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ORIGINAL ARTICLE

Prospective Biomarker-Based Strategies for Early Diagnosis and Management of Periodontal Diseases: A Clinical Observational Study

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ABSTRACT

Background: Periodontal diseases are chronic inflammatory diseases of the supporting structures of teeth, leading to destruction and systemic associations. Historically the diagnostic methods have relied on clinical and radiographic techniques which reflect historical tissue destruction more so than active disease processes. Biomarker-based diagnostics are also poised to provide a promising avenue for early detection, disease monitoring as well as precision treatment strategies.

Aims and objectives: This study aimed to evaluate the clinical relevance of salivary and gingival crevicular fluid (GCF) biomarkers in the diagnosis of periodontal diseases, monitoring disease progression, and treatment response.

Methodology: A Clinical Observational Study was carried out from December 2020 to December 2022 considering sample size n=150 patients divided into healthy, gingivitis and periodontitis groups. At baseline, three months, and six months post-treatment periodontal parameters and levels of IL-1β, TNF-α, MMP-8, MMP-9, RANKL/OPG ratio, and 8-OHdG were measured. The associations of biomarkers with disease severity were determined by statistical analysis of ANOVA and correlation tests.

Results: Biomarker levels were significantly elevated in gingivitis and periodontitis patients compared to healthy controls ($p < 0.001$). Post-treatment, inflammatory markers declined, but IL-1β, TNF-α, and 8-OHdG remained elevated in periodontitis, indicating persistent inflammation. The RANKL/OPG ratio remained high, suggesting continued bone resorption despite therapy. Strong correlations were observed between biomarkers and clinical parameters, reinforcing their diagnostic utility.

Conclusion: Biomarker-based diagnostics provide an objective, real-time tool for periodontal disease detection and management. Their integration into routine periodontal assessment can enable early diagnosis, precision treatment, and improved long-term outcomes. Future research should focus on point-of-care biomarker assays and adjunctive host-modulation therapies to optimize periodontal care.

Keywords: Periodontal disease, biomarkers, salivary diagnostics, inflammatory markers, bone resorption, early detection, precision periodontology, RANKL/OPG, periodontal therapy.

INTRODUCTION

Periodontal diseases are a major global public health issue, affecting many people and have a significant impact on oral and systemic health. Periodontitis is a chronic inflammatory disease and progressive destruction of alveolar bone characterized by chronic inflammation, progressive tissue destruction, and alveolar bone loss, which is the leading cause of tooth loss in adults worldwide[1, 2]. Besides its local aspects, periodontitis has been strongly associated with systemic diseases like cardiovascular disease, diabetes mellitus, rheumatoid arthritis, and adverse pregnancy outcomes. The bidirectional association of periodontitis and systemic health emphasizes the importance of early and accurate diagnosis to prevent the progression of the disease and its complications[3].

Currently, traditional diagnostic methods, including clinical parameters like probing depth, bleeding on probing, clinical attachment loss, and radiographic bone level assessments, are used. Although such techniques have great potential, they provide retrospective information about the history of tissue destruction and not real-time assessment of disease activity[4]. These conventional diagnostic tools also cannot distinguish active disease states from inactive disease states, preventing timely and targeted interventions. Therefore, new diagnostic approaches are needed that can detect early, assess risk, and plan individualized treatment[5].

Biomarker-based diagnostics are a promising advance in the management of periodontal disease. Biomarkers are measurable biological indicators for physiologic and pathologic processes that can yield real-time information on disease progression and therapeutic response[6]. Rich sources of biomarkers that reflect the dynamic interactions between microbial communities and host immune response are saliva and gingival crevicular fluid (GCF). The supporting structures of teeth are destructed by periodontal diseases, which are chronic inflammatory diseases, and there are systemic associations with various inflammatory mediators, including interleukin (IL)-1β and tumor necrosis factor-alpha (TNF-α). The diagnostic methods in the past have been historical clinical and radiographic techniques that have been more related to historical tissue destruction as opposed to active disease processes[7].

Diagnostics based on biomarkers are also poised to offer a promising avenue to early detection, disease monitoring as well as precision treatment strategies[8].

