Diagnostic accuracy of gingival bleeding on brushing, salivary activated MMP-8 and a self-reported questionnaire in early detection of periodontitis: abridged secondary publication

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KEY MESSAGES

1. The modified questionnaire of the Centers for Disease Control and Prevention and the American Academy of Periodontology is useful for self-detecting periodontal diseases, and its integration with demographic/lifestyle variables further improves diagnostic performance.
2. The oral rinse activated MMP-8 point-of-care test can identify different severities of periodontitis, and combining it with individual and lifestyle profiles enhances diagnostic performance.
3. Self-reported gingival bleeding on brushing is a sign of periodontal diseases, particularly gingival inflammation.
4. The haemoglobin levels of saliva/toothpaste slurry could be useful for identifying generalised severe periodontitis.
5. Salivary biomarkers of certain adipose metabolism–related and ageing-related proteins as well as inflammatory cytokines may potentially identify patients with severe periodontitis.

Hong Kong Med J 2025;31(Suppl 7):S32-5
HMRF project number: 07182796

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Introduction

Periodontal diseases, including gingivitis and periodontitis, are among the most common pathological conditions in humans.[1] Periodontitis results from dysbiotic biofilm-induced dysregulated immunoinflammatory responses in susceptible individuals. It is a major cause of severe tooth loss in adults and substantially affects oral health and quality of life. Periodontitis is closely associated with systemic comorbidities such as diabetes mellitus and cardiovascular disease.[2,3] Periodontal status can reflect host susceptibility to various systemic inflammatory comorbidities.[4,5] Gingival bleeding on brushing (GBoB) is a sign of periodontal disease.[6] Salivary biomarkers such as salivary activated MMP-8 (aMMP-8), haemoglobin, and pro-inflammatory cytokines can be used for periodontal assessment and diagnosis.[7-9] This study aimed to assess the diagnostic performance of: (1) GBoB as a sentinel sign, (2) salivary aMMP-8 and other oral fluid-based periodontal biomarkers, and (3) a validated questionnaire alone and/or in combination for the early detection of periodontitis.

Methods

The study protocol was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (references: UW19-188 and UW22-132). Oral informed consent was obtained from all participants.

In the development cohort, consecutive patients at the Prince Philip Dental Hospital were recruited from July 2019 to August 2020 to undergo three screening tests: (1) a self-administered, eight-item, validated Chinese version of the questionnaire of the US Centers for Disease Control and Prevention (CDC) and the American Academy of Periodontology (AAP),[10,11] (2) an oral rinse aMMP-8 point-of-care test (POCT),[7,8] and (3) the GBoB test with subsequent detection of salivary haemoglobin levels in saliva/toothpaste slurry samples (TPS) using ultraviolet-visible spectroscopy. All participants then underwent a full-mouth periodontal examination by a single examiner, and periodontal diagnosis was made according to the current classification.[12,13] Oral samples were stored at -80°C for further analysis.

In the external validation cohort, a representative community sample was recruited using random sampling. Individuals with edentulism, pregnancy, periodontal treatment in the previous 12 months, or antibiotic treatment in the last 3 months were excluded. Participants underwent (1) a self-administered and modified 13-item CDC-AAP questionnaire, (2) collection of an unstimulated

whole saliva sample for quantitation of haemoglobin levels using enzyme-linked immunosorbent assay (ELISA) and assessment of a cluster of inflammatory protein biomarkers by Cytokine ELISA Plate Arrays and MILLIPLEX immunoassays, (3) the oral rinse aMMP-8 POCT, (4) the GBoB test with detection of salivary haemoglobin levels in the TPS using ultraviolet-visible spectroscopy and a high-sensitivity ELISA, and (5) full-mouth periodontal and radiographic examinations by two well-calibrated examiners. Periodontal diagnosis was then made following the current classification.[12,13] Oral samples were stored at -70°C for further assessment.

