Salivary matrix metalloproteinase (MMP)-8 as a biomarker for periodontitis

A PRISMA-compliant systematic review and meta-analysis

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Abstract
Background: Salivary matrix metalloproteinase (MMP)-8 is currently considered to be one of the most promising biomarkers for early diagnosis of periodontitis, however, several recent studies showed conflicting results.

Objective: To determine the salivary matrix metalloproteinase (MMP)-8 levels between periodontitis patients and healthy individuals, and to assess its diagnostic value in periodontitis.

Methods: Literatures were searched on PubMed and Embase databases up to August 2017, for articles reporting salivary MMP-8 levels between periodontitis patients and health controls with the data of means ± standard deviation (SD). Methodological quality was assessed by the Newcastle Ottawa scale (NOS). Standard mean differences (SMDs), heterogeneity, and publication bias were assessed by Stata 13.0 software.

Results: A total of 10 studies including 485 periodontitis patients and 379 healthy controls that met the preset inclusion criteria were included, the qualities of these studies were either good (n = 7) or moderate (n = 3). Eight studies showed salivary MMP-8 levels were higher in periodontitis patients compared with healthy controls (P < .05), while 2 studies showed opposite results (P > .05). The pooled SMD was 1.195 (95% CI: 0.720–1.670), with P of 89.3%, indicating high heterogeneity. Funnel plot showed publication bias existed.

Conclusion: Our meta-analysis showed that salivary MMP-8 levels were significantly higher in periodontitis patients compared with healthy controls overall. Due to the heterogeneity and publication bias of included studies, further high quality studies are still needed to verify the conclusion.

Abbreviations: IL = interleukin, MMP = matrix metalloproteinase, NOS = Newcastle Ottawa scale, PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses, SD = standard deviation, SMDs = standard mean differences, TNF-α = tumor necrosis factor-α.

Keywords: biomarker, matrix metalloproteinase (MMP)-8, meta-analysis, periodontitis, salivary

1. Introduction

Periodontitis is an inflammatory disease caused by bacterial infection, with the characteristics of periodontal damage, alveolar bone resorption, and eventually tooth loss.\textsuperscript{[1]} It is generally considered to be one of the most common diseases worldwide, with a prevalence of 15% to 20%.\textsuperscript{[2]} Of greater concern, periodontitis has been shown to be associated with other serious diseases, such as coronary heart disease, head and neck carcinoma, and chronic obstructive pulmonary disease.\textsuperscript{[3–5]} Therefore, early detection and intervention of periodontitis is of great importance. Differentiating destructive periodontitis patients from healthy individuals is simple at professional level, which mainly rely on clinical diagnostic criteria such as probing depth, attachment level, bleeding on probing, plaque index, and radiographic assessment.\textsuperscript{[6]} However, the early stage of initiation and/or progression remains a challenge for dentist based on the above clinical diagnostic criteria.\textsuperscript{[7]} Saliva has the advantages of being easily and noninvasively collected, thus biomarkers from saliva for early detection of periodontitis are desirable.

In the past few years, great efforts have been made to explore these biomarkers. As periodontitis is an inflammatory response, the inflammatory process will lead to increased secretion of pro-inflammatory cytokines such as interleukin (IL)-1α, IL-1β, IL-6, and tumor necrosis factor-α (TNF-α).\textsuperscript{[8]} Following this, neutrophils release a variety of enzymes such as matrix metalloproteinase (MMP), and inflammatory mediators. Biomarker detections from saliva are noninvasive, easily accessible, and economically friendly, and several types of salivary biomarkers have been shown to be associated with both oral diseases and systemic diseases.\textsuperscript{[9]} Salivary biomarkers such as IL-1, IL-6, and MMP-8 have been reported to be significantly elevated in periodontitis patients compared with healthy controls.\textsuperscript{[10]}

MMPs are key proteases involved in periodontitis and associated with periodontal status.\textsuperscript{[11,12]} Type I collagen accounts for large quantities of periodontal extracellular matrix, thus
special attention has been paid to collagenases and gelatinase such as MMP-8, MMP-13, MMP-2, and MMP-9 in periodontitis. Because type I collagen represents the bulk component of periodontal extracellular matrix, thus special attention has been paid to collagenases. Among them, MMP-8 is the main collagenase in periodontitis; moreover, 90% to 95% of collagenolytic activity in gingival crevicular fluid originated from MMP-8. Thus MMP-8 is currently considered to be one of the most promising biomarkers for periodontitis in oral fluids.

While some studies showed higher levels of salivary MMP-8 in periodontitis patients compared with healthy individuals, other studies showed opposite or contradictory results. While some studies showed higher levels of salivary MMP-8 in periodontitis patients compared with healthy individuals, other studies showed opposite or contradictory results.