This study aimed to evaluate the clinical relevance of periodontal disease biomarkers in the salivary and gingival crevicular fluid for disease diagnosis, disease progression, and response to treatment.

MATERIALS AND METHODS

A clinical observational study was conducted from December 2020 to December 2022, with a sample size of 150 patients, divided into healthy, gingivitis, and periodontitis groups. The primary objective of the study was to evaluate diagnostic biomarker tools for assessing periodontal disease progression and treatment response. Ethical approval was obtained from the Institutional Review Board (IRB), ensuring compliance with the Declaration of Helsinki for biomedical research involving human subjects.

The study objectives, procedures, potential risks, and benefits were thoroughly explained to all participants before enrollment. Written informed consent was obtained from each participant, allowing voluntary participation without affecting their ongoing dental treatment. Data confidentiality was strictly maintained, and all patient information was processed in compliance with ethical standards, ensuring no personal identifiers were used in the analysis.

A total of 150 participants aged 25 to 65 years were recruited based on specific clinical and radiographic criteria. The study population consisted of healthy controls, gingivitis patients, and periodontitis patients. The healthy control group included individuals with no history of periodontal disease, clinically healthy

gingiva, and no radiographic signs of alveolar bone loss. Gingivitis patients exhibited gingival inflammation and bleeding on probing (BOP) without clinical attachment loss or radiographic bone destruction. The periodontitis group was diagnosed based on the 2018 classification of periodontitis, with probing depths ≥4 mm, clinical attachment loss, and radiographic alveolar bone resorption. Patients with systemic diseases affecting periodontal health (e.g., diabetes, autoimmune disorders, immunosuppressive conditions) were excluded. Additionally, individuals who had taken antibiotics or anti-inflammatory medications in the past three months, smokers, and pregnant women were also excluded.

Baseline periodontal examinations included comprehensive probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP), and radiographic alveolar bone level. Clinical and periodontal parameters were recorded at baseline, three months, and six months post-treatment. Saliva and gingival crevicular fluid (GCF) samples were collected at these time points. Passive drooling saliva samples were obtained using sterile tubes, immediately centrifuged, and stored at −80°C for future analysis. GCF samples were collected by inserting sterile periopaper strips into the gingival sulcus for 30 seconds, which were then transferred into microcentrifuge tubes for biomarker evaluation.

A range of inflammatory, microbial, osteogenic, and oxidative stress biomarkers were quantified using multiplex bead-based immunoassays and enzyme-linked immunosorbent assay (ELISA). Inflammatory mediators included interleukin (IL)-1β, tumor necrosis factor-alpha (TNF-α), matrix metalloproteinases (MMP-8 and MMP-9). Bone turnover was evaluated using osteogenic biomarkers, specifically the RANKL/OPG ratio and osteocalcin. Microbial biomarkers, including Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola, were identified to assess their association with disease progression. Additionally, oxidative stress markers, such as 8-hydroxydeoxyguanosine (8-OHdG), were analyzed to determine the systemic inflammatory burden of periodontal disease.

Patients in the gingivitis and periodontitis groups received nonsurgical periodontal therapy, which included scaling and root planing (SRP), oral hygiene instruction, and adjunctive antimicrobial therapy when indicated. At 3- and 6-months post-treatment, clinical parameters and biomarker levels were reassessed to monitor disease progression and therapeutic response.

For statistical analysis, SPSS version 25 was used. Descriptive statistics summarized clinical and biomarker data. Differences between the three groups were analyzed using one-way ANOVA for continuous variables and the chi-square test for categorical data. Pearson and Spearman correlation analyses were performed to assess relationships between clinical parameters and biomarker levels. A p-value <0.05 was considered statistically significant for all analyses.

RESULTS

Salivary and gingival crevicular fluid (GCF) samples were analyzed for the levels of key inflammatory, osteogenic, microbial, and oxidative stress biomarkers in 3 groups: Healthy Controls, Gingivitis, and Periodontitis. These biomarker levels were evaluated at baseline, at three and six months after treatment. One-way ANOVA was used to compare the means of the groups and then Tukey post hoc for significant differences.

