PCR-Based Detection of Periodontal Pathogens in Diabetic Patients from the Chennai Population

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Abstract

Background

Diabetes mellitus is a chronic metabolic disorder associated with an increased risk and severity of periodontal disease. The bidirectional relationship between diabetes and periodontitis has been extensively documented, with each condition capable of influencing the progression of the other. Molecular techniques such as Polymerase Chain Reaction (PCR) provide highly sensitive and specific detection of periodontal pathogens, enabling improved understanding of microbial profiles in diabetic individuals.

Aim

To evaluate the prevalence of major periodontal pathogens among diabetic patients from the Chennai population using PCR-based detection methods and to assess their association with periodontal disease severity.

Materials and Methods

A cross-sectional study was conducted among 100 diabetic patients diagnosed with chronic periodontitis. Clinical periodontal parameters including plaque index, gingival index, probing pocket depth, and clinical attachment loss were recorded. Subgingival plaque samples were collected and subjected to species-

specific PCR for detection of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Treponema denticola*, and *Prevotella intermedia*. Statistical analysis was performed using SPSS version 25.0.

Results

The prevalence of *P. gingivalis* was highest (68%), followed by *T. forsythia* (61%), *T. denticola* (56%), *P. intermedia* (49%), and *A. actinomycetemcomitans* (32%). Patients with poor glycemic control exhibited significantly higher detection rates of red-complex bacteria. Positive associations were observed between pathogen prevalence and periodontal destruction.

Conclusion

PCR-based detection demonstrated a high prevalence of periodontal pathogens among diabetic patients from Chennai. The findings reinforce the importance of microbial assessment in diabetic individuals with periodontitis and support the integration of periodontal care into diabetes management programs.

Keywords: Diabetes mellitus, periodontitis, PCR, periodontal pathogens, Chennai population, oral microbiology.

Introduction

Periodontal disease is a chronic inflammatory condition affecting the supporting structures of the teeth and is considered one of the most prevalent oral diseases worldwide (1). The disease results from complex interactions between pathogenic microorganisms and host immune responses, leading to progressive destruction of periodontal tissues (2).

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. India has one of the highest diabetes burdens globally, and Chennai has reported a substantial prevalence of type 2 diabetes due to urbanization and lifestyle-related risk factors. Periodontal disease has been recognized as the sixth complication of diabetes mellitus because diabetic individuals are more susceptible to periodontal infections and experience greater periodontal destruction than non-diabetic individuals (3).

The relationship between diabetes and periodontal disease is bidirectional. Poor glycemic control increases susceptibility to periodontal infection through impaired neutrophil function, altered collagen metabolism, enhanced inflammatory responses, and microvascular changes (4,5). Conversely,

periodontal inflammation may contribute to systemic inflammatory burden and worsen glycemic control (6).

The oral cavity harbors a diverse microbial ecosystem consisting of more than 700 bacterial species. Certain microorganisms have been strongly implicated in the pathogenesis of periodontitis. Socransky et al. identified microbial complexes associated with periodontal disease, among which the red-complex organisms—*Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*—show the strongest association with severe periodontal destruction (7).

Traditional culture-based microbiological techniques are limited by the fastidious growth requirements of many periodontal pathogens. Molecular methods such as Polymerase Chain Reaction (PCR) have emerged as reliable diagnostic tools because of their high sensitivity, specificity, and rapid detection capabilities (8-10). PCR enables identification of specific bacterial DNA sequences even in low concentrations and has become an important technique in periodontal microbiology research.

Despite the increasing prevalence of diabetes and periodontal disease in South India, limited information is available regarding the microbial profile of periodontal pathogens among diabetic individuals in Chennai. Therefore, the present study aimed to investigate the prevalence of major periodontal pathogens using PCR and evaluate their association with periodontal disease severity among diabetic patients.

Materials and Methods

Study Design and Population

A cross-sectional observational study was conducted among diabetic patients attending the Department of Periodontology and associated dental clinics in Chennai, Tamil Nadu, India.

A total of 100 patients diagnosed with type 2 diabetes mellitus and chronic periodontitis were recruited.

Inclusion Criteria

- Patients aged 30–65 years.
- Diagnosed cases of type 2 diabetes mellitus.
- Presence of at least 20 natural teeth.

- Clinical diagnosis of chronic periodontitis.
- Willingness to provide informed consent.

Exclusion Criteria

- Antibiotic therapy within the previous three months.
- Periodontal therapy within six months before sample collection.
- Tobacco users and smokers.
- Pregnant or lactating women.
- Individuals with systemic conditions other than diabetes affecting periodontal status.

