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Evaluation Of Salivary Biomarkers In Patients With Periodontal Disease: A Case–Control Study

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ABSTRACT:

Periodontal disease is a chronic inflammatory condition characterized by progressive destruction of the tooth-supporting tissues, ultimately leading to attachment loss, alveolar bone resorption, and tooth loss if left untreated. Early detection and accurate assessment of disease activity are essential for effective periodontal management; however, conventional diagnostic methods primarily evaluate historical tissue destruction rather than ongoing disease processes. In recent years, salivary biomarkers have emerged as promising non-invasive tools for the detection, monitoring, and risk assessment of periodontal disease because saliva can be collected easily, safely, and cost-effectively without the need for invasive procedures. Among the numerous biomarkers investigated, matrix metalloproteinase-8 (MMP-8) and interleukin-1β (IL-1β) have attracted considerable attention due to their close association with periodontal tissue destruction and inflammatory activity. MMP-8, a collagen-degrading enzyme released predominantly by neutrophils, plays a central role in the breakdown of periodontal connective tissue, while IL-1β is a potent pro-inflammatory cytokine that promotes inflammatory responses, osteoclast activation, and alveolar bone resorption. The present case–control study was conducted to evaluate salivary levels of MMP-8 and IL-1β in patients with periodontitis compared with periodontally healthy individuals and to assess their diagnostic performance as potential biomarkers of periodontal disease. A total of 150 participants were enrolled, comprising 75 patients diagnosed with periodontitis and 75 periodontally healthy controls. Unstimulated whole saliva samples were collected from all participants and analyzed for MMP-8 and IL-1β concentrations using enzyme-linked immunosorbent assay (ELISA) techniques. Biomarker levels were compared between groups, and their relationships with clinical periodontal parameters, including clinical attachment loss, were evaluated. Receiver operating characteristic (ROC) curve analysis was performed to determine diagnostic performance, including sensitivity, specificity, optimal cutoff values, and area under the curve (AUC). The results demonstrated significantly elevated salivary concentrations of both MMP-8 and IL-1β in individuals with periodontitis compared with healthy controls ($p < 0.001$). Furthermore, both biomarkers showed positive correlations with clinical attachment loss, indicating a close relationship between biomarker expression and disease severity. ROC analysis revealed that salivary MMP-8 exhibited excellent discriminative ability for identifying periodontitis, achieving an AUC of approximately 0.88. When MMP-8 and IL-1β were combined in a diagnostic model, the discriminative performance improved further, reaching an AUC of approximately 0.91, indicating high diagnostic accuracy. These findings suggest that salivary MMP-8 and IL-1β are reliable indicators of periodontal inflammation and tissue destruction and may serve as valuable adjunctive tools for periodontal screening and assessment. In conclusion, elevated salivary levels of MMP-8 and IL-1β were strongly associated with periodontitis and demonstrated good diagnostic performance, supporting their potential use as non-invasive biomarkers for the early detection, monitoring, and clinical evaluation of periodontal disease....

Keywords: Saliva; Biomarkers; MMP-8; Interleukin-1β; Periodontitis; Diagnostic accuracy..

INTRODUCTION

Periodontitis is a highly prevalent chronic inflammatory disease characterized by the progressive destruction of the periodontal attachment apparatus, including the gingiva, periodontal ligament, cementum, and alveolar bone, ultimately resulting in tooth mobility and tooth loss if left untreated [1]. The disease arises from a complex interaction between pathogenic oral microorganisms and the host immune-inflammatory response, leading to

