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Photodynamic therapy response of *Filifactor alocis* and *Fretibacterium fastidiosum*: Diabetic versus non-diabetic chronic periodontitis patients

Jitendra Jasutkar, Sumedha Srivastva, Veena Kalburgi*, Neha Jaiswal, Harsimran Kaur & Sahaj Samaiya

Department of Periodontology, People's College of Dental Sciences and Research Centre, People's University, Bhopal, Madhya Pradesh, India; *Corresponding author

Affiliation URL:
https://www.peoplesuniversity.edu.in/dental-college-bhopal/

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Author contact:
Jitendra Jasutkar - Email: jitendrajasutkar@gmail.com
Sumedha Srivastva - Email: srivastavasumi88@gmail.com
Veena Kalburgi - Email: drveenakalburgi1@gmail.com
Neha Jaiswal - Email: neha.malviya89@gmail.com
Harsimran Kaur - Email: hsksasan@gmail.com
Sahaj Samaiya - Email: sahajsamaiya236@gmail.com

Abstract:
Chronic generalized periodontitis is associated with emerging periodontal pathogens, posing greater challenges for microbial control in patients with diabetes. Therefore, it is of interest to evaluate the prevalence of *Filifactor alocis* and *Fretibacterium fastidiosum* in healthy individuals and diabetic and non-diabetic patients with chronic generalized periodontitis. Thirty patients underwent clinical and microbiological assessment before and after scaling and root planing with adjunctive photodynamic therapy. Adjunctive photodynamic therapy reduced bacterial prevalence and significantly improved plaque index, gingival inflammation, probing depth and clinical attachment level. Clinical improvements were high in non-diabetic patients, supporting photodynamic therapy as a valuable adjunct in the management of chronic periodontitis.

Keywords: Chronic generalized periodontitis; *Filifactor alocis; Fretibacterium fastidiosum;* photodynamic therapy; diabetes mellitus

Background:
Periodontitis is a chronic inflammatory disease affecting the components of the teeth's support and one of the major causes of tooth loss in the world. The three micro-organisms pathogenic to periodontal disease, *Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola* were previously associated with periodontal disease **[1]**. Thanks to recent advances in molecular biology and sequencing, however, several new microbes have been identified that cause periodontal disease **[2]**. *Filifactor alocis*, an emerging pathogen, is being highlighted. *F. alocis* is a Gram-positive obligate anaerobe that is often present in deep periodontal spaces and inflammatory tissues **[3]**. Recently, *Fretibacterium fastidiosum*, an anaerobic pathogen of the Synergistetes, was discovered. It is found in advanced periodontal lesions and represents periodontal dysbiosis and chronic inflammation, with periodontitis being a systemic risk associated with diabetes **[4]**. Chronic hyperglycemia alters immune function, increasing inflammatory mediators and altering the subgingival microbiota, thereby exacerbating periodontal damage **[5, 6]**. Diabetics with chronic periodontitis more likely to have emerging pathogens like *F. aloc.is* **[7, 8]**. Therefore, it is of interest to evaluate the prevalence of *F. alocis* and *F. fastidiosum* and their responses to PDT, in diabetic and non-diabetic CGP patients, given their increasing significance.

Methodology:
Research design and research setting:
A clinical and microbiological non-randomised, parallel-group study was conducted in the Department of Periodontics, People's College of Dental Sciences and Research Centre, Bhopal. The study's objectives were to evaluate both clinical and microbiological effects of PDT in chronic generalised periodontitis patients without and with T2DM and to compare with healthy subjects. The trial lasted long enough to allow recruitment, intervention and follow-up evaluations of patients at baseline and at 1 and 3 months. The study was conducted after obtaining ethical clearance from the Institutional Ethics Committee.

Study cohort and sample size:
There were 30 participants in the study, divided into three groups of 10 each. Group I consisted of people with healthy gums without any periodontal disease and served as controls. Group II included those patients with chronic generalised periodontitis associated with type 2 diabetes mellitus and Group III included patients with chronic generalised periodontitis without diabetes mellitus. The patients were selected from the Department of Periodontics, outpatient division of People's College of Dental Sciences and Research Centre, Bhopal.

Criteria for inclusion:
Patients included in the study ranged in age from 25 to 50 years with moderate to severe generalised periodontitis (GPP) with more than 30% of sites affected. Participants included people with type 2 diabetes mellitus and healthy subjects (no systemic diseases) who fulfilled the clinical criteria. Assessment was limited to vital, asymptomatic teeth.

Criteria for exclusion:
Smokers, tobacco chewers, chronic alcohol drinkers, pregnant or lactating women and those using immunosuppressive drugs were excluded. Patients who had received periodontal treatment, systemic/local antibiotic therapy within the last 6 months and patients who had had a reaction to methylene blue were excluded.

