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Research Article

Molecular Biomarkers of Periodontal Health: Biological Significance of Salivary and Gingival Crevicular Fluid Markers in Periodontal Homeostasis: A Cross-Sectional Study

Dania Hassan[1], Momena Rashid[2*], Ayesha Mehboob[3], Sidra Azeem Malik[4], Syed Ibrar Hussain Humayun[5], Sobia Salam[6]

[1]Senior Lecturer, Department of Oral Biology, Peshawar Dental College, Peshawar, Pakistan.
[2*]Assistant Professor, Department of Oral Biology, Rehman College of Dentistry, Peshawar, Pakistan.
[3]Demonstrator, Department of Dental Materials, Rawal Institute of Health Sciences, Islamabad, Pakistan.
[4]Assistant Professor, Department of Periodontology, Margalla Institute of Health Sciences, Rawalpindi, Pakistan.
[5]Assistant Professor, Department of Periodontology, Lahore Medical & Dental College, Lahore, Pakistan.
[6]Assistant Professor, Department of Oral Biology, Rehman College of Dentistry, Peshawar, Pakistan.

Corresponding Author: Dr. Momena Rashid
Assistant Professor, Department of Oral Biology, Rehman College of Dentistry, Peshawar, Pakistan.
Email: idrmominarasheed@gmail.com

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ABSTRACT

Background: Periodontal diseases are chronic inflammatory conditions characterized by alterations in host immune responses and progressive destruction of tooth-supporting tissues. Salivary and gingival crevicular fluid biomarkers, particularly interleukin-1β (IL-1β), interleukin-6 (IL-6), and matrix metalloproteinase-8 (MMP-8), have emerged as promising indicators of periodontal inflammation and tissue degradation.

Objective: To evaluate the relationship between salivary and gingival crevicular fluid concentrations of IL-1β, IL-6, and MMP-8 and clinical periodontal parameters among individuals with periodontal health, gingivitis, and periodontitis.

Methods: An analytical cross-sectional study was designed for Peshawar Dental College and Khyber Medical University, Peshawar, from March 2025 to April 2026. The proposed sample comprised 87 adults, equally distributed among periodontally healthy participants (n=29), patients with gingivitis (n=29), and patients with periodontitis (n=29). Clinical parameters included plaque index, gingival index, probing pocket depth, clinical attachment loss, and bleeding on probing. Salivary and gingival crevicular fluid concentrations of IL-1β, IL-6, and MMP-8 were to be determined using enzyme-linked immunosorbent assay. Group comparisons and correlations were planned using appropriate statistical tests, with $p<0.05$ considered significant.

Results: In the analysis of 87 participants, 29 (33.3%) were classified as periodontally healthy, 29 (33.3%) had gingivitis, and 29 (33.3%) had periodontitis. The mean age was 34.64 ± 8.09 years. Clinical periodontal parameters, including plaque index, gingival index, probing pocket depth, clinical attachment loss, and bleeding on probing, differed significantly among the groups ($p<0.001$). Median salivary IL-1β concentrations increased from 57.5 pg/mL in healthy participants to 145.8 pg/mL in gingivitis and 226.1 pg/mL in periodontitis ($p<0.001$). Corresponding IL-6 concentrations were 14.3, 40.2, and 48.5 pg/mL, while MMP-8 concentrations were 14.5, 34.0, and 42.2 ng/mL, respectively (both $p<0.001$). Gingival crevicular fluid biomarkers also differed significantly among the groups ($p<0.001$). Salivary IL-1β demonstrated strong positive correlations with probing pocket depth ($r=0.78$) and bleeding on probing ($r=0.77$), while IL-6 and MMP-8 were positively associated with clinical periodontal parameters.

Conclusion: IL-1β, IL-6, and MMP-8 are biologically relevant candidates for evaluating periodontal inflammation and tissue degradation. Their clinical diagnostic utility requires confirmation through validated biomarker measurements and analysis alongside established periodontal examination parameters.

Keywords: Periodontitis, Gingivitis, Interleukin-1β, Interleukin-6, Matrix Metalloproteinase-8, Saliva, Gingival Crevicular Fluid.