All questionnaire responses were dichotomised; missing or unanswered items were excluded from analysis. Periodontal conditions were categorised as periodontal health, gingivitis, stages I/II periodontitis, and stages III/IV (severe) periodontitis.[13] Inter-group differences were compared using the Chi-squared test or Fisher's exact test. Univariate associations were examined using binary logistic regression. Logistic regression models were then constructed for identifying periodontal diseases (gingivitis and periodontitis), periodontitis, stages I/II periodontitis, and stages III/IV periodontitis. Variables with P values ≥0.20 were selected for the multivariable models. The backward stepwise selection method was used, and the area under the receiver operating characteristic curve (AUROC), sensitivity, and specificity were calculated. Model 1 included each question from the modified CDC-AAP questionnaire, whereas model 2 consisted of demographic/lifestyle variables (eg, age, sex, tobacco smoking, and diabetes). Model 3 was constructed by integrating models 1 and 2. These models were validated by the algorithms in the development cohort, then used to identify periodontitis and severe periodontitis in the external validation cohort. Discriminative power was evaluated using AUROC, sensitivity, specificity, and positive and negative predictive values.

Results

In the development cohort, 408 individuals (189 men and 219 women) aged 18 to 86 (mean±standard deviation, 41±18) years were included. Of these, 7.8% were current smokers. Overall, 68.6% had periodontitis (15.9% stage I, 15.9% stage II, 29.7% stage III, and 7.1% stage IV), whereas the remaining 31.4% had no periodontitis (16.2% periodontal health and 15.2% gingivitis). The questionnaire exhibited moderate-to-high accuracy for detecting periodontal diseases (gingivitis and periodontitis), periodontitis, and stages III/IV periodontitis, with AUROC of 0.837, 0.803, and 0.870, respectively. Model 3, incorporating the self-reported CDC-AAP questionnaire and demographic/lifestyle variables, exhibited the highest accuracy for detecting periodontitis, particularly severe periodontitis (AUROC=0.953, sensitivity=95.7%, and specificity=89.0%).

The results of the aMMP-8 POCT were correlated with periodontal parameters and periodontitis after adjustment for confounders, with 33.2% sensitivity and 93.0% specificity for periodontitis detection (threshold: >10 ng/mL). However, the aMMP-8 POCT was less effective at differentiating among stages of periodontitis or distinguishing periodontal health from gingivitis. The aMMP-8 level adjusted by the number of teeth present improved periodontitis detection, yielding 67.1% sensitivity and 68.8% specificity. It predicted stage IV periodontitis at a threshold of 0.4312 ng/mL (AUROC=0.856, sensitivity=89.7%, and specificity=73.6%). Further integration with age and tobacco smoking enhanced diagnostic performance (AUROC=0.883, sensitivity=82.5%, and specificity=84.4%).

Of the 408 participants, 37.1% reported having GBoB; they exhibited more severe gingival inflammation than those without GBoB (median % of bleeding on probing: 25.0% vs 13.5%; P<0.001). Self-reported GBoB enhanced diagnostic performance for gingival inflammation and periodontal diseases (odds ratios=3-8), but it exhibited relatively low sensitivity for discriminating gingivitis from periodontitis. The haemoglobin level in TPS was positively correlated with the number of bleeding sites (r=0.409 for concentration, r=0.520 for total amount; P<0.001) and the number of periodontal pockets; the strongest correlations were observed with the number of bleeding pockets ≥6 mm (r=0.538, P<0.001), as well as the total amount of haemoglobin and number of bleeding sites (r=0.520, P<0.001). Participants who self-reported having GBoB exhibited higher haemoglobin levels than those without GBoB (205.69 μg/mL vs 62.98 μg/mL). A concentration of 90.58 μg/mL of haemoglobin or 0.51 μL of blood volume enabled visual detection of GBoB (AUROC=0.848). Moreover, haemoglobin levels were significantly higher in patients with gingivitis and periodontitis than in individuals with periodontal health. The absence of self-reported GBoB and low haemoglobin levels exhibited high predictive values (93%-98%) for periodontal health.

In the external validation cohort, 384 individuals (146 men and 238 women) aged 18 to 84 (mean±standard deviation, 55.3±16.6) years were included; 89.1% were non-smokers. Of these, 6.3% were periodontally healthy, 19.3% had gingivitis, and 74.4% had periodontitis (4.2% stage I, 21.7% stage II, 59.1% stage III, and 15.0% stage IV). The severity of periodontal diseases significantly increased with age and the prevalences of cardiovascular disease and diabetes.