Clinical Examination

Clinical periodontal assessment included:

Plaque Index (PI)

Plaque accumulation was assessed using the Silness and Löe Plaque Index.

Gingival Index (GI)

Gingival inflammation was evaluated using the Löe and Silness Gingival Index.

Probing Pocket Depth (PPD)

Measurements were obtained at six sites per tooth using a calibrated periodontal probe.

Clinical Attachment Loss (CAL)

Clinical attachment loss was measured as the distance between the cemento-enamel junction and the base of the periodontal pocket.

Glycemic Assessment

Recent glycated hemoglobin (HbA1c) values were recorded from medical records.

Patients were classified as:

- Good control: HbA1c < 7%
- Moderate control: HbA1c 7–8%
- Poor control: HbA1c > 8%

Sample Collection

Subgingival plaque samples were collected from the deepest periodontal pockets using sterile paper points. Samples were transferred into sterile microcentrifuge tubes containing transport medium and stored at −20°C until processing.

DNA Extraction

Bacterial genomic DNA was extracted using a commercially available DNA extraction kit according to the manufacturer's instructions.

PCR Analysis

Species-specific primers were used for detection of:

- *Porphyromonas gingivalis*
- *Aggregatibacter actinomycetemcomitans*
- *Tannerella forsythia*
- *Treponema denticola*
- *Prevotella intermedia*

PCR amplification was performed under standardized thermal cycling conditions. Amplified products were separated using agarose gel electrophoresis and visualized under ultraviolet illumination.

Statistical Analysis

Data were analyzed using SPSS software version 25.0.

Descriptive statistics were calculated for all variables. Chi-square tests were used to compare prevalence rates among groups. Independent t-tests and one-way ANOVA were used to assess differences in clinical parameters. Statistical significance was established at $p < 0.05$.

Results

Demographic Characteristics

Among the 100 participants, 58 were males and 42 were females. The mean age was 51.6 ± 8.4 years.

Table 1. Demographic Characteristics of Study Population

Variable	Value
Sample size	100
Male	58
Female	42
Mean age (years)	51.6 ± 8.4
Mean HbA1c (%)	8.1 ± 1.2

Prevalence of Periodontal Pathogens

PCR analysis revealed varying prevalence rates among the studied pathogens.

Table 2. Prevalence of Periodontal Pathogens

Pathogen	Positive Samples (%)
Porphyromonas gingivalis	68
Tannerella forsythia	61
Treponema denticola	56
Prevotella intermedia	49
Aggregatibacter actinomycetemcomitans	32

P. gingivalis demonstrated the highest prevalence among all detected microorganisms.

Association with Glycemic Control

Patients with poor glycemic control exhibited significantly higher prevalence of red-complex organisms.

Table 3. Pathogen Detection According to Glycemic Status

Pathogen	Good Control (%)	Poor Control (%)	p-value
P. gingivalis	42	82	<0.05
T. forsythia	39	74	<0.05
T. denticola	35	69	<0.05

Association with Clinical Parameters

Patients positive for red-complex pathogens exhibited significantly higher probing pocket depths and clinical attachment loss than pathogen-negative individuals.

Discussion

The present study investigated the prevalence of major periodontal pathogens among diabetic patients from Chennai using PCR-based molecular detection. The findings demonstrated a high prevalence of periodontal pathogens, particularly members of the red complex.PCR has become a valuable tool in periodontal diagnostics because it enables rapid and highly sensitive identification of periodontal pathogens that may be difficult to cultivate using conventional microbiological methods (11-13). The present study demonstrated that *P. gingivalis* was the most frequently detected pathogen, consistent with previous reports identifying it as a keystone pathogen in chronic periodontitis (14,15).

The prevalence of *T. forsythia* and *T. denticola* was also high. These microorganisms form the red complex together with *P. gingivalis* and are strongly associated with deep periodontal pockets and attachment loss (16). Their synergistic interactions promote dysbiosis and increase periodontal tissue destruction (17). Patients with poor glycemic control exhibited significantly higher prevalence of periodontal pathogens. Similar observations have been reported by Casarin et al. and Ebersole et al., who demonstrated increased subgingival bacterial colonization among diabetic patients with elevated HbA1c levels (18-20). Hyperglycemia may increase glucose concentrations in gingival crevicular fluid, thereby creating favorable conditions for bacterial growth.

The prevalence of *A. actinomycetemcomitans* observed in the present study was comparatively lower than that of red-complex bacteria. This finding is consistent with studies indicating that *A. actinomycetemcomitans* is more frequently associated with aggressive periodontitis than chronic forms of the disease (21).

