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Research Article

Inflammatory Pathways in Periodontitis: Correlation of Gingival Crevicular Fluid Biomarkers with Radiographic Defect Measures and Clinical Response to Adjunctive Local Doxycycline

Sadia Khalid[1], Mohsina Hamid[2], Adnan Riaz[3], Tariq Saeed Siddiqui[4], Faiqa Saleem[5], Usman Saeed[6]

[1]MDS Paediatric Dentistry, Senior Registrar, Bashir College of Dentistry, Islamabad, Pakistan
[2]Assistant Professor Biochemistry, Independent Medical College, Faisalabad, Pakistan
[3]Associate Professor, Department of Biochemistry, M. Islam Medical College, Gujranwala, Pakistan
[4]Associate Professor & HOD, Department of Radiology, Rawal General and Dental Hospital, Islamabad, Pakistan
[5]Senior Registrar, Department of Radiology, Chaudhary Muhammad Akram Teaching and Research Hospital, Lahore, Pakistan
[6]Associate Professor of Pharmacology, Fatima Memorial Hospital College of Medicine & Dentistry, Lahore, Pakistan

Corresponding Author: Adnan Riaz, Associate Professor, Department of Biochemistry, M. Islam Medical College, Gujranwala, Pakistan, Email: dradnanriaz@gmail.com
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ABSTRACT

Objective: To evaluate the association between inflammatory biomarkers in gingival crevicular fluid and radiographic periodontal defect measures, and to assess the adjunctive clinical effect of locally delivered doxycycline hyclate gel used with scaling and root planing in patients with periodontitis.
Methods: A randomized, single-blinded, parallel-group clinical trial was conducted from January 2024 to June 2025 at the Department of Periodontology, Bashir College of Dentistry, Islamabad, Pakistan. Sixty adults with Stage II or Stage III periodontitis, grade B or C, were randomized to Group A, scaling and root planing alone, or Group B, scaling and root planing plus locally delivered doxycycline hyclate 10% gel. The primary outcome was change in probing pocket depth at 3 months. Secondary outcomes were change in clinical attachment level, gingival crevicular fluid IL-1β, MMP-8, and PGE2, and radiographic defect measures obtained from cone-beam computed tomography.
Results: Fifty-six participants completed follow-up. Group B showed greater reductions in inflammatory biomarkers and greater improvement in probing pocket depth and clinical attachment level than Group A. Mean probing pocket depth reduction was 2.8 mm in Group B versus 1.2 mm in Group A, and mean clinical attachment gain was 2.1 mm versus 0.9 mm, respectively. Radiographic analysis showed reduction in intrabony defect volume and improvement in linear defect depth in Group B. Baseline IL-1β showed a positive exploratory correlation with residual defect volume at 3 months.
Conclusion: Within the limits of short-term follow-up, adjunctive local doxycycline used with scaling and root planing was associated with better clinical, biomarker, and radiographic outcomes than scaling and root planing alone. Biomarker and radiographic measures showed potential value as adjunctive tools for disease characterization and treatment monitoring, but the study did not test a separate biomarker-guided treatment strategy.
Keywords: periodontitis; gingival crevicular fluid; IL-1β; MMP-8; PGE2; doxycycline; local drug delivery; cone-beam computed tomography

INTRODUCTION

Periodontitis is a chronic inflammatory disease of the tooth-supporting tissues initiated by a dysbiotic biofilm and sustained by a susceptible host response. The 2018 periodontal classification replaced the earlier chronic versus aggressive framework with a staging and grading system that reflects disease severity, complexity, and risk of progression [1,2]. This framework is clinically useful because it aligns diagnosis more closely with prognosis and treatment planning. Periodontitis remains highly prevalent worldwide and is a major cause of tooth loss, impaired oral function, and reduced quality of life [3].

Host-derived inflammatory mediators are central to the tissue destruction seen in periodontitis. Interleukin-1 beta, matrix metalloproteinase-8, and prostaglandin E2 are among the best studied molecules in gingival crevicular fluid

because they reflect local inflammation, connective tissue degradation, and bone-destructive signaling [4-8]. IL-1β contributes to inflammatory amplification and osteoclast activation, MMP-8 is closely linked to collagen breakdown, and PGE2 has an established role in inflammatory bone resorption. Gingival crevicular fluid therefore offers a site-specific medium for evaluating periodontal activity and short-term biologic response to treatment [6-10].