Overall, there were significant differences in

multiple responses among groups with different periodontal conditions. Most periodontally healthy individuals reported that they had no gum disease and frequently used dental floss. An increased number of individuals experienced chewing difficulty with the onset and progression of gum disease from periodontal health to gingivitis and severe periodontitis. Some questions aimed at identifying periodontal diseases and periodontitis were validated in the external validation cohort, which is representative of the Hong Kong community. The proposed questionnaire-based algorithm for predicting periodontitis achieved higher accuracy in the external validation cohort than in the development cohort.

Increased accuracy was observed for the oral rinse aMMP-8 POCT alone in differentiating among stages of periodontitis. Considering the two thresholds of the aMMP-8 test (10 ng/mL and 20 ng/mL), the external validation data were consistent with the development data. The ratio of aMMP-8 level to the number of teeth present was useful for identifying the patients with severe periodontitis. The aMMP-8 test could differentiate the most susceptible patients at high risk of periodontitis from those at low to moderate risk. Moreover, this test could differentiate localised from generalised form of periodontitis. Integration of demographic/lifestyle factors with the aMMP-8 POCT demonstrated improved diagnostic performance.

The haemoglobin levels in TPS from patients with generalised severe periodontitis were significantly higher than those from individuals with localised gingivitis and generalised stages I/II periodontitis. Overall, haemoglobin level was more effective in predicting generalised stage III/IV periodontitis than other stages or extents of periodontitis. Moreover, detectable haemoglobin was present in unstimulated saliva samples mainly from patients with generalised severe periodontitis. Patients with severe periodontitis had significantly increased levels of several salivary biomarkers (eg, adipose metabolism factors). Collectively, these findings highlighted the potential utility of biomarkers in unstimulated saliva and haemoglobin in TPS for assessing periodontal inflammation and disease progression.

Discussion

In the development cohort, the self-reported CDC-AAP questionnaire was useful for detecting periodontitis; combining this questionnaire with demographic/lifestyle variables (eg, smoking) could further enhance the identification of patients with severe periodontitis. The aMMP-8 POCT was also useful for screening to identify periodontitis, particularly when combined with demographic/lifestyle variables such as smoking. Self-reported GBoB, which can be visually detected after minor blood loss, could serve as a sentinel sign of periodontal diseases, particularly in the presence of gingival inflammation. It may be useful for proactively promoting disease prevention and timely oral/periodontal healthcare.

In the external validation cohort, the modified CDC-AAP questionnaire was useful for identifying periodontitis. It validated several questions for self-detection of periodontal diseases and periodontitis, such as self-perception of gum disease and self-reported loose teeth. These findings could contribute to public education regarding oral/periodontal health, proactive prevention of periodontal diseases, and the promotion of oral healthcare in clinical practice. The aMMP-8 POCT enabled identifying individuals with and without periodontitis. Its integration with individual and lifestyle profiles further enhanced diagnostic performance in clinical settings. The advanced assays involving the featured panels of biomarkers showed significantly increased levels of certain adipose metabolism–related and ageing-related factors, as well as inflammatory cytokines, in unstimulated saliva from patients with severe periodontitis, compared with individuals without periodontitis. These salivary biomarkers may be useful for evaluating periodontal status and tracking disease progression.

Conclusion

The multi-faceted and integrated approaches of self-reported questionnaires and tests as well as oral fluid-based diagnostic biomarkers may facilitate preventive oral/periodontal healthcare and clinical interventions.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#07182796). The full report is available from the Health and Medical Research Fund website (https://rfs2.healthbureau.gov.hk).

Disclosure

The results of this research have been previously published in:

1. Deng K, Pelekos G, Jin L, Tonetti M. Diagnostic accuracy of self-reported measures of periodontal disease: a clinical validation study using the 2017 case definitions. J Clin Periodontol 2021;48:1037-50.
2. Deng K, Pelekos G, Jin L, Tonetti M. Diagnostic accuracy of a point-of-care aMMP-8 test in the discrimination of periodontal health and disease. J Clin Periodontol 2021;48:1051-65.
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Acknowledgements

We thank Dr Ke Deng, Dr Lut Ting Lam, Dr Choi Ka Kung, Dr Tianfan Cheng, and Ms Tingting Lin for their contributions.

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