To the best of our knowledge, the diagnostic value of salivary MMP-8 in periodontitis has not been systematically evaluated with all currently available data yet. Therefore, we aim to do a systematic review and meta-analysis to determine its diagnostic value between periodontitis patients and healthy controls.

2. Materials and methods

2.1. Focused question

The focused question was “Do salivary MMP-8 level differ significantly between periodontitis patients and healthy controls?” If answer is “yes,” we could use MMP-8 as a potential biomarker for early diagnosis of periodontitis.

2.2. Search strategy

We searched PubMed and Embase electric database (up to August 2017) for the studies reporting salivary MMP-8 level between periodontitis patients and healthy controls with the data of means ± standard deviation (SD) and sample size (number of patients included).

The following search terms were used: (1) “matrix metalloproteinase-8” OR “matrix metalloproteinase 8” OR “MMP-8” OR “MMP8”; (2) “salivary” OR “saliva”; (3) “periodontitis” OR “periodontal disease.” Then the above three parts were connected by Boolean operator “AND.” Articles published in other languages except English, in-vitro studies and animal studies were excluded, and no other filter was set. We also searched reference lists from original articles or reviews to include more related studies. The authors were contacted for details if needed.

2.3. Study selection

The included studies should fulfill the following criteria: (1) clinical trials, either cross-sectional or observational studies in human; (2) presence of periodontitis patients compared with healthy controls; (3) evaluated salivary MMP-8 in relation to periodontitis; (4) studies that presented with numerical values of sample size and mean ± SD of MMP-8 levels, or it could be calculated from available data of the study.

2.4. Data extraction and quality assessment

Two authors (LZ and LH) independently identified included studies and extracted the data for further analysis. The data were tabulated by the study participants, inclusion criteria for periodontitis patients and healthy controls, assay used for detecting MMP-8, regions of the study performed, salivary MMP-8 levels (mean ± SD) with sample size, and statistical significance (P value). Discrepancy was resolved by consensus meeting with other co-authors to arrive at consensus. The whole process of literature selection was summarized in Figure 1, according to PRISMA guidelines.

Methodological quality assessment was done by 2 authors based on Newcastle Ottawa scale (NOS) grading system, the details of each item in this grading system were described previously, which was used for quality evaluation of observational studies and nonrandomized studies.

2.5. Statistical analyses

Meta-analysis was conducted to the primary outcome: mean salivary MMP-8 level (mean ± SD) between periodontitis patients and healthy controls. Forest plot was produced reporting standardized mean differences (SMDs) and 95% confidence interval (CI), which were calculated for each study. SMD is the mean divided by the SD of a difference in each study between patients and controls. It can be seen as the mean difference if all data were transformed to a scale where SD within groups was equal to 1.0. Funnel plot was used for the evaluation of publication bias.

Heterogeneity was assessed using Higgins I², Tau-square, and Chi-square tests, with I²>75% indicating relevant heterogeneity. When the heterogeneity test was statistically significant, random-effects model was used. Otherwise fix-effects model was used. Publication bias was assessed by funnel plot. All the above statistical analyses were performed with Stata 13.0 software (Stata Corporation, College Station, TX). A P value <.05 was considered to be statistically significant.

2.6. Ethical review

This study was a systematic review and meta-analysis, and did not involve patient consent. Thus ethical approval could be waived.

3. Results

3.1. Identification of studies

The searches yielded 275 relevant articles for consideration primarily. After reviewing the titles and abstracts, 251 articles were excluded because they did not fulfill the inclusion criteria. Around 25 studies were selected for full-text review, and 15 studies were excluded in this step (Fig. 1). A total of 10 studies, including 485 periodontitis patients and 379 health controls that met the preset inclusion criteria were included for meta-analysis, the characteristics of the 10 studies were summarized in Table 1.

3.2. Quality assessment

Study quality as assessed by the NOS was summarized in Table 2, the quality varied across the studies. Among the 10 included studies, 7 studies were graded as good quality and 3 studies were graded as moderate quality. All 10 studies met the NOS criteria for case definition, and had good representativeness.

3.3. Data synthesis and meta-analysis

The salivary MMP-8 levels from each independent study of both periodontitis patients and health controls were summarized in Table 1. The study of Gursoy et al enrolled patients with MMP-8 detected by 2 different methods (immuno-fluorometric
assay [IFMA] and enzyme linked immunosorbent assay [ELISA]), with separate data of MMP-8 levels (mean ± SD), respectively, thus the 2 set of data were analyzed separately. The range of MMP-8 levels (mean ± SD) varied greatly among different studies, from 2.95 ± 0.66 (n=27) to 888.6 ± 990.1 (n=84) in periodontitis patients, and from 2.51 ± 0.81 (n=18) to 309.4 ± 183.4 (n=81) in healthy controls. The pooled SMD was 1.195 (95% CI: 0.720–1.670), with the forest plot drawn in Figure 2. For heterogeneity testing, Chi-square was 93.66 (P < .05), and $I^2$ (variation in SMD attributable to heterogeneity) was 89.3%. The estimate of between-study variance Tau-square was 0.5373. Test of SMD = 0, $z$ = 4.93 (P < .05). Thus the variability in difference was significant. Funnel plot was shown in Figure 3, indicating publication bias.