INTRODUCTION

Periodontal diseases are among the most prevalent chronic inflammatory conditions affecting oral health worldwide and constitute a substantial public health concern. These diseases involve inflammation of the gingiva and supporting periodontal structures, with clinical manifestations ranging from reversible gingival inflammation to irreversible loss of periodontal attachment and alveolar bone. Periodontitis is a major contributor to tooth loss, impaired mastication, functional limitations, and deterioration in oral health-related quality of life. The global burden of periodontal disease remains considerable, particularly among adults, and is influenced by aging populations, socioeconomic disparities, inadequate oral hygiene, and limited access to preventive dental services. Early identification of inflammatory changes and timely clinical management are therefore essential for preserving periodontal health and preventing progressive tissue destruction [1, 2].

The pathogenesis of periodontal disease involves a complex interaction between microbial dysbiosis and the host immune-inflammatory response. Accumulation of pathogenic dental biofilms stimulates innate and adaptive immune mechanisms, leading to the production of inflammatory cytokines, chemokines, and proteolytic enzymes. Although the inflammatory response initially serves a protective role against microbial invasion, excessive or persistent immune activation may contribute to destruction of the periodontal ligament, connective tissue, and alveolar bone. Altered inflammatory signaling, neutrophil activation, and impaired resolution of inflammation are important components of periodontal tissue damage. Consequently, molecular mediators associated with these pathways have attracted increasing attention as potential indicators of periodontal disease activity and tissue breakdown [3-5].

Among the inflammatory biomarkers associated with periodontal pathology, interleukin-1β (IL-1β) and interleukin-6 (IL-6) are particularly relevant because of their involvement in inflammatory regulation and host defense. IL-1β participates in leukocyte recruitment, inflammatory amplification, and osteoclast-mediated bone resorption, whereas IL-6 contributes to immune-cell differentiation, inflammatory signaling, and regulation of bone metabolism. Matrix metalloproteinase-8 (MMP-8), a collagen-degrading enzyme predominantly associated with neutrophil activity, plays an important role in the degradation of periodontal connective tissue. Increased concentrations of these mediators may reflect inflammatory activity and structural tissue damage. However, their expression is influenced by individual biological variability, microbial factors, systemic conditions, and the severity of periodontal inflammation. Assessment of these biomarkers alongside clinical periodontal indices may offer additional information regarding the underlying biological processes associated with periodontal disease [6, 7].

Saliva and gingival crevicular fluid (GCF) have emerged as useful biological specimens for investigating periodontal inflammatory mediators because of their accessibility and relatively noninvasive collection procedures. Saliva contains a diverse range of proteins, enzymes, cytokines, and other molecular constituents derived from oral tissues and secretions, offering opportunities for periodontal screening and disease monitoring. Gingival crevicular fluid provides a more localized representation of the periodontal microenvironment and contains inflammatory mediators released during host responses to microbial challenges. Advances in immunoassay techniques, particularly enzyme-linked immunosorbent assay, have facilitated quantitative evaluation of these biomarkers in oral fluids. Nevertheless, variations in specimen collection, laboratory protocols, and biomarker concentrations highlight the importance of standardized analytical methods and interpretation alongside conventional clinical examination [8-10].

Despite growing interest in molecular diagnostics, conventional periodontal assessment continues to rely primarily on clinical parameters such as probing pocket depth, clinical attachment loss, bleeding on probing, and radiographic evaluation. These measurements remain essential for diagnosis and assessment of accumulated periodontal destruction, but they do not directly quantify the molecular activity underlying inflammatory tissue responses. Combining clinical parameters with inflammatory and tissue-degradation biomarkers may provide a more comprehensive understanding of periodontal conditions. However, differences in biomarker performance, sampling techniques, and diagnostic thresholds continue to limit their routine application, particularly in resource-constrained clinical settings. Further research

using accessible laboratory techniques and commonly investigated biomarkers is therefore warranted. The present study aimed to evaluate the association of salivary and gingival crevicular fluid concentrations of IL-1β, IL-6, and MMP-8 with clinical periodontal parameters among periodontally healthy individuals and patients with gingivitis and periodontitis.

MATERIALS AND METHODS

Study Design and Setting

This analytical cross-sectional study was designed to evaluate the association of salivary and gingival crevicular fluid (GCF) inflammatory biomarkers with clinical periodontal health and disease. The study setting comprised Peshawar Dental College (PDC), Peshawar, in collaboration with Khyber Medical University (KMU), Peshawar, Pakistan. The study period extended from March 2025 to April 2026, covering 14 months. The investigation focused on three commonly studied inflammatory biomarkers, interleukin-1β (IL-1β), interleukin-6 (IL-6), and matrix metalloproteinase-8 (MMP-8), to evaluate their relationship with periodontal inflammation, tissue destruction, and clinical periodontal parameters.