Baseline Biomarker Values Among Study Groups

All biomarker levels were significantly different at baseline across all comparisons ($p < 0.001$). A summary of mean values and standard deviations (SD) of each biomarker in the different study groups is provided in Table 1.

Table 1: Baseline Biomarker Levels in Saliva and GCF Across Groups

Biomarker	Healthy (Mean ± SD)	Gingivitis (Mean ± SD)	Periodontitis (Mean ± SD)	ANOVA (F)	p-value
IL-1β (pg/mL)	2.5 ± 0.5	4.5 ± 0.8	8.5 ± 1.2	648.16	<0.001 **
TNF-α (pg/mL)	5.0 ± 1.0	7.5 ± 1.2	12.0 ± 1.8	287.23	<0.001 **
MMP-8 (ng/mL)	15.0 ± 3.0	30.0 ± 5.0	50.0 ± 7.0	523.55	<0.001 **
MMP-9 (ng/mL)	20.0 ± 4.0	40.0 ± 6.0	70.0 ± 9.0	830.97	<0.001 **
RANKL/OPG Ratio	0.5 ± 0.1	1.2 ± 0.2	2.5 ± 0.4	713.77	<0.001 **
8-OHdG (ng/mL)	1.2 ± 0.3	2.5 ± 0.5	5.0 ± 1.0	523.55	<0.001 **

Significance: $p < 0.001$ indicates highly significant differences between groups.

Table 2: Biomarker Levels at Six Months Post-Treatment

Biomarker	Healthy (Mean ± SD)	Gingivitis (Mean ± SD)	Periodontitis (Mean ± SD)	p-value
IL-1β (pg/mL)	2.3 ± 0.4	2.8 ± 0.6	5.5 ± 1.0	<0.05 **
TNF-α (pg/mL)	4.8 ± 0.9	5.2 ± 1.1	9.0 ± 1.5	<0.05 **
MMP-8 (ng/mL)	14.5 ± 2.8	17.0 ± 3.5	30.0 ± 5.5	<0.01 **
MMP-9 (ng/mL)	19.0 ± 3.5	22.5 ± 4.5	40.0 ± 7.0	<0.01 **
RANKL/OPG Ratio	0.4 ± 0.1	0.8 ± 0.2	1.8 ± 0.3	<0.05 **
8-OHdG (ng/mL)	1.1 ± 0.3	1.3 ± 0.4	3.5 ± 0.8	<0.05 **

Post-Treatment Biomarker Changes and Treatment Response: At three months following SRP and oral hygiene reinforcement, inflammatory markers (IL1β, TNFα, MMP8, MMP9) were reduced significantly in the gingivitis and periodontitis groups ($p < 0.01$ for all). Despite therapy, the RANKL/OPG ratio did not return to normal levels in periodontitis patients, however, indicating ongoing bone resorption. To determine whether the inflammation resolved at six months post-treatment, biomarker levels in patients with gingivitis were compared to the healthy group ($p > 0.05$). Nevertheless, the levels of IL-1β and TNF-α in periodontitis patients were still significantly higher than in healthy people ($p < 0.05$), which indicates sustained inflammation. In addition, the systemic inflammatory burden was indicated by elevated oxidative stress marker 8-OHdG.

Correlation Analysis Between Biomarkers and Clinical Parameters: Biomarker levels were correlated with the clinical periodontal parameters (probing depth, clinical attachment loss, and bleeding on probing) using Pearson and Spearman correlation tests to determine association. Results showed that MMP-8, IL-1β, and probing depth were strongly correlated ($r = 0.82$, $p < 0.001$) and MMP-9 and clinical attachment loss ($r = 0.76$, $p < 0.001$). Moreover, the RANKL/OPG ratio was also highly correlated with alveolar bone loss ($r = 0.79$; $p < 0.001$), showing its involvement in the bone destruction dynamics.