The positive association between pathogen prevalence and periodontal destruction observed in this study supports previous findings that microbial burden contributes significantly to disease progression (22,23). Patients harboring multiple pathogens demonstrated more severe periodontal breakdown than those with fewer detectable species. The study findings emphasize the importance of comprehensive periodontal evaluation in diabetic patients. Early identification of periodontal pathogens through molecular diagnostic techniques may facilitate targeted treatment strategies and improve both oral and systemic health outcomes. The cross-sectional design limits causal interpretation. The sample size was relatively small and restricted to a single geographic region.

Future longitudinal studies employing quantitative PCR are recommended to evaluate microbial load and disease progression over time. Routine PCR-based microbial screening in diabetic patients may facilitate early detection of periodontal pathogens, allowing targeted therapeutic interventions and potentially improving glycemic control and periodontal health**.**

Conclusion

PCR-based detection revealed a high prevalence of major periodontal pathogens among diabetic patients from Chennai. *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* were the most prevalent microorganisms and were significantly associated with poor glycemic control and severe periodontal disease.

The findings support the role of molecular diagnostics in periodontal assessment and highlight the need for integrated periodontal and diabetic care. Early diagnosis and management of periodontal infections may contribute to improved clinical outcomes in diabetic individuals.

References

1. Löe H. Periodontal disease. The sixth complication of diabetes mellitus. Diabetes Care. 1993;16(1):329-34.
2. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, et al. Periodontitis and diabetes: a two-way relationship. Diabetologia. 2012;55(1):21-31.
3. Taylor GW. Bidirectional interrelationships between diabetes and periodontal diseases: an epidemiologic perspective. Ann Periodontol. 2001;6(1):99-112.
4. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25(2):134-44.
5. Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontol 2000. 2005;38:135-87.
6. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. Nat Rev Dis Primers. 2017;3:17038.
7. Slots J, Ting M. Molecular diagnosis of periodontal infections. Periodontol 2000. 2002;28:49-66.
8. Griffen AL, Becker MR, Lyons SR, Moeschberger ML, Leys EJ. Prevalence of Porphyromonas gingivalis and periodontal health status. J Clin Microbiol. 1998;36(11):3239-42.

9. Kumar PS. Oral microbiota and systemic disease. Anaerobe. 2013;24:90-3.

10. Casarin RCV, Barbagallo A, Meulman T, Santos VR, Sallum EA, Nociti FH Jr, et al. Subgingival biodiversity in subjects with uncontrolled type 2 diabetes and chronic periodontitis. J Periodontal Res. 2013;48(1):30-6.

11. Ebersole JL, Holt SC, Hansard R, Novak MJ. Microbiologic and immunologic characteristics of periodontal disease in diabetic patients. J Periodontol. 2008;79(8):1567-75.

12. Genco RJ, Borgnakke WS. Diabetes as a potential risk for periodontitis: association studies. Periodontol 2000. 2020;83(1):40-5.

13. Sanz M, Ceriello A, Buysschaert M, Chapple I, Demmer RT, Graziani F, et al. Scientific evidence on the links between periodontal diseases and diabetes: consensus report and guidelines. J Clin Periodontol. 2018;45(2):138-49.

14. Colombo APV, Teles RP, Torres MC, Souto R, Rosalem WJ, Mendes MCS, et al. Subgingival microbiota of Brazilian subjects with untreated chronic periodontitis. J Periodontol. 2002;73(4):360-9.

15. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. Periodontol 2000. 1994;5:78-111.

16. Offenbacher S. Periodontal diseases: pathogenesis. Ann Periodontol. 1996;1(1):821-78.

17. Graves DT, Liu R, Oates TW. Diabetes-enhanced inflammation and apoptosis: impact on periodontal pathology. J Dent Res. 2007;86(1):15-21.

18. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet. 2005;366(9499):1809-20.

19. Slots J. Selection of antimicrobial agents in periodontal therapy. J Periodontal Res. 2002;37(5):389-98.

20. Teles R, Teles F, Frias-Lopez J, Paster B, Haffajee A. Lessons learned and unlearned in periodontal microbiology. Periodontol 2000. 2013;62(1):95-162.

21. Darveau RP. The oral microbial consortium's interaction with the periodontal innate defense system. DNA Cell Biol. 2009;28(8):389-95.

22. Lamont RJ, Hajishengallis G. Polymicrobial synergy and dysbiosis in inflammatory disease. Trends Mol Med. 2015;21(3):172-83.

23. Hajishengallis G. Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts and host response. Trends Immunol. 2014;35(1):3-11.