Sample Size and Sampling Technique

The study sample comprised 87 adult participants, divided into three equal groups of 29 individuals each: periodontally healthy participants, patients with gingivitis, and patients with periodontitis. A non-probability consecutive sampling technique was selected for participant recruitment. A total of 87 participants were included in the study and allocated equally into three groups comprising 29 periodontally healthy individuals, 29 patients with gingivitis, and 29 patients with periodontitis. The selection of the study population and biomarkers was informed by previously published Pakistani research evaluating salivary IL-1β, IL-6, and MMP-8 in periodontal diseases[11]. Equal allocation among the three groups facilitated comparisons of biomarker concentrations and clinical periodontal characteristics across different periodontal conditions.

Eligibility Criteria and Clinical Classification

Adults aged 18 years and above were eligible for inclusion. Participants were classified into three groups according to periodontal examination findings and the 2017 World Workshop classification of periodontal and peri-implant diseases and conditions. Periodontal health was defined by the absence of clinical attachment loss attributable to periodontitis, probing pocket depths of 3 mm or less, and bleeding on probing at fewer than 10% of examined sites. Gingivitis was characterized by bleeding on probing at 10% or more of sites without clinical attachment loss attributable to periodontitis. Periodontitis was classified according to interdental clinical attachment loss and other accepted diagnostic criteria, including probing pocket depth and radiographic bone loss where indicated. Individuals with uncontrolled systemic inflammatory diseases, pregnancy, recent periodontal therapy, or antibiotic or anti-inflammatory medication use within the preceding three months were excluded to minimize potential influences on biomarker concentrations.

Clinical Periodontal Examination

Periodontal examinations were standardized using a calibrated periodontal probe. Clinical parameters included plaque index, gingival index, probing pocket depth (PPD), clinical attachment loss (CAL), and bleeding on probing (BOP). Plaque accumulation and gingival inflammation were assessed using established clinical indices. Probing pocket depth was measured in millimeters from the gingival margin to the base of the periodontal pocket, while clinical attachment loss was measured relative to the cementoenamel junction. Periodontal probing was performed at six sites per tooth, excluding third molars. Bleeding on probing was recorded as the percentage of examined sites demonstrating bleeding following gentle probing. Demographic and relevant clinical information, including age, sex, smoking status, and oral hygiene practices, was recorded using a structured data collection form.

Saliva and Gingival Crevicular Fluid Collection

Unstimulated whole saliva was selected for biomarker assessment because of its noninvasive collection procedure and suitability for evaluating inflammatory changes in the oral environment. Participants were instructed to avoid eating, drinking, smoking, and performing oral hygiene procedures for at least one hour before specimen collection. Saliva was collected by passive drooling into sterile, labeled collection tubes before the periodontal examination. Samples were centrifuged to remove cellular debris, and the clarified supernatant was transferred into sterile microcentrifuge tubes for subsequent analysis.

Gingival crevicular fluid samples were collected using sterile absorbent paper strips inserted gently into the gingival sulcus or periodontal pocket after isolation of the sampling area and removal of supragingival plaque. Paper strips contaminated with blood or saliva were excluded. Samples were handled under standardized conditions to minimize protein degradation and contamination. Specimens were maintained under appropriate cold-chain conditions and stored at −80°C until laboratory analysis, with repeated freeze–thaw cycles avoided.

Laboratory Assessment of Inflammatory Biomarkers

Salivary and GCF concentrations of IL-1β, IL-6, and MMP-8 were assessed using enzyme-linked immunosorbent assay (ELISA), a commonly employed laboratory technique for quantitative inflammatory biomarker measurement. IL-1β and IL-6 were selected as indicators of inflammatory activity, whereas MMP-8 was included as a marker associated with collagen degradation and periodontal connective tissue destruction. Laboratory procedures followed the analytical requirements of the respective commercially available ELISA kits, including calibration using standard solutions, incubation, washing, substrate development, and spectrophotometric measurement. Biomarker concentrations were calculated from standard calibration curves and expressed in appropriate concentration units. Quality-control measures included the use of assay controls and duplicate measurements where supported by the testing protocol. Salivary and GCF measurements were considered separately because their biomarker concentrations may reflect different aspects of periodontal inflammation.