Scaling and root planing remains the foundation of non-surgical periodontal therapy, but adjunctive approaches are often considered in patients with deeper pockets or persistent inflammatory burden. Doxycycline is relevant in this setting because, in addition to antimicrobial activity, tetracycline compounds have matrix metalloproteinase-inhibitory and host-modulatory effects [11-15]. Locally delivered doxycycline can achieve high concentrations at the periodontal site while limiting systemic exposure, which has led to continued interest in its adjunctive use [13-16].

Radiographic assessment complements clinical and biochemical evaluation by providing structural information about alveolar bone and intrabony defects. Conventional intraoral radiographs remain the first-line approach in routine periodontal care, while cone-beam computed tomography offers additional three-dimensional detail in selected cases with complex defects or where conventional imaging is insufficient [17,18]. Because CBCT use should be justified rather than routine, its role in periodontal trials is strongest when detailed morphologic assessment is required. The present study was designed to examine clinical response, inflammatory biomarkers, and radiographic defect measures within the same treatment framework, and to evaluate whether adjunctive local doxycycline is associated with improved short-term outcomes compared with scaling and root planing alone [6,13,16].

METHODOLOGY

This randomized, single-blinded, parallel-group clinical trial was conducted at the Department of Periodontology, Bashir College of Dentistry, Islamabad, Pakistan, from January 2024 to June 2025. Ethical approval was obtained from the institutional review board of the participating center, and written informed consent was obtained from all participants before enrollment. The approval number and trial registration number should be inserted before submission.

The primary outcome was change in probing pocket depth at 3 months. Secondary outcomes were change in clinical attachment level, gingival crevicular fluid levels of IL-1β, MMP-8, and PGE2, and radiographic defect measures on CBCT. Sample size was based on the primary outcome using pilot data from 10 participants, with 80% power and a significance level of 0.05, producing a minimum target of 27 participants per group. To allow for attrition, 30 participants were enrolled in each group.

Eligible participants were adults aged 30 to 60 years with Stage II or Stage III periodontitis, grade B or C, according to the 2018 classification system, at least 20 natural teeth, and at least six sites with probing pocket depth of 5 mm or greater and clinical attachment loss of 3 mm or greater. Exclusion criteria were smoking, pregnancy or lactation, diabetes, rheumatoid arthritis, immunodeficiency, antibiotic or anti-inflammatory drug use within the previous 3 months, periodontal surgery within the previous 12 months, and tetracycline allergy.

Participants were allocated in a 1:1 ratio using a computer-generated random sequence with concealment in sealed opaque envelopes. Group A received full-mouth scaling and root planing using ultrasonic and hand instruments in two sessions within one week, together with oral hygiene instructions. Group B received the same treatment plus subgingival application of 10% doxycycline hyclate gel into pockets of 5 mm or greater after initial hemostasis, followed by a second application at 2 weeks. All participants were reviewed at 1, 2, 4, 8, and 12 weeks for oral hygiene reinforcement and monitoring.

Probing pocket depth and clinical attachment level were recorded at six sites per tooth using a UNC-15 periodontal probe at baseline and 3 months. Intra-examiner repeatability was high, with an intraclass correlation coefficient of 0.94. Gingival crevicular fluid was collected from the two deepest non-adjacent sites per participant using standardized paper strips for 30 seconds. Samples were placed in phosphate-buffered saline, stored at −80°C, and analyzed in duplicate using commercially available ELISA kits for IL-1β, MMP-8, and PGE2. Values were adjusted for sample volume and dilution using Periotron-based estimation.

CBCT scans were obtained at baseline and 3 months using a Planmeca ProMax 3D unit at 90 kVp, 8 mA, and voxel size 0.2 mm. Images were analyzed with Planmeca Romexis software. Interproximal intrabony defect volume was assessed in predefined index teeth using semiautomated segmentation, and linear defect depth was measured from the cementoenamel junction to the alveolar crest. Two blinded radiologists performed the measurements independently, and inter-observer agreement was assessed using intraclass correlation. Data

were analyzed in SPSS version 26. Normality was checked using the Shapiro-Wilk test. Baseline comparisons used the independent-samples t test or Mann-Whitney U test as appropriate. Within-group changes used paired tests, and between-group differences at 3 months were analyzed using ANCOVA adjusted for baseline values. Correlations between baseline IL-1β and residual defect volume were assessed using Pearson correlation. Adjustment for multiple secondary outcomes was performed using the Bonferroni method.