4. Discussion
This meta-analysis systematically evaluated the salivary MMP-8 levels between periodontitis patients and healthy controls of 10 independent studies from different countries. In general, our results showed that MMP-8 level was significantly higher in periodontitis patients than in health controls, although the heterogeneity exists among different studies. MMPs are proteolytic enzymes belonging to zinc protease super family involved in physiological degradation of extracellular matrix proteins and basement membranes, and they can be categorized into several groups.[13] MMP-8 belongs to collagenase group, which exhibits a unique ability to decompose type I and III collagen.[13] MMP-8 levels have been found to correlate with the levels of type I collagen degradation products, overcoming the protective shield of tissue inhibitors of MMP in disease active sites compared with inactive sites from periodontitis patients and healthy controls.[13] Therefore, it can be hypothesized that MMP-8 acts as a biomarker in periodontitis. The salivary level of MMP-8 varied greatly between different studies and the SD was also relatively variable in some studies,[10,17,21,22] which may partly be explained by the variation in salivary flow rate, use of antimicrobial agents, and smoking habits. These factors may cause interference in salivary analysis to some extent. On the other hand, different detection methods (such as ELISA, IFMA, and Luminex) may also contribute to the variability. SMD was calculated in order to reduce discrepancy. Our results showed that $I^2$ = 89.3%, indicating substantial heterogeneity. Thus random-effects model was used for SMD estimation. The heterogeneity may be due to different detection methods used, such as IFMA versus ELISA, which may be explained by the different specificity of antibodies used in the 2 methods.[24] The heterogeneity may also be induced by the unstandardized diagnosis criteria for periodontitis, different enrolling criteria for healthy controls, different study population (gender, age), and different study designs. Funnel plot analysis indicated publication bias existed, which may be partially explained by 3 studies with relatively small sample size locating far away from the funnel’s
Table 1: Characteristics of the 10 studies included in this study.

| Study (author, year) | Detection method | Periodontitis vs control | Salivary MMP-8 levels (mean ± SD) (ng/mL) | Criteria for periodontitis | Criteria for controls |
|---------------------|------------------|--------------------------|------------------------------------------|---------------------------|-----------------------|
| Miller et al., 2006 | ELISA USA        | P < .0005                | Periodontitis: 408.6 ± 423.3 (n = 28)   | Healthy adults of similar age, race and sex who had BOP < 10% of their periodontal sites, had <2% periodontal sites of PD > 5mm, had <1% periodontal sites of CAL > 2mm, and had no radiographic bone loss evident in posterior vertical bitewing films. | Subjects with no teeth with PD > 4mm |
| Gursoy et al., 2010 (1) | FMA Finland | P < .05                  | Periodontitis: 888.6 ± 900.1 (n = 84)   | Subjects with no teeth with PD > 4mm |
| Gursoy et al., 2010 (2) | ELISA USA        | P < .05                  | Periodontitis: 89.8 ± 53.1 (n = 84)     | Patients with chronic periodontitis, which was assessed on the basis of PPD, CAL, gingival index (GI), and plaque index (PI). | Subjects with no teeth with PD > 4mm |
| Emonds et al., 2013 | ELISA USA        | P < .05                  | Periodontitis: 283.47 ± 203.4 (n = 50)  | In good general health and periodontal health, PPD > 5mm in <2% of sites, no PD > 5mm, CAL of > 2mm in <1% of sites. | Subjects with no teeth with PD > 4mm |
| Mechiari et al., 2013 | ELISA Brazil    | P < .05                  | Periodontitis: 2.95 ± 0.66 (n = 27)     | Periodontally healthy subjects, with the following exclusion criteria: any periodontal surgery within 6 month; any chronic diseases, infectious diseases, immunological diseases, pregnancy, lactation, smoking, and use of medication. | Subjects with intact periodontium |
| Kushinskii et al., 2011 | Enzyme immuno assay | P < .05               | Periodontitis: 298.37 ± 234.81 (n = 82) | Patients with chronic generalized periodontitis | Subjects with intact periodontium |
| Gupta et al., 2015 | ELISA India     | P < .05                  | Periodontitis: 348.26 ± 202.1 (n = 20)  | Patients with chronic periodontitis, which was assessed on the basis of PPD, CAL, gingival index (GI), and plaque index (PI). | Subjects with intact periodontium |
| Johnson et al., 2016 | Luminex USA     | P < .011                 | Periodontitis: 129.8 ± 89.1 (n = 31)    | Subjects with BOP, <2% sites with PD > 3mm, and no sites with PD > 5mm |
| Rangbulla et al., 2017 | ELISA India     | P < .001                 | Periodontitis: 672.18 ± 411.0 (n = 30)  | Clinically healthy periodontium. Exclusion criteria: Patients with systemic diseases; Patients undergoing or who have undergone organ transplantation; Patients on corticosteroid medications or cytotoxic drugs; Pregnant and lactating patients. | Subjects with intact periodontium |
| Noack et al., 2017 | Time-resolved immunoassay | P < .05                | Periodontitis: 100.39 ± 15.62 (n = 20)  | Periodontally healthy subjects |
| Germany | P < .05         |                           | Control: 26.11 ± 28.40 (n = 20)          | Had at least 20 teeth without clinical attachment loss and symptoms of gingival inflammation. | Subjects with intact periodontium |
| Martinez et al., 2017 | ELISA Sweden    | P < .05                  | Periodontitis: 333.6 ± 66.7 (n = 28)    | At least 20 own teeth with no interproximal attachment loss and without clinical signs and symptoms of gingival inflammation. | Subjects with intact periodontium |
| Control: 66.7 ± 33.3 (n = 7) |                           |                           | Had at least 20 teeth excluding third molars. Exclusion criteria: systemic diseases and medications; Treatment with antibiotics <8 weeks before the study, pregnancy or breast feeding. | Subjects with intact periodontium |