Statistical Analysis

Data analysis was planned using IBM SPSS Statistics version 26. Continuous variables were summarized as mean ± standard deviation or median with interquartile range according to their distribution, whereas categorical variables were expressed as frequencies and percentages. Normality was assessed using the Shapiro–Wilk test. Comparisons of normally distributed continuous variables among the three periodontal groups were performed using one-way analysis of variance, while the Kruskal–Wallis test was applied to non-normally distributed variables. The chi-square test or Fisher's exact test, as appropriate, was used to evaluate associations between categorical variables. Spearman's rank correlation coefficient was employed to determine relationships between inflammatory biomarker concentrations and clinical periodontal parameters, including plaque index, gingival index, probing pocket depth, clinical attachment loss, and bleeding on probing. Where relevant, post hoc pairwise comparisons were conducted with correction for multiple testing. Statistical significance was established at a p-value below 0.05.

RESULTS

A total of 87 participants were distributed equally among three periodontal groups, comprising 29 periodontally healthy individuals, 29 patients with gingivitis, and 29 patients with periodontitis. The overall mean age was 34.64 ± 8.09 years. Participants with periodontitis were older than those in the other two groups ($p<0.001$). There were 44 males (50.6%) and 43 females (49.4%). Gender distribution and smoking status did not differ significantly among the groups ($p=0.550$ and $p=0.501$, respectively).

Table 1. Demographic Characteristics of Study Participants (N=87)

Variable	Healthy (n=29)	Gingivitis (n=29)	Periodontitis (n=29)	p-value
Age (years), mean ± SD	27.7 ± 5.4	33.8 ± 5.3	42.4 ± 5.5	<0.001
Male, n (%)	14 (48.3)	17 (58.6)	13 (44.8)	0.550
Female, n (%)	15 (51.7)	12 (41.4)	16 (55.2)	
Smokers, n (%)	6 (20.7)	10 (34.5)	8 (27.6)	0.501
Non-smokers, n (%)	23 (79.3)	19 (65.5)	21 (72.4)	

Clinical periodontal parameters demonstrated significant differences across the three groups. Mean probing pocket depth increased from 1.79 mm in healthy participants to 4.66 mm in those with periodontitis. Similarly, bleeding on probing increased from 9.0% to 63.3%. Clinical attachment loss was present in the periodontitis group, with a mean of 3.47 mm. Differences in plaque index, gingival index, probing pocket depth, clinical attachment loss, and bleeding on

probing were statistically significant (all p<0.001).

Table 2. Comparison of Clinical Periodontal Parameters Among Study Groups

Clinical parameter	Healthy	Gingivitis	Periodontitis	p-value
Plaque index	0.54 ± 0.19	1.43 ± 0.18	2.09 ± 0.25	<0.001
Gingival index	0.38 ± 0.22	1.21 ± 0.22	1.93 ± 0.22	<0.001
Probing pocket depth (mm)	1.79 ± 0.38	3.19 ± 0.38	4.66 ± 0.38	<0.001
Clinical attachment loss (mm)	0.00	0.00	3.47 ± 1.20	<0.001
Bleeding on probing (%)	9.0 ± 6.8	32.5 ± 7.8	63.3 ± 7.4	<0.001

Values presented as mean ± SD. Overall comparisons performed using the Kruskal–Wallis test.

Salivary inflammatory biomarker concentrations were higher in participants with periodontal disease. Median IL-1β increased from 57.5 pg/mL in healthy participants to 226.1 pg/mL in those with periodontitis. A similar increase was observed in IL-6 and MMP-8 concentrations. Overall differences among the three groups were statistically significant for all three salivary biomarkers (p<0.001).

Table 3. Comparison of Salivary Biomarker Concentrations Among Periodontal Groups

Salivary biomarker	Healthy (n=29)	Gingivitis (n=29)	Periodontitis (n=29)	p-value
IL-1β (pg/mL)	57.5 (47.5–79.9)	145.8 (122.4–212.5)	226.1 (160.7–287.3)	<0.001
IL-6 (pg/mL)	14.3 (10.7–18.1)	40.2 (31.1–47.3)	48.5 (38.0–67.9)	<0.001
MMP-8 (ng/mL)	14.5 (11.8–17.4)	34.0 (27.2–45.4)	42.2 (35.6–54.2)	<0.001

Values presented as median (interquartile range). Kruskal–Wallis test.