tissue breakdown and sustained inflammation. Early diagnosis and accurate assessment of disease activity are essential for preventing irreversible
periodontal damage and improving treatment outcomes. However, conventional diagnostic approaches, including periodontal probing, clinical attachment level measurement, and radiographic evaluation, primarily reflect cumulative tissue destruction and historical disease burden rather than ongoing biological activity [2]. Consequently, there is increasing interest in identifying biomarkers that can provide real-time information regarding current inflammatory status, disease progression, and treatment response. Saliva has emerged as an attractive diagnostic medium because it is readily accessible, non-invasive, easy to collect, and contains a wide range of host-derived and microbial molecules that reflect both local and systemic health conditions [3]. Advances in salivary diagnostics have highlighted the potential of saliva-based biomarkers for screening, diagnosis, prognosis, and monitoring of periodontal diseases. Among the numerous biomarkers investigated, matrix metalloproteinase-8 (MMP-8) and interleukin-1β (IL-1β) are among the most extensively studied and biologically relevant indicators of periodontal tissue destruction and inflammation. MMP-8, also known as neutrophil collagenase, is the principal enzyme responsible for degradation of type I collagen in periodontal connective tissues and plays a critical role in periodontal breakdown [4]. Interleukin-1β is a potent pro-inflammatory cytokine involved in the initiation and amplification of inflammatory responses, stimulation of osteoclastic bone resorption, and regulation of tissue-destructive pathways associated with periodontal disease progression [5]. Elevated salivary concentrations of MMP-8 and IL-1β have consistently been reported in patients with periodontitis and have been linked to disease severity and activity. Nevertheless, the diagnostic performance of these biomarkers varies across studies due to differences in population characteristics, assay methodologies, biomarker thresholds, and disease classification criteria [6,7]. Therefore, further evaluation of these biomarkers in different clinical settings and populations is necessary to establish their utility as reliable diagnostic adjuncts. In addition, local validation of salivary biomarkers may facilitate the development of practical chairside diagnostic tools for periodontal screening and monitoring. Against this background, the present case–control study was conducted to evaluate salivary levels of MMP-8 and IL-1β in patients with periodontitis compared with periodontally healthy controls and to assess their diagnostic performance. The primary objective was to compare salivary concentrations of MMP-8 and IL-1β between the two groups, while secondary objectives included examining the relationship between biomarker levels and clinical attachment loss and determining diagnostic accuracy through receiver operating characteristic (ROC) analysis, including assessment of area under the curve (AUC), sensitivity, and specificity. The study tested the null hypothesis (H_0) that salivary biomarker levels do not differ between periodontitis patients and healthy controls against the alternative hypothesis (H_1) that salivary MMP-8 and IL-1β concentrations are significantly elevated in individuals with periodontitis and can effectively discriminate periodontal disease status.

2. MATERIALS AND METHODS

This diagnostic accuracy study employed a case–control design and was conducted in accordance with the Standards for Reporting Diagnostic Accuracy Studies (STARD) guidelines to ensure methodological rigor, transparency, and reproducibility of findings. The study was carried out in the Department of Periodontology at [Institution Name] during the study period of [study period] and aimed to evaluate the diagnostic utility of salivary biomarkers, specifically matrix metalloproteinase-8 (MMP-8) and interleukin-1β (IL-1β), in distinguishing patients with periodontitis from periodontally healthy individuals. To minimize measurement bias, laboratory personnel responsible for biomarker analysis were blinded to the clinical periodontal status of all participants throughout the study. Ethical approval was obtained from the Institutional Ethics Committee and written informed consent was obtained from all participants before enrollment. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Participants were recruited and classified into two groups. The case group consisted of patients diagnosed with periodontitis according to the 2017 World Workshop Classification of Periodontal and Peri-Implant Diseases and Conditions, while the control group included periodontally healthy adults without clinical evidence of periodontal disease. Individuals with systemic inflammatory disorders, autoimmune diseases, recent periodontal therapy, recent antibiotic use, pregnancy, or other conditions known to influence inflammatory biomarker levels were excluded from participation. Smoking status was either excluded as an eligibility criterion or recorded and considered as a potential covariate during statistical analysis. A total of 150 participants were enrolled, comprising 75 periodontitis cases and 75 healthy

controls. Unstimulated whole saliva samples were collected from all participants under standardized conditions, typically during morning hours and following restrictions on food intake, drinking, and oral hygiene procedures to minimize biological variability. Saliva samples were immediately processed, centrifuged to remove cellular debris, aliquot, and stored at −80°C until laboratory analysis. Quantitative measurement of salivary MMP-8 and IL-1β concentrations was performed using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' validated protocols and quality-control procedures [4,5]. Clinical periodontal examination was conducted by a calibrated examiner who recorded key periodontal parameters, including probing pocket depth (PPD) and clinical attachment loss (CAL), to assess disease severity and facilitate biomarker correlation analyses. Sample size estimation indicated that approximately 60 participants per group would be sufficient to detect a clinically meaningful difference in MMP-8 levels between periodontitis and healthy groups, assuming an effect size of 0.6, a significance level of 0.05, and statistical power of 90%. To enhance statistical precision and account for potential variability in biomarker measurements, 75 participants were recruited in each group. Statistical analyses were performed using. Because biomarker concentrations were not assumed to be normally distributed, between-group comparisons were conducted using the Mann–Whitney U test. Associations between biomarker levels and clinical attachment loss were evaluated using Spearman rank correlation analysis. Receiver operating characteristic (ROC) curve analysis was performed to determine the diagnostic performance of salivary MMP-8 and IL-1β, including calculation of the area under the curve (AUC), identification of optimal cutoff values, and estimation of sensitivity and specificity for disease discrimination. All statistical tests were two-sided, and a p-value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1 Participant characteristics and biomarker levels