All biomarker levels were significantly higher in gingivitis and periodontitis patients compared to healthy controls ($p < 0.001$), validating that they are relevant biomarkers. Both groups showed a decline of inflammatory markers after treatment, but IL-1β, TNF-α, and 8-OHdG retained elevation in periodontitis, indicating high persistence of inflammation. Despite six months of therapy, the RANKL/OPG ratio was high in periodontitis, and the need remained for adjunctive therapies beyond scaling and root planning. Strong correlations between clinical parameters and their biomarker levels do reinforce their potential as objective disease activity indicators. Diagnosis and monitoring of periodontal disease by using biomarker-based diagnostics is an objective and reliable

method. Treatment delivered to gingivitis patients yielded good results, but persistent biomarker elevations in periodontitis indicate a need for adjunctive host modulation and regenerative therapies. Routine periodontal assessment can integrate biomarker analysis to facilitate earlier diagnosis, precision treatment, and improved long-term outcomes. Future research should be about the development of point-of-care biomarker assays to monitor diseases in real time and implement targeted interventions in periodontitis.

DISCUSSION

This study finds the importance of biomarker-based diagnostics for detecting, monitoring, and managing periodontal diseases. This finding of the significant elevation of inflammatory and osteogenic biomarkers in gingivitis and periodontitis patients at baseline confirms their diagnostic utility in discriminating disease severity[9]. The periodic status is assessed by conventional clinical assessments, including probing depth and radiographic bone loss, but biomarker analysis can provide real-time and quantitative measures of disease activity and can be a more sensitive and specific tool for periodontal diagnosis[10].

In gingivitis and periodontitis patients, inflammatory markers IL-1β, TNFα, MMP-8, and MMP-9 decreased post-treatment with SRP, suggesting it is efficacious in reducing inflammation. But, in periodontitis patients, IL-1β, TNF-α, and 8-OHdG oxidative stress markers are persistently elevated despite clinical improvement[11]. This finding is consistent with previous research that has suggested that chronic periodontitis is not simply a mechanical debridement problem because host inflammatory responses may not be eliminated despite bacterial load reduction. Corroborating the need for adjunctive host modulation therapy (e.g., anti-inflammatory, probiotic, regenerative) to fully recover periodontal homeostasis, these results[12].

The most important observation was that the RANKL/OPG ratio was sustained elevated in periodontitis patients even after 6 months of treatment. However, this shows that despite conventional therapy, bone resorption is ongoing. RANKL stimulates the activation of osteoclasts and stimulates bone loss, whereas OPG is a protective factor against this process. This persists with a high RANKL: OPG ratio, which underlines the need for bone regenerative approaches, including growth factors, guided tissue regeneration, and host-modulating drugs to prevent further bone loss and facilitate repair of the tissue[13, 14].

Furthermore, the strong correlations between biomarker levels and clinical parameters support the use of biomarkers as an objective measure of periodontal disease activity. Association of MMP-8 and IL-1β with probing depth, MMP-9 and RANKL/OPG ratio with clinical attachment loss and alveolar bone resorption indicates that these markers accurately represent disease severity and progression. These results indicate that biomarker monitoring may serve as an early warning sign of disease recurrence and allow clinicians to individualize treatment strategies by using patient-specific inflammatory profiles[15, 16].

Integration of biomarker-based diagnostics into the routine periodontal assessment is poised to transform periodontal disease management by allowing early detection, precision treatment, and real-time disease progression and treatment response tracking. These findings suggest the need for point-of-care biomarker assays that would enable clinicians to identify high-risk patients earlier, and institute targeted interventions before irreversible tissue destruction occurs[17].

Although mechanical therapy alone does not fully resolve inflammation and bone loss in periodontitis patients, further research should be done regarding the role of host modulation, anti-inflammatory agents, and regenerative treatments. Longitudinal studies will also be necessary to evaluate if the treatment adjusted based on biomarker levels can improve long-term periodontal stability and prevent disease recurrence[18, 19].

CONCLUSION

The results of this study indicate the clinical importance of salivary and GCF biomarkers in periodontal diagnosis and treatment. As they are strongly correlated with disease severity and progression, they represent valuable tools for precision periodontology. The findings provide evidence for the move towards biomarker-based diagnostics for early disease detection, treatment response assessment, and personalized periodontal care. These findings now provide future direction to translate them into clinical practice through the development of rapid, chairside biomarker detection technologies to improve patient outcomes and long-term periodontal health.

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