Gingival crevicular fluid biomarker concentrations also demonstrated significant variation according to periodontal status. Median GCF IL-1β was 78.9 pg/mL in healthy participants and increased to 231.9 pg/mL in the periodontitis group. Likewise, IL-6 and MMP-8 concentrations were highest among participants with periodontitis. All three biomarkers showed significant overall differences across the periodontal groups (p<0.001).

Table 4. Comparison of Gingival Crevicular Fluid Biomarkers Among Periodontal Groups

GCF biomarker	Healthy (n=29)	Gingivitis (n=29)	Periodontitis (n=29)	p-value
IL-1β (pg/mL)	78.9 (54.4–92.4)	178.6 (125.5–227.7)	231.9 (195.1–337.8)	<0.001
IL-6 (pg/mL)	18.4 (15.2–24.3)	44.1 (35.7–56.0)	66.5 (53.6–78.7)	<0.001
MMP-8 (ng/mL)	20.4 (16.9–24.5)	39.5 (32.5–55.7)	58.7 (50.7–72.9)	<0.001

Values presented as median (interquartile range). Kruskal–Wallis test.

Spearman correlation analysis demonstrated significant positive associations between salivary inflammatory biomarkers and periodontal clinical parameters. IL-1β was positively correlated with probing pocket depth (r=0.78), bleeding on probing (r=0.77), and gingival index (r=0.76). Salivary IL-6 showed a positive correlation with plaque index and bleeding on probing (r=0.78 for both). MMP-8 was also positively associated with probing pocket depth (r=0.72) and bleeding on probing (r=0.70). All reported correlations were statistically significant (p<0.001).

Table 5. Correlation of Salivary Biomarkers with Clinical Periodontal Parameters

Clinical parameter	IL-1β (r)	IL-6 (r)	MMP-8 (r)
Plaque index	0.75	0.78	0.69
Gingival index	0.76	0.75	0.68
Probing pocket depth	0.78	0.75	0.72
Clinical attachment loss	0.59	0.59	0.50

Bleeding on probing	0.77	0.78	0.70

Spearman correlation coefficients (r). All p-values <0.001.

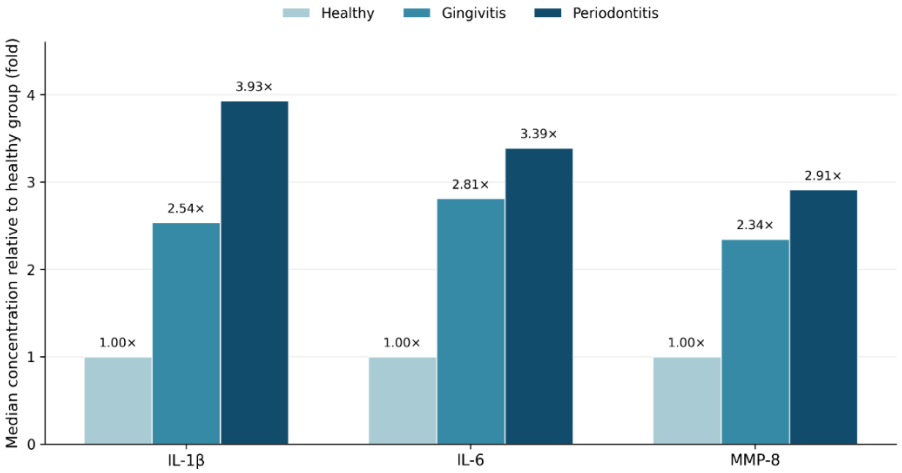


Fig. 1: Comparison of Median Salivary IL-1β, IL-6, and MMP-8 Levels Among Periodontally Healthy Participants and Patients with Gingivitis and Periodontitis. Values are Expressed as Fold Changes Relative to the Healthy Group to Accommodate Differences in Biomarker Measurement Units.

DISCUSSION

Periodontal diseases represent a complex interaction between microbial dysbiosis, host inflammatory responses, and progressive destruction of tooth-supporting tissues. The transition from periodontal health to gingivitis and subsequently to periodontitis involves alterations in immune regulation, cytokine production, and extracellular matrix degradation. Among the inflammatory mediators involved in these processes, interleukin-1β (IL-1β), interleukin-6 (IL-6), and matrix metalloproteinase-8 (MMP-8) have gained particular importance because of their association with periodontal inflammation and connective tissue breakdown. Their detection in saliva and gingival crevicular fluid provides insight into host-mediated periodontal pathology and may complement conventional clinical examination. Increased biomarker concentrations generally reflect heightened inflammatory activity, although their expression may vary according to disease severity, individual immune responses, and local periodontal conditions. The use of molecular biomarkers alongside clinical indices may therefore improve the biological assessment of periodontal health and disease [12-14].