Conduct initial assessment and clinical evaluation:
A comprehensive case history and clinical assessment were conducted for all subjects. Clinical periodontal parameters were documented at baseline, one month and three months by a single calibrated examiner utilizing a UNC-15 periodontal probe. The evaluated parameters comprised the Plaque Index (PI) as per Silness and Löe, the Gingival Index (GI) according to Löe

and Silness, the Modified Sulcular Bleeding Index (mSBI), Probing Depth (PD) and Clinical Attachment Level (CAL). Standard infection control protocols were adhered to during the evaluation. Subgingival plaque specimens were obtained from designated periodontal locations with probing depths of ≥5 mm with sterile Gracey curettes. The sampling locations were segregated to avert saliva contamination. Samples were placed in decreased transport fluid and conveyed to the microbiological laboratory under anaerobic conditions. Enriched culture media with suitable additives were utilized for the isolation of anaerobic periodontal pathogens. Colony-forming unit (CFU) quantification and microbiological identification were conducted to ascertain the presence of *Filifactor alocis* and *Fretibacterium fastidiosum*. Subgingival plaque specimens were obtained from designated periodontal locations with probing depths of ≥5 mm with sterile Gracey curettes. The sampling locations were segregated to avert saliva contamination. Samples were placed in decreased transport fluid and conveyed to the microbiological laboratory under anaerobic conditions. Enriched culture media with suitable additives were utilized for the isolation of anaerobic periodontal pathogens. Colony-forming unit (CFU) quantification and microbiological identification were conducted to ascertain the presence of *Filifactor alocis* and *Fretibacterium fastidiosum*.

Photodynamic therapy protocol:
Photodynamic treatment was provided to Groups II and III. A 1% methylene blue solution served as the photosensitising agent and was administered into periodontal pockets. Following sufficient incubation, activation was performed utilizing an 810-nm soft tissue diode laser equipped with a fiber-optic tip in accordance with established clinical protocol. Laser irradiation was meticulously administered within the periodontal pocket to guarantee optimal activation of the photosensitizer and elimination of microbes, while minimizing tissue damage.

Follow up evaluation:
At 1 month and 3 months after the treatment, patients were re-evaluated. Periodontal parameters and microbiological findings were reassessed at every appointment. The improvement in periodontal status and the reduction in microbial load were the key outcome measures ures. A comparative evaluation was conducted among healthy, diabetic and non-diabetic periodontitis patients.

Statistical examination:
The data obtained were entered into microcomputers and analysed using the Statistical Package for Social Sciences (SPSS) Software version 26.0. One-Way ANOVA with Tukey's test was used for intergroup comparisons and Repeated-measures ANOVA was used for intragroup comparisons over time intervals. A p-value ≤ 0.05 was considered statistically significant.

Results:
The study included 30 participants, divided into three groups: healthy controls; patients with chronic generalised periodontitis and type 2 diabetes mellitus; and patients with chronic generalised periodontitis without diabetes mellitus (n = 10 per group). Clinical periodontal parameters and microbiological findings were evaluated at baseline, one month and three months. At baseline, both periodontitis groups showed significantly poorer periodontal health than the healthy control group. Following scaling and root planing combined with adjunctive antimicrobial photodynamic therapy (aPDT), both periodontitis groups showed notable improvements in plaque index, gingival inflammation, probing pocket depth and clinical attachment level at three months **(Table 1).** The non-diabetic periodontitis group demonstrated marginally greater clinical improvement than the diabetic periodontitis group. *Filifactor alocis* and *Fretibacterium fastidiosum* were predominantly detected in patients with periodontitis, particularly in those with diabetes mellitus. Following adjunctive aPDT, significant reductions were observed in both bacterial prevalence and mean microbial load at three months **(Table 2).** Despite microbiological improvement in both treatment groups, residual bacterial levels remained higher in patients with diabetes than in those without diabetes. Overall, chronic generalised periodontitis was associated with a higher prevalence of emerging periodontal pathogens, including *F. alocis* and *F. fastidiosum*, particularly among individuals with type 2 diabetes mellitus. Significant clinical and microbiological improvements were observed three months after treatment. These findings suggest that aPDT may provide beneficial adjunctive effects when combined with scaling and root planing in the management of chronic generalized periodontitis.

Table 1: Comparison of clinical periodontal parameters among study groups

Parameter	Healthy Controls (n=10)	Diabetic Periodontitis + PDT (n=10)	Non-Diabetic Periodontitis + PDT (n=10)	p-value
Plaque Index (Baseline)	0.72 ± 0.18	2.41 ± 0.31	2.29 ± 0.28	<0.001*
Plaque Index (3 Months)	0.70 ± 0.17	1.12 ± 0.24	0.96 ± 0.21	<0.001*
Gingival Index (Baseline)	0.64 ± 0.15	2.32 ± 0.29	2.18 ± 0.26	<0.001*
Gingival Index (3 Months)	0.60 ± 0.13	0.94 ± 0.18	0.82 ± 0.16	<0.001*
Probing Depth (mm) Baseline	1.8 ± 0.4	6.4 ± 0.8	5.9 ± 0.7	<0.001*
Probing Depth (mm) 3 Months	1.7 ± 0.3	3.7 ± 0.6	3.2 ± 0.5	<0.001*
Clinical Attachment Level (mm) Baseline	0.0 ± 0.0	6.8 ± 0.9	6.3 ± 0.8	<0.001*
Clinical Attachment Level (mm) 3 Months	0.0 ± 0.0	4.5 ± 0.7	3.9 ± 0.6	<0.001*

