report, we wish to stress upon the role periodontopathogens play in the direct and indirect activation of matrix metalloproteinases (MMPs) via microbial proteinases from potent dysbiotic periodontopathogens to elaborate a local and systemic inflammatory and tissue destructive immune response in the pathogenesis of periodontitis (Hajishengallis & Chavakis, 2021; Sorsa et al., 2016). An attempt was also made to relate this to the modern point-of-care diagnostics for periodontitis, its new clinical classification as well as related systemic diseases such as prediabetes/diabetes which has an established bidirectional relationship with periodontitis (Deng et al., 2021; Grigoriadis et al., 2019, 2021; Sanz et al., 2018; Sorsa et al., 2020).

There exists evidence in the literature to support the fact that potent dysbiotic periodontopathogens can elaborate microbial proteases which can activate latent forms of MMP-1, MMP-8, and MMP-9 by direct proteolysis along with instigating their secretion not only from inflammatory neutrophils but also from fibroblasts of gingival origin and oral keratinocytes (Nieminen et al., 2018; Sorsa et al., 1992, 1995).

proMMPs have been evidenced to be activated by the proteases and virulence factors derived from *T. denticola* (*T.d.*) (Ding et al., 1996; Sorsa et al., 1992, 1995). The outer membrane protein of *T.d.* has been known to incite the release of cathepsin G and elastase from polymorphonuclear leukocytes (PMNs) (Ding et al., 1996). The *T.d.* microbial proteinases may also potentiate the activation of MMPs via the oxidative pathway (Ding et al., 1996). *P. gingivalis* (*P.g.*), *F. nucleatum* (*F.n.*), and *T.d.* have been demonstrated to be phagocytosed as whole cells by PMNs in relatively similar numbers, however, significant differences were observed in the PMN degranulation which each produced (Ding et al., 1997).

Periodontitis and dysbiotic periodontopathogens such as *T.d.* have been observed to play an important role in the induction and promotion of carcinogenesis at both oral and extraoral sites (Heikkilä et al., 2018; Nieminen et al., 2018). This can be related to the fact that the enhanced proteolysis, chiefly mediated by the host MMPs, has been demonstrated to play an important role in both cancer development and progression (Nieminen et al., 2018; Sorsa et al., 1992). Nevertheless, it remains questionable if the proteinases elaborated by periodontopathogens are released in quantities equitable with the tissue degradation observed in periodontal and its related systemic diseases (Hajishengallis & Chavakis, 2021; Nieminen et al., 2018; Sorsa et al., 1992, 1995, 2016).

Over time, however, it has become clear that proteinases responsible for causing matrix degradation of which collagenases in particular found in the diseased periodontium, are chiefly obtained from PMNs (Sorsa et al., 2016). Upon release by selective degranulation from PMNs, the latent forms of these MMPs are converted to their activated forms by means of their interaction with reactive oxygen species or by proteolytic cleavages (Sorsa et al., 2016). Indeed, PMN-derived collagenolytic aMMP-8 activity and immunoreactivity are elevated and activated in the gingival tissue, gingival crevicular fluid (GCF), saliva, and mouth rinse of patients suffering from periodontitis and can hence, be diagnostically utilized by chair-side/point-of-care (PoC) lateral flow immunoassays (Alassiri et al., 2018; Deng et al., 2021; Lee et al., 1995; Räisänen et al., 2021; Sorsa et al., 2016, 2020).

The rapidity with which the degranulation of PMNs progresses along with the levels of enzymes released upon microbial phagocytosis further strengthens the credence of this cascade as a major mechanism in the pathophysiology of periodontitis (Sorsa et al., 2016). It seems well established that PMNs serve as the major source for MMPs and serine-type proteases (Sorsa et al., 2016). It is also interesting to note that reactive oxygen species scavenging compounds such as tetracyclines, chlorhexidine, and ascorbate can prevent the activation of latent MMPs (Gendron et al., 1999; Sorsa et al., 2016; Suomalainen et al., 1991). The maintenance of MMP-8 levels within physiological limits forms a significant part and principle of therapeutics (Sorsa et al., 2016).