IL-1β is an important proinflammatory cytokine involved in the initiation and amplification of periodontal inflammation. It contributes to leukocyte recruitment, inflammatory mediator production, and osteoclast activation, thereby participating in periodontal connective tissue and alveolar bone destruction. Elevated IL-1β concentrations in oral fluids may reflect an intensified host response to periodontal pathogens and are frequently associated with increased gingival inflammation, periodontal pocket depth, and clinical attachment loss. IL-6 also contributes to periodontal inflammatory responses through its effects on immune-cell activation, acute-phase signaling, and osteoclast differentiation. Although both cytokines are involved in periodontal tissue responses, their biological functions are not identical, suggesting that simultaneous assessment may provide more comprehensive information than either marker alone. The interpretation of cytokine concentrations should nevertheless consider biological variation, smoking, systemic inflammation, and other potential confounding factors [15, 16].

MMP-8 is a collagen-degrading enzyme primarily released by activated neutrophils and is directly involved in periodontal extracellular matrix degradation. Its role in the breakdown of type I collagen makes it particularly relevant to destructive periodontal disease. Increased MMP-8 concentrations in saliva and gingival crevicular fluid may indicate collagenolytic activity associated with periodontal inflammation and tissue destruction. The active form of MMP-8 is of particular diagnostic interest because it more directly reflects ongoing enzymatic activity than total MMP-8 concentration. However, biomarker concentrations are influenced by sample collection procedures, laboratory assays, and the distinction between total and active enzyme

forms. Accordingly, MMP-8 measurements should be interpreted in conjunction with probing pocket depth, bleeding on probing, clinical attachment loss, and radiographic findings rather than used as an independent diagnostic criterion [17-19].

Saliva and gingival crevicular fluid offer important opportunities for noninvasive or minimally invasive periodontal biomarker assessment. Saliva collection is convenient, inexpensive, and generally acceptable to patients, making it suitable for repeated assessment and potential chairside screening. However, whole saliva represents a mixture of secretions and biological constituents originating from multiple oral tissues, which may reduce the specificity of individual molecular measurements. Gingival crevicular fluid, in contrast, reflects biological changes occurring near the gingival sulcus and periodontal pocket and may provide more localized information regarding inflammatory activity. Differences between these fluids emphasize the need for standardized sample collection, storage, and laboratory processing. Combining inflammatory cytokines with tissue-degradation markers may improve the identification of periodontal disease, although diagnostic thresholds and clinical applicability require further validation before routine implementation [20-22].

The clinical significance of periodontal biomarkers extends beyond identifying existing inflammation and includes their potential application in periodontal risk assessment, monitoring therapeutic responses, and supporting personalized preventive strategies.

Limitation of Study

Nevertheless, several methodological limitations require consideration. Cross-sectional biomarker measurements cannot establish temporal relationships or determine whether elevated inflammatory mediators precede periodontal tissue destruction. Biomarker levels may also vary with age, smoking, oral hygiene, systemic inflammatory conditions, sampling methods, and assay characteristics. Furthermore, the absence of universally accepted diagnostic thresholds limits direct comparison across clinical settings. Future longitudinal investigations involving larger populations, standardized biomarker assessment, and repeated measurements before and after periodontal treatment are needed to determine the predictive value and clinical utility of IL-1β, IL-6, and MMP-8. Integration of molecular findings with conventional periodontal indices may ultimately support more accurate and biologically informed periodontal assessment.

CONCLUSION

IL-1β, IL-6, and MMP-8 are biologically relevant inflammatory biomarkers associated with periodontal inflammation and tissue degradation. Their assessment in saliva and gingival crevicular fluid offers a promising complementary approach to conventional periodontal examination. IL-1β and IL-6 provide information regarding host inflammatory activity, whereas MMP-8 reflects collagen breakdown associated with periodontal tissue destruction. Combined biomarker assessment may improve the biological characterization of periodontal conditions and support future diagnostic applications. However, standardized laboratory procedures, validated diagnostic thresholds, and longitudinal clinical investigations remain essential before these biomarkers can be recommended as independent diagnostic tools in routine periodontal practice.

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