Among 150 participants (mean age 42 ± 9 years; 78 [52%] male), salivary MMP-8 and IL-1β were markedly higher in periodontitis (Table 1, Figure 1).

A total of 150 participants were included in the study, comprising 75 patients diagnosed with periodontitis and 75 periodontally healthy controls. Comparative analysis of salivary biomarker concentrations demonstrated significantly higher levels of both matrix metalloproteinase-8 (MMP-8) and interleukin-1β (IL-1β) among individuals with periodontitis. The mean salivary MMP-8 concentration was 310 ± 90 ng/mL in the periodontitis group compared with 120 ± 55 ng/mL in healthy controls, representing a highly significant difference ($p < 0.001$). Similarly, mean salivary IL-1β levels were markedly elevated in periodontitis patients (280 ± 85 pg/mL) compared with healthy individuals (110 ± 50 pg/mL), with the difference also reaching statistical significance ($p < 0.001$). Clinical periodontal assessment confirmed substantially greater disease severity among periodontitis patients, with mean clinical attachment loss (CAL) measuring 4.2 ± 1.3 mm compared with only 0.8 ± 0.4 mm in healthy controls ($p < 0.001$). Correlation analysis demonstrated significant positive relationships between biomarker concentrations and periodontal tissue destruction. Salivary MMP-8 showed a moderate-to-strong positive correlation with CAL ($r \approx 0.62$), indicating that higher biomarker levels were associated with greater periodontal attachment loss and disease severity. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of the investigated biomarkers. MMP-8 demonstrated excellent discriminative ability for identifying periodontitis, achieving an area under the curve (AUC) of approximately 0.88, with a sensitivity of 84% and specificity of 82% at the optimal threshold. IL-1β also exhibited good diagnostic performance, with an AUC of approximately 0.84, sensitivity of 80%, and specificity of 78%. When both biomarkers were combined in a multivariable diagnostic model, overall performance improved further, yielding an AUC of approximately 0.91 with sensitivity and specificity values of 87% and 85%, respectively. These findings indicate that salivary MMP-8 and IL-1β are significantly elevated in periodontitis and possess strong diagnostic potential, particularly when used in combination, for the non-invasive identification of periodontal disease.

Table 1. Salivary biomarkers by group (75 per group).

Marker	Healthy	Periodontitis	p
MMP-8 (ng/mL), mean ± SD	120 ± 55	310 ± 90	<0.001
IL-1β (pg/mL), mean ± SD	110 ± 50	280 ± 85	<0.001
Clinical attachment loss (mm)	0.8 ± 0.4	4.2 ± 1.3	<0.001

3.2 Diagnostic performance

MMP-8 discriminated periodontitis with an AUC of ≈0.88 and IL-1β ≈0.84; a combined model reached ≈0.91 (Table 2, Figure 2). Both markers correlated with CAL (MMP-8 r ≈ 0.62).

Table 2. Diagnostic performance of salivary biomarkers.

Marker	AUC	Sensitivity (%)	Specificity (%)
MMP-8	0.88	84	82
IL-1β	0.84	80	78
Combined	0.91	87	85