This study aimed to investigate the relationship between periodontal dysbiotic bacteria (*T.d.* and its dentilisin proteinase), prediabetes (HbA1c ≥5.7%), and active matrix metalloproteinase-8 (aMMP-8) in the pathogenesis and various phases, i.e., stages and grades of periodontitis (Grigoriadis et al., 2021; Tonetti et al., 2018). 150 Greek adult patients attending a Periodontology University Clinic and fulfilling the criteria for testing according to the screening questionnaire of the Centers for Disease Control and Prevention (CDC) were enrolled in this study in accordance with a previous description (Grigoriadis et al., 2019, 2021; Sorsa et al., 2020). The study was approved by the Ethical Committee of the School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece (#64, 12/June/2018). Chair-side assessment of HbA1c in capillary blood was measured using the Cobas Roche b101® diagnostic system (Grigoriadis et al., 2019, 2021). Levels of active MMP-8 (aMMP-8) were analyzed quantitatively by the chair-side/PoC PerioSafe® lateral flow mouthrinse immunotest accompanied by the digital reader ORALyzer® according to the manufacturer's instructions (Deng et al., 2021; Räisänen et al., 2021; Sorsa et al., 2020). Total/latent salivary MMP-8 levels were measured by R&D Systems ELISA kits (Räisänen et al., 2021). Western immunoblots utilizing specific polyclonal antibody for MMP-8 were performed for the verification of proMMP-8 activation by *T.d.* dentilisin (Nieminen et al., 2018; Sorsa et al., 1992, 1995). Clinical periodontal and oral health parameters were assessed for six surfaces of each tooth using an automated probe (Florida probe, Florida Probe Corporation) and the patients were stratified according to the new classification for periodontitis (31 healthy, 15 stage I, 81 stage II, and 23 stage III patients) (Tonetti et al., 2018). The results thus obtained were subjected to statistical analyses with SPSS Statistics, version 27 (IBM Corp). The association between aMMP-8, total/latent MMP-8 along with the stage and grade of periodontitis (extent/severity and rate of disease progression, respectively) and prediabetes were assessed by the Kruskal–Wallis test and pairwise post hoc comparisons (Dunn-Bonferroni test). Statistical significance was determined with p-values ≤ 0.05.

Figure 1a depicts statistically significant differences in aMMP-8 levels between the stage (disease severity) of periodontitis as described previously (Sorsa et al., 2020). The lowest aMMP-8 levels were detected among the healthy and stage I periodontitis patients and the highest aMMP-8 levels among patients with the stage II and III periodontitis (Sorsa et al., 2020). Noteworthy, as Figure 1b depicts, similar differences in total/latent MMP-8 levels could not be observed in the same healthy and periodontitis patients ($p = 0.180$). Upon activation by *T.d.* dentilisin latent 75 kDa proMMP-8 was converted to 65 kDa active, and fragmented lower molecular size MMP-8 species, in a time-dependent manner, as illustrated in Figure 1c. Finally, patients with rapid rate of periodontitis progression (grade C) and prediabetes exerted serially increasing and highest aMMP-8 levels (Figure 2).

Thus, an increase in aMMP-8 levels, but not in total/latent MMP-8 levels, was reflective of progressive disease activity (stage [severity] and grade [rate of progression] of periodontitis) particularly with prediabetes but also in its absence. Prediabetes/diabetes has an established bidirectional relationship with periodontitis, which have been shown to increase the risk of poor glycemic control in patients with diabetes mellitus (DM), and related risk of associated diabetes complications and morbidity, due to the increased production of bacterial products and inflammatory mediators eventually ending up into the bloodstream from periodontal pockets (Sanz et al., 2018). In this study, Western blot revealed that *T.d.* dentilisin activated latent proMMP-8 to active and fragmented low molecular weight MMP-8 species. These same aMMP-8 forms and species are specifically detected by the antibody utilized by the aMMP-8 PoC test (Deng et al., 2021; Grigoriadis et al., 2019, 2021; Räisänen et al., 2021; Sorsa et al., 2016, 2020). The results in the present study are in agreement with previous studies which indicate that such selective assays and antibodies for aMMP-8, but not for total/latent MMP-8, are beneficial in periodontal disease diagnostics (Alassiri et al., 2018;