BOP = bleeding on probing; CAL = clinical attachment loss; ELISA = enzyme linked immunosorbent assay; GI = gingival index; FMA = fluorescence immunoassay; MMP = matrix metalloproteinase; PI = plaque index; PD = probing depth; PPD = probing pocket depth.
margin. Therefore, the findings in this meta-analysis should be analyzed with caution. Thus further high-quality studies with robust design and larger sample size are highly recommended.

In conclusion, our meta-analysis results suggested that, salivary MMP-8 levels were significantly higher in periodontitis patients than in healthy controls. Substantial heterogeneity existed among these included studies, thus prospective studies and randomized designs with larger sample size are still needed to verify our results in the future.

References

[1] Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005;366:1809–20.
[2] Kassebaum NJ, Bernabe E, Dahiya M, et al. Global burden of severe periodontitis in 1990–2010: a systematic review and meta-regression. J Dent Res 2014;93:1045–53.
[3] Leng WD, Zeng XT, Kwong JS, et al. Periodontal disease and risk of coronary heart disease: an updated meta-analysis of prospective cohort studies. Int J Cardiol 2015;201:469–72.
Zeng XT, Deng AP, Li C, et al. Periodontal disease and risk of head and neck cancer: a meta-analysis of observational studies. PLoS One 2013;8:e79017.

Zeng XT, Tu ML, Liu DY, et al. Periodontal disease and risk of chronic obstructive pulmonary disease: a meta-analysis of observational studies. PLoS One 2012;7:e46508.

Gupta N, Gupta ND, Gupta A, et al. Role of salivary matrix metalloproteinase-8 (MMP-8) in chronic periodontitis diagnosis. Front Med 2015;9:72–6.

Ghiabi E, Weerasinghe S. The periodontal examination profile of general dentists in Nova Scotia, Canada. J Periodontol 2011;82:33–40.

Silwual N, Singh AK, Arunz B, et al. Human resistin stimulates the pro-inflammatory cytokines TNF-alpha and IL-12 in macrophages by NF-kappaB-dependent pathway. Biochem Biophys Res Commun 2005;334:1092–101.

Rathnayake N, Akerman S, Klinge B, et al. Salivary biomarkers for detection of systemic diseases. PLoS One 2013;8:e61356.

Ebersole JL, Schuster JL, Stevens J, et al. Patterns of salivary analytes provide diagnostic capacity for distinguishing chronic adult periodontitis from health. J Clin Immunol 2013;33:271–9.

Rathnayake N, Gustafsson A, Norhammar A, et al. Salivary matrix metalloproteinases and myeloperoxidase in relation to coronary heart and periodontal diseases: a subgroup report from the PAROK-RANK study (periodontitis and its relation to coronary artery disease). PLoS One 2015;10:e0126370.

Franco C, Patricia HR, Tino S, et al. Matrix metalloproteinases as regulators of periodontal inflammation. Int J Mol Sci 2017;18:pu: E440.

Rangbull V, Nirola A, Gupta M, et al. Salivary IgA, interleukin-1beta and MMP-8 as salivary biomarkers in chronic periodontitis patients. Chin J Dent Res 2017;20:43–51.

Noack B, Kipping T, Tervahartiala T, et al. Association between serum and oral matrix metalloproteinase-8 levels and periodontal health status. J Periodontal Res 2017;52:824–31.