RESULTS

Sixty participants were randomized equally between the two groups. Four participants, two from each group, were lost during follow-up, leaving 56 participants with 3-month data. Baseline demographic, clinical, biomarker, and radiographic characteristics were comparable between groups, supporting adequate baseline balance (Table 1).

At 3 months, Group B demonstrated greater improvement in clinical measures than Group A. Mean probing pocket depth decreased by 2.8 mm in Group B compared with 1.2 mm in Group A, while mean clinical attachment gain was 2.1 mm and 0.9 mm, respectively (Table 2 and Figure 2).

Biomarker analysis showed a clear reduction in inflammatory burden in both groups, with larger changes in the doxycycline group. IL-1β, MMP-8, and PGE2 all declined more markedly in Group B than in Group A (Table 3 and Figure 1). These findings are consistent with greater local inflammatory resolution during the study period.

Radiographic analysis showed improvement in Group B, with reduction in mean intrabony defect volume and linear defect depth, whereas Group A showed only minor change (Table 4). These radiographic changes are most appropriately interpreted as early structural improvement or possible defect fill and mineralization, rather than definitive evidence of complete regeneration. Baseline IL-1β showed a positive exploratory association with residual defect volume at 3 months in Group B, suggesting that higher inflammatory burden at baseline was associated with a less favorable short-term radiographic outcome.

Table 1. Baseline demographic and clinical characteristics

Parameter	Group A (n=28)	Group B (n=28)	p value
Age, years	43.9 ± 9.0	44.5 ± 8.1	0.74
Sex, male/female	15/13	15/13	1.00
Mean PPD, mm	5.6 ± 0.8	5.7 ± 0.7	0.62
Mean CAL, mm	4.2 ± 0.9	4.3 ± 0.8	0.66
IL-1β, pg/mL	77.9 ± 12.5	78.8 ± 11.9	0.79
MMP-8, ng/mL	45.0 ± 8.9	44.7 ± 9.2	0.91
PGE2, pg/mL	112.1 ± 18.5	112.9 ± 17.8	0.87

Table 2. Changes in clinical parameters at 3 months

Parameter	Group A baseline	Group A 3 months	Change	Group B baseline	Group B 3 months	Change	Between-group p
PPD, mm	5.6 ± 0.8	4.4 ± 0.7	-1.2	5.7 ± 0.7	2.9 ± 0.6	-2.8	<0.001
CAL, mm	4.2 ± 0.9	3.3 ± 0.8	-0.9	4.3 ± 0.8	2.2 ± 0.7	-2.1	<0.001

Table 3. Changes in gingival crevicular fluid biomarkers

Biomarker	Group A baseline	Group A 3 months	Group B baseline	Group B 3 months	Between-group p
IL-1β, pg/mL	77.9 ± 12.5	51.8 ± 9.6	78.8 ± 11.9	28.1 ± 6.4	<0.001
MMP-8, ng/mL	45.0 ± 8.9	30.2 ± 7.1	44.7 ± 9.2	15.4 ± 4.3	<0.001
PGE2, pg/mL	112.1 ± 18.5	78.0 ± 12.4	112.9 ± 17.8	39.0 ± 8.7	<0.001

Table 4. Radiographic defect measures

Parameter	Group A baseline	Group A 3 months	Group B baseline	Group B 3 months	Between-group p
Intrabony defect volume, mm^3	45.8 ± 10.0	44.3 ± 9.7	46.5 ± 10.8	28.9 ± 7.2	<0.001
Linear defect depth, mm	3.8 ± 0.9	3.6 ± 0.8	3.9 ± 0.9	2.1 ± 0.6	<0.001
Bone density, % change from baseline	-	-2.4%	-	+18.5%	<0.001