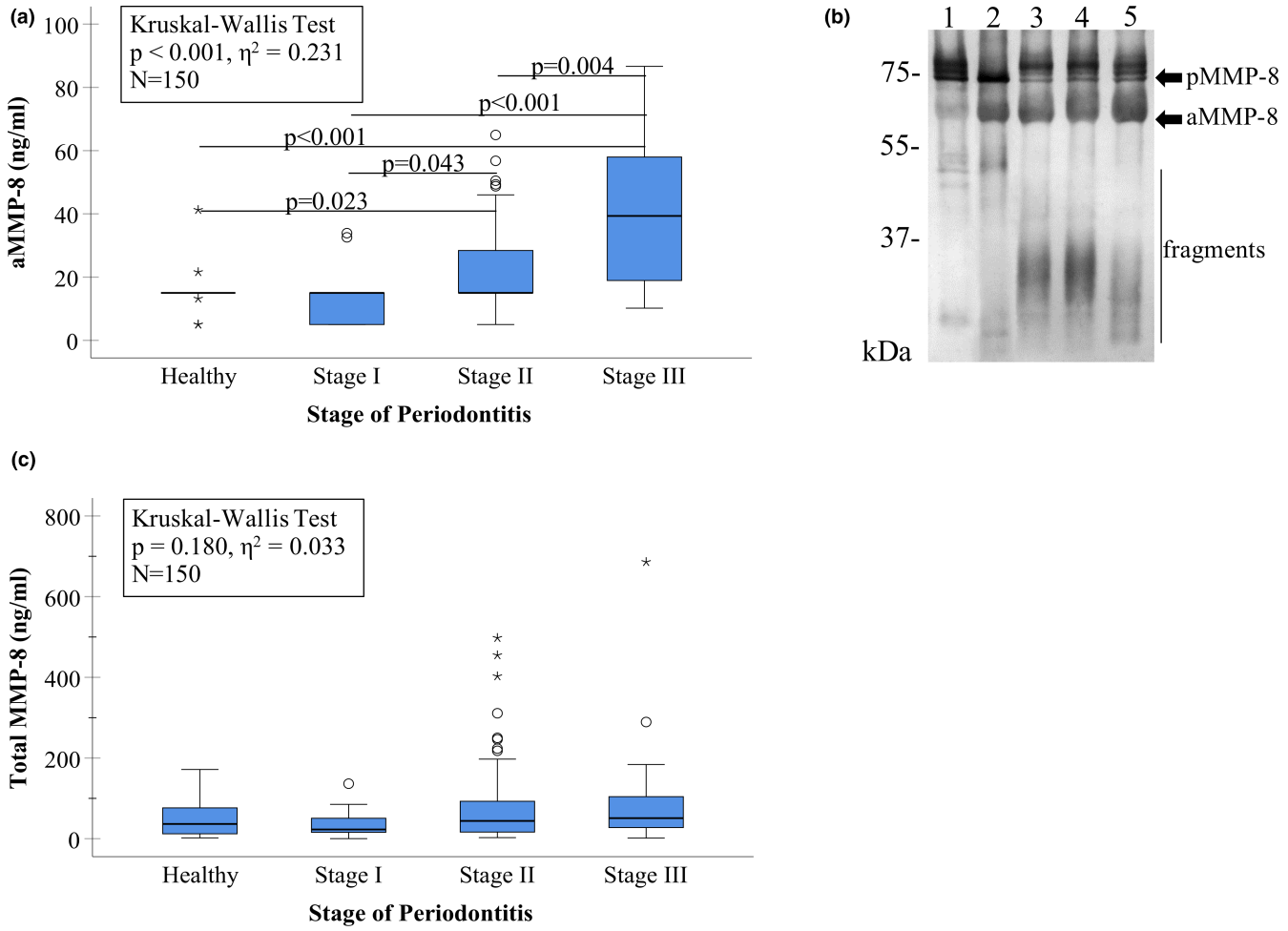

FIGURE 1 Box plot of concentrations of matrix metalloproteinase-8 (MMP-8) (ng/ml) of 150 Greek adults as described in Sorsa et al. (2020) were measured by (a) the quantitative active MMP-8 (aMMP-8) lateral flow mouth rinse point-of-care technology (POCT) (PerioSafe/ORALyzer combination) and (b) total (latent & active) salivary MMP-8 (ELISA, Quantikine, R&D Systems) categorized by stage of periodontitis. The figure depicts statistically significant differences in aMMP-8 and total MMP-8 levels between groups assessed by the Kruskal–Wallis test, effect sizes (η^2), and pairwise post hoc comparisons (Dunn–Bonferroni test). (c) Western blot revealing conversion of latent human proMMP-8 (pMMP-8) (lane 1) to active and fragmented MMP-8 species by *Treponema denticola* protease dentilisin (chymotrypsin-like protease) (lanes 2–5) indicated by arrows. Mobilities of molecular weight markers are indicated on left. According to Deng et al. (2021) aMMP-8 lateral flow POCT can detect these active and fragmented MMP-8 species. Panel A from Sorsa et al. (2020) is reproduced under the terms and conditions of the Creative Commons Attribution (CC BY) license https://creativecommons.org/licenses/by/4.0/

Lee et al., 1995; Sorsa et al., 2016, 2020). A prospective study by Lee et al. (1995) was one of the first to demonstrate longitudinally, the important role active MMP-8 played in the progression of periodontitis. Furthermore, concentrations of active and fragmented MMP-8 species in saliva have been longitudinally demonstrated to correlate well not only with salivary *T.d.