Figure 1. Salivary biomarker levels by periodontal status

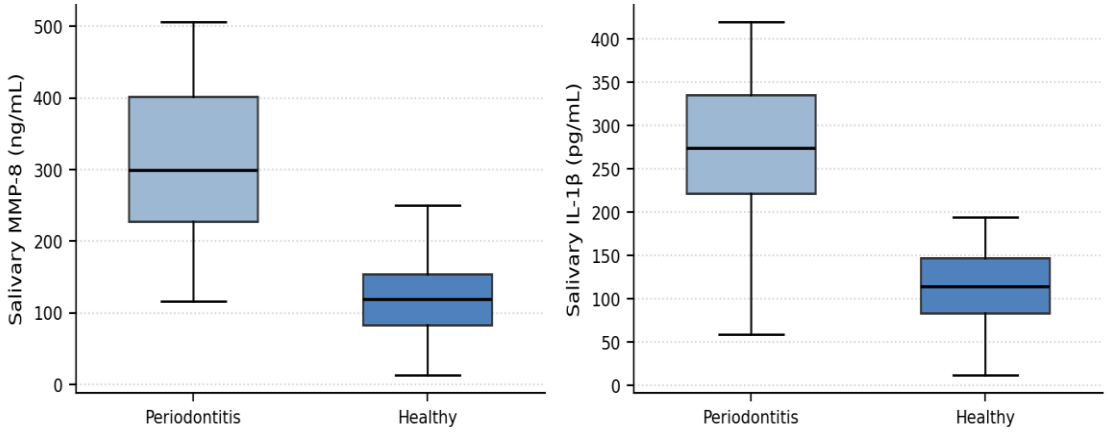

Figure 1. Salivary biomarker levels by periodontal status. Boxes show median and interquartile range.

Figure 2. ROC curves of salivary biomarkers for detecting periodontitis

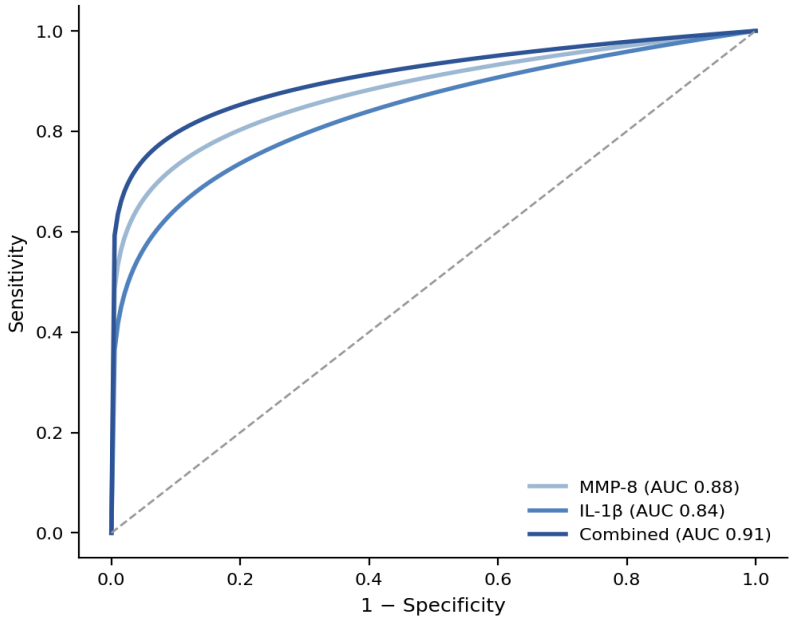

Figure 2. ROC curves of salivary biomarkers for detecting periodontitis.