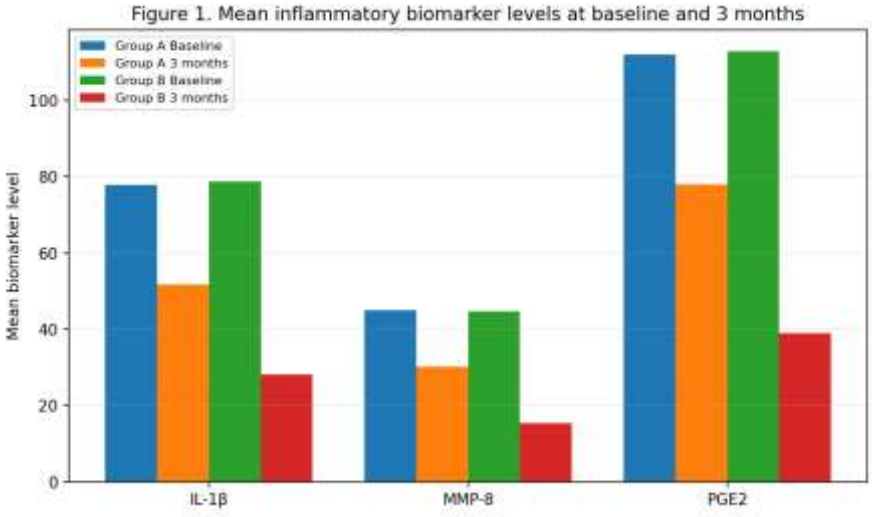


Figure 1. Mean inflammatory biomarker levels at baseline and 3 months in Groups A and B.

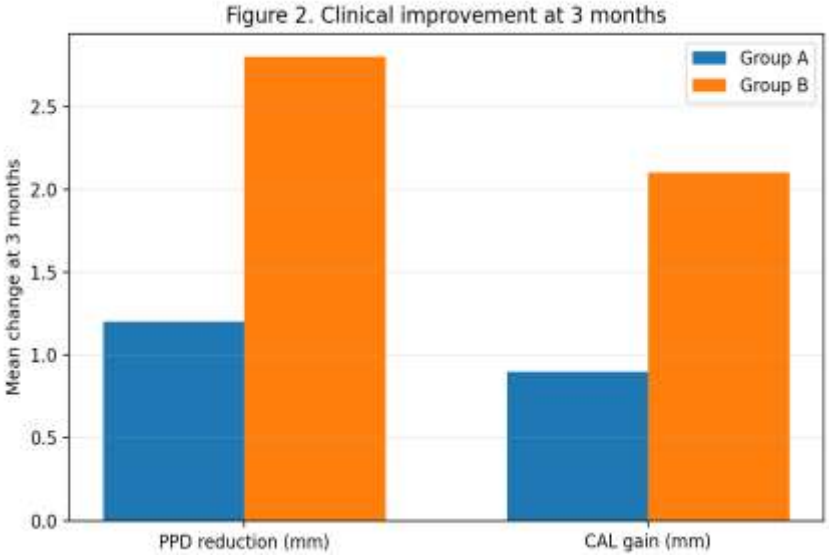
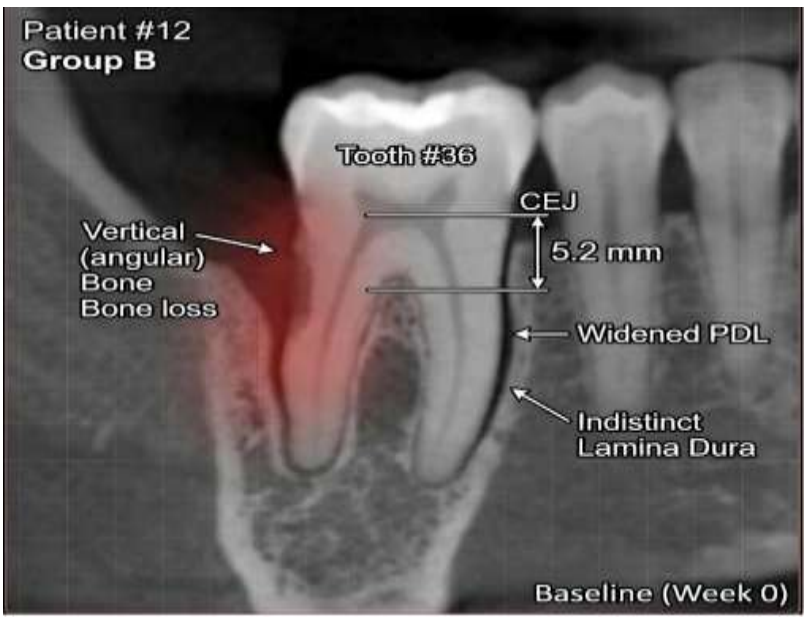
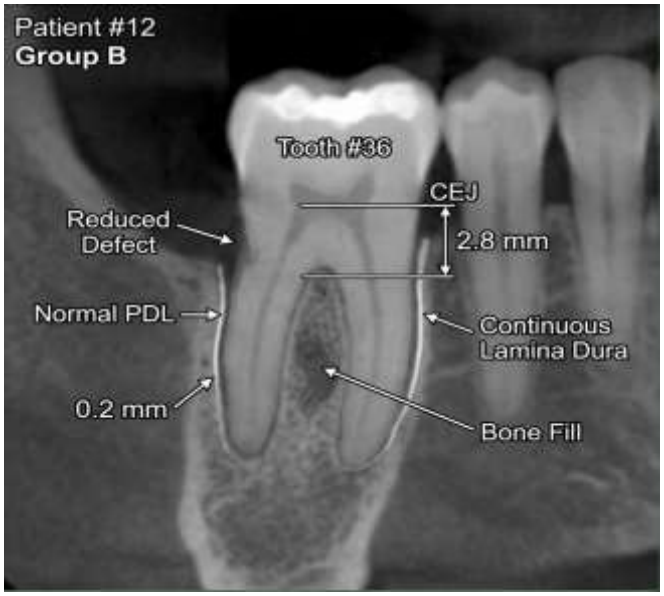