* concentrations but also with the progression of periodontitis (Gürsoy et al., 2018). The aMMP-8 PoC test correlates strongly with standard laboratory methods, ELISA and IFMA, with the same antibody and has been validated in various countries in both adolescent and adult populations as a means to define periodontal disease at both the site- and patient-levels, assess prognosis and evaluate patients in the treatment and maintenance phases (Alassiri et al., 2018; Deng et al., 2021; Räisänen et al., 2021; Sorsa et al., 2016, 2020). As the results in the present study indicate, elevated aMMP-8 levels are reflective of oral triggered host response, and hence, as a biomarker, increased disease progression. The highest aMMP-8 levels were detected in patients with prediabetes and grade C of periodontitis (rapid rate of disease progression). This may be the result of an alteration of the pathogenic dysbiotic potential of periodontal microbiota and/or modification of the host inflammatory response to the microbial challenge that may further be potentiated with prediabetes (Grigoriadis et al., 2019, 2021; Hajishengallis & Chavakis, 2021; Nieminen et al., 2018; Sorsa et al., 1992).

Smoking is a major behavioral risk factor for periodontitis associated with host inflammatory response and progression of periodontitis (Leite et al., 2018). Previous studies have reported that it can both up- and down-regulated aMMP-8 levels in smokers compared with