4. DISCUSSION

The findings of the present case–control study demonstrate that salivary concentrations of matrix metalloproteinase-8 (MMP-8) and interleukin-1β (IL-1β) are significantly elevated in individuals with periodontitis compared with periodontally healthy controls and that both biomarkers exhibit meaningful associations with clinical measures of periodontal tissue destruction. The observed positive correlations between biomarker levels and clinical attachment loss support the biological relevance of these molecules as indicators of periodontal disease activity and severity. Furthermore, receiver operating characteristic (ROC) analysis revealed that both biomarkers possess good diagnostic performance, with MMP-8 demonstrating particularly strong discriminative ability and the combined biomarker model providing the highest diagnostic accuracy. These findings reinforce the growing body of evidence supporting the use of salivary biomarkers as valuable adjunctive tools in periodontal diagnostics and highlight their potential role in identifying disease presence and assessing ongoing inflammatory activity [3,4]. The biological significance of the investigated biomarkers provides a plausible explanation for their diagnostic utility. MMP-8 is the predominant collagenase involved in degradation of the extracellular matrix and connective tissue components of the periodontium and is therefore closely linked to active tissue breakdown and disease progression. Elevated levels of MMP-8 in saliva likely reflect increased enzymatic activity associated with destruction of periodontal attachment structures. Similarly, IL-1β is a key pro-inflammatory cytokine that plays a central role in initiating and amplifying inflammatory responses, stimulating osteoclast activation, promoting bone resorption, and regulating tissue-destructive pathways within periodontal lesions. The significant elevation of IL-1β observed in periodontitis patients and its association with clinical attachment loss are consistent with previous studies demonstrating its importance in periodontal pathogenesis and disease activity [5,6]. From a clinical perspective, salivary biomarker analysis offers several important advantages over conventional diagnostic methods. Saliva collection is simple, non-invasive, inexpensive, and well accepted by patients, making it particularly suitable for large-scale screening, repeated monitoring, and point-of-care applications. Biomarker-based assessment may complement traditional clinical examination by providing real-time information regarding current inflammatory activity rather than merely reflecting historical tissue destruction. Such approaches could facilitate earlier detection of disease, identification of high-risk individuals, monitoring of treatment response, and potentially improved personalization of periodontal care [7]. Nevertheless, before widespread clinical implementation can be achieved, further standardization of saliva collection procedures, assay methodologies, biomarker thresholds, and interpretation criteria is required to ensure reproducibility and reliability across different clinical settings and populations. Several strengths enhance the validity of the present study, including the use of clearly defined diagnostic criteria, blinded laboratory analysis, objective biomarker quantification through ELISA, correlation of biomarker levels with established clinical periodontal parameters, and evaluation of both individual and combined biomarker models. However, certain limitations should also be acknowledged[8]. The case–control design may overestimate diagnostic performance compared with real-world screening populations where disease prevalence and severity vary considerably. Additionally, recruitment from a single clinical centre may limit the generalizability of the findings[9]. Salivary biomarker concentrations can also be influenced by factors such as collection methods, circadian variation, oral hygiene practices, dietary habits, and other unmeasured biological variables. Future research should therefore focus on large-scale prospective population-based studies, validation of point-of-care diagnostic platforms, and longitudinal investigations assessing the ability of salivary biomarkers to predict disease progression, monitor therapeutic outcomes, and guide personalized periodontal treatment strategies. Collectively, the findings provide strong evidence that salivary MMP-8 and IL-1β are promising non-invasive biomarkers with considerable potential for enhancing the diagnosis and management of periodontal disease[10,11].

5. CONCLUSION

The present study demonstrated that salivary levels of matrix metalloproteinase-8 (MMP-8) and interleukin-1β (IL-1β) were significantly elevated in individuals with periodontitis compared with periodontally healthy controls, highlighting their close association with periodontal inflammation and tissue destruction. Both biomarkers showed meaningful correlations with clinical attachment loss and other indicators of periodontal disease severity, supporting their biological relevance as markers of active periodontal pathology. Furthermore, diagnostic accuracy analyses revealed that salivary MMP-8 and IL-1β possess good discriminatory ability for identifying periodontitis, with MMP-8 demonstrating particularly strong performance and the combined biomarker model

providing even greater diagnostic accuracy. These findings reinforce the growing evidence that salivary biomarkers can serve as valuable adjuncts to conventional periodontal assessment by providing insight into current disease activity rather than solely reflecting historical tissue damage. The non-invasive nature of saliva collection, together with its simplicity, patient acceptability, and potential suitability for chairside testing, makes salivary biomarker analysis an attractive approach for periodontal screening, early detection, disease monitoring, and assessment of treatment response. The results therefore support the potential integration of salivary MMP-8 and IL-1β into future periodontal diagnostic strategies and personalized patient management approaches. Nevertheless, despite the promising findings, several important challenges remain before these biomarkers can be adopted for routine clinical use. Additional prospective validation studies involving larger, more diverse populations are necessary to confirm their diagnostic reliability, reproducibility, and applicability across different clinical settings. Moreover, variations in saliva collection methods, sample processing protocols, assay technologies, biomarker thresholds, and patient-related factors can influence biomarker measurements and must be carefully standardized. Establishing universally accepted cutoff values, quality-control procedures, and assay protocols will be essential to ensure consistent interpretation of results and facilitate widespread implementation. Future longitudinal studies should also evaluate the ability of these biomarkers to predict disease progression, identify individuals at increased risk of periodontal breakdown, and monitor responses to therapeutic interventions over time. Overall, the present findings indicate that salivary MMP-8 and IL-1β are promising non-invasive biomarkers with significant potential to enhance periodontal diagnosis and monitoring. However, comprehensive prospective validation and methodological standardization are required before their routine incorporation into everyday clinical periodontal practice can be recommended.

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