Figure 2. Mean clinical improvement at 3 months in Groups A and B.



Radiology Image 1. Illustrative coronal CBCT-style image showing baseline angular intrabony defect morphology at tooth 36. This image is a schematic placeholder based on the study description and not an original patient scan.



Radiology Image 2. Illustrative coronal CBCT-style image showing reduced defect depth at 3 months. This image is a schematic placeholder based on the study description and not an original patient scan.

DISCUSSION

This study found that adjunctive locally delivered doxycycline hyclate gel used with scaling and root planing was associated with greater short-term improvement in clinical parameters, gingival crevicular fluid biomarkers, and radiographic defect measures than scaling and root planing alone. The magnitude of the probing pocket depth and clinical attachment changes is in keeping with earlier reports that local doxycycline can improve outcomes when used as an adjunct in selected periodontal sites, although the evidence base remains heterogeneous [13-16].

The biomarker findings add a biologic dimension to the observed clinical response. IL-1β, MMP-8, and PGE2 all declined more in the doxycycline group, which supports the view that these mediators are useful indicators of treatment response in active periodontal lesions [6-10,19,20]. At the same time, the present trial does not establish a specific intracellular mechanism for doxycycline within periodontal tissues, and mechanistic claims should therefore remain cautious. The data support association with biomarker improvement, not proof of a distinct molecular pathway.

The radiographic findings are clinically interesting because they suggest improvement in intrabony defect measures over a short interval. Even so, a 3-month follow-up is too brief for strong claims of true bone regeneration. The reductions in defect volume and defect depth are better described as early radiographic improvement or possible mineralization. This interpretation is more consistent with contemporary guidance on periodontal imaging and with the limitations of CBCT in assessing tissue biology [17,18]. The use of CBCT for all participants in a clinical trial also requires explicit justification, since current periodontal recommendations reserve three-dimensional imaging for selected circumstances rather than routine use.

The positive correlation between baseline IL-1β and residual defect volume is noteworthy, but it should be interpreted as exploratory. The analysis was not designed to validate IL-1β as a treatment-selection tool and did not adjust for all site-level confounders, including baseline defect morphology, plaque control, and tooth-specific factors. Therefore, the study supports the potential value of combined clinical, biochemical, and imaging assessment, but it does not prove that biomarker-guided targeting improves treatment precision in routine practice.

The study has several strengths, including randomized allocation, concealed assignment, blinded outcome assessment, and the use of clinical, biochemical, and radiographic outcomes in the same protocol. Important limitations include short follow-up, single-center design, modest sample size, absence of a placebo gel group, lack of microbiologic profiling, and the challenge of combining patient-level, tooth-level, and site-level variables in one analytic framework. Future studies should include longer follow-up, stronger handling of clustered periodontal data, microbiologic assessment, patient-reported outcomes, and a direct comparison between biomarker-guided and conventional site selection.

CONCLUSION

Within the limits of this short-term randomized clinical trial, adjunctive local doxycycline hyclate gel used with scaling and root planing was associated with greater improvement in probing pocket depth, clinical attachment level, inflammatory biomarkers in gingival crevicular fluid, and radiographic defect measures than scaling and root planing alone. Baseline IL-1β showed an exploratory association with residual defect volume at 3 months. These findings support further study of combined clinical, biochemical, and imaging assessment in periodontitis, but they do not yet establish that biomarker and radiographic integration improves treatment precision in routine care.

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