Table 2: Comparison of microbiological findings among study groups

Microbiological Variable	Healthy Controls (n=10)	Diabetic Periodontitis + PDT (n=10)	Non-Diabetic Periodontitis + PDT (n=10)	p-value
***Filifactor alocis* Positive – Baseline**	1 (10%)	9 (90%)	8 (80%)	<0.001*
***Filifactor alocis* Positive – 3 Months**	1 (10%)	3 (30%)	2 (20%)	0.021*
***Fretibacterium fastidiosum* Positive – Baseline**	0 (0%)	8 (80%)	7 (70%)	<0.001*
***Fretibacterium fastidiosum* Positive – 3 Months**	0 (0%)	2 (20%)	1 (10%)	0.034*
Mean Bacterial Load (CFU ×10^5) Baseline	0.4 ± 0.2	8.9 ± 1.8	7.8 ± 1.5	<0.001*
Mean Bacterial Load (CFU ×10^5) 3 Months	0.3 ± 0.1	2.6 ± 0.8	1.9 ± 0.6	<0.001*

- **Chi-square/Fisher's exact test – presence or absence of bacterial species**
- **Repeated Measures ANOVA and One-Way ANOVA – bacterial load and quantitative parameters**

Discussion:

The present study aimed to evaluate the prevalence of *Filifactor alocis* and *Fretibacterium fastidiosum* among healthy individuals, patients with chronic generalised periodontitis and type 2 diabetes mellitus and non-diabetic patients with chronic generalised periodontitis. It also evaluated the effect of adjunctive antimicrobial photodynamic therapy (aPDT). The findings revealed significantly poorer periodontal health and higher microbial loads among patients with periodontitis, particularly those with diabetes mellitus. These results support the current understanding that diabetes intensifies periodontal inflammation and may alter the subgingival microbiota towards a more pathogenic profile. At baseline, the diabetic and non-diabetic periodontitis groups demonstrated significantly higher plaque index, gingival index, probing pocket depth and clinical attachment loss than healthy individuals. Patients with diabetes also exhibited greater periodontal destruction than non-diabetic patients. This finding is consistent with recent studies indicating that hyperglycaemia increases inflammatory cytokine production and oxidative stress while impairing immune function and wound healing. These mechanisms may accelerate periodontal tissue destruction and increase disease severity **[9, 10]**. Microbiological analysis showed a significantly higher prevalence of *F. alocis* and *F. fastidiosum* in both chronic periodontitis groups, with the highest detection rates observed among patients with diabetes. At baseline, *F. fastidiosum* was detected in 90% of diabetic patients with periodontitis and 80% of non-diabetic patients with periodontitis. These findings are consistent with previous studies identifying *F. alocis* as an emerging periodontal pathogen strongly associated with severe periodontitis. It may also interact synergistically with other periodontal pathogens, thereby contributing to dysbiosis and disease progression **[11, 12]**. Significant improvements in all clinical periodontal parameters and microbial counts were observed three months after scaling and root planing combined with adjunctive aPDT. Both treatment groups showed reductions in probing pocket depth and gains in clinical attachment level. However, patients with diabetes showed less clinical improvement than non-diabetic patients. This difference may be explained by chronic metabolic dysregulation, an exaggerated inflammatory response and delayed tissue healing associated with diabetes mellitus. Previous studies have reported improvements in periodontal parameters following adjunctive aPDT, although the magnitude of benefit may depend on disease severity and the patient's glycaemic control **[13–16]**. The significant reductions in *F. alocis* and *F. fastidiosum* following aPDT may be attributed to the antibacterial action of photoactivated methylene blue. Photoactivation generates reactive oxygen species that damage bacterial cell membranes and other essential cellular components. The combination of mechanical debridement and photodynamic bacterial inactivation may therefore explain the favourable clinical and microbiological outcomes observed in this study. Overall, the findings support the potential role of emerging periodontal pathogens in chronic generalised periodontitis, particularly among patients with type 2 diabetes mellitus. They also suggest that aPDT may be a beneficial adjunct to conventional non-surgical periodontal therapy, especially in patients with a high microbial burden. However, these findings should be interpreted with caution given the small sample size and short follow-up period.

Conclusion:

Patients with chronic generalised periodontitis were more likely to have *Filifactor alocis* and *Fretibacterium fastidiosum*, particularly in patients with diabetes. Adjunctive PDT with S+RP resulted in significant clinical improvement and reduction in the microbial load. Thus, we show that PDT could be used as an effective adjunct treatment for chronic periodontitis associated with periodontal infection.

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