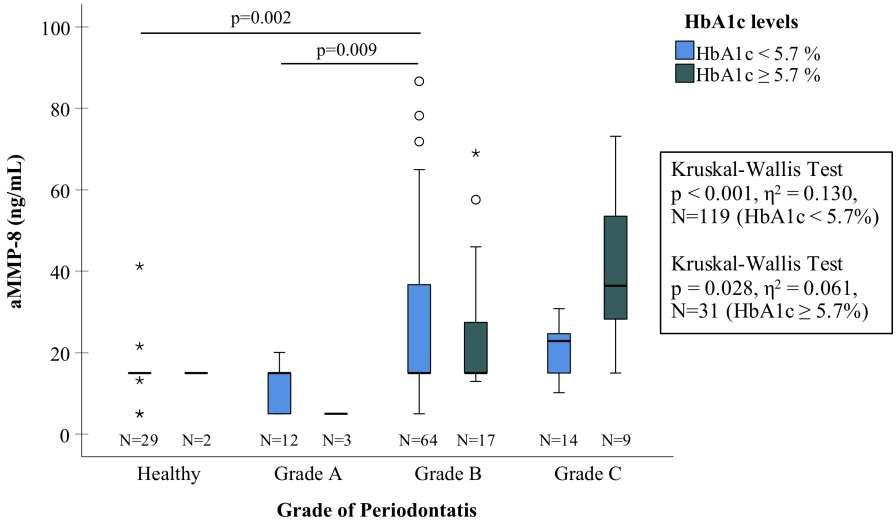

FIGURE 2 Box plot of aMMP-8 levels (ng/mL) as measured by the quantitative, online, and real-time aMMP-8 PoC mouthrinse test (PerioSafe/ORALyzer) and categorized by grade of periodontitis and HbA1c levels (<5.7% & ≥5.7%; $n = 150$). The figure depicts statistically significant differences in aMMP-8 levels between groups assessed by the Kruskal–Wallis test, effect size (η^2), and pairwise post hoc comparisons (Dunn–Bonferroni test)

nonsmokers and former smokers (Liede et al., 1999; Mäntylä et al., 2006). In this study, smokers and nonsmokers grouped together showed that elevated aMMP-8 levels were linked to increased severity and progression of periodontitis, which is in agreement with and extends previous findings (Johnson et al., 2016). This suggests that the effectiveness of the type of aMMP-8 PoC testing utilized in this study seems to not be adversely affected by smoking, yet we recognize that further research in larger populations is required to confirm this.

In the present study, only one microbial protease (*T.d.* dentilisin) was investigated. The positive results obtained here suggest that further research may be extended to comprise of a wider selection of periodontopathogenic bacteria and microbial proteases to investigate their potential relationship with collagenolytic activity and periodontitis progression in other larger populations to confirm and extend these results. Further, incorporation of such activating microbial proteinase(s) from potent dysbiotic periodontopathogens, such as *Treponema denticola's* dentilisin (Nieminen et al., 2018; Sorsa et al., 1992, 1995) into aMMP-8 PoC technologies may eventually strengthen and improve the oral fluid diagnostics of periodontal disease (Deng et al., 2021; Räisänen et al., 2021; Sorsa et al., 2020).

CONFLICT OF INTEREST

Prof Timo Sorsa is the inventor of U.S. 5,652,223, 5,736,341, 5,864,632, 6,143,476 and US 2017/0023571A1 (issued June 6, 2019), WO 2018/060553 A1 (issued May 31, 2018), 10,488,415 B2, and US 2017/0023671A1, Japanese Patent 2016–554676 and South Korean patent 10–2016–7025378. Dirk-Rolf Gieselmann is the inventor of US patent 2017/0023571A1 and a Japanese patent 2016–554676. The other authors report no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

AUTHOR CONTRIBUTIONS

Shipra Gupta: Conceptualization; Data curation; Investigation; Writing-original draft; Writing-review & editing. **Vaibhav Sahni:** Conceptualization; Data curation; Investigation; Visualization; Writing-original draft; Writing-review & editing. **Ismo T. Räisänen:** Data curation; Investigation; Methodology; Visualization; Writing-original draft; Writing-review & editing. **Andreas Grigoriadis:** Data curation; Investigation; Methodology; Writing-review & editing. **Dimitra Sakellari:** Conceptualization; Data curation; Methodology; Supervision; Writing-review & editing. **Dirk-Rolf Gieselmann:** Data curation; Methodology; Visualization; Writing-review & editing. **Timo Sorsa:** Conceptualization; Data curation; Funding acquisition; Methodology; Supervision; Writing-original draft; Writing-review & editing.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/odi.14008.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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