ORIGINAL ARTICLE

Porphyromonas gingivalis in Dental Caries: Prevalence and Association with Diabetes and Smoking

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ABSTRACT

Background and objectives: *Porphyromonas gingivalis* is an intraoral anaerobic bacterium that causes periodontal disease. Diabetes and smoking are thought to be the main associated risk factors. The aim of this study was to assess the prevalence of *P. gingivalis* in periodontitis patients and to determine its association with diabetes and smoking. **Methods:** This three - month cross-sectional study was conducted in the department of Microbiology, Sharda Hospital, Greater Noida. 38 plaque samples were collected and cultured anaerobically to look for the growth of *Porphyromonas gingivalis*. **Results:** *P. gingivalis* growth was detected in 27 of 38 samples, accounting for a 71.05% prevalence rate. Diabetic smokers were responsible for 66% of *P. gingivalis* development, which was statistically significant (p value 0.05). **Conclusion:** Diabetic smokers are more vulnerable to developing periodontal infections, especially due to *P. gingivalis*. So, regular dental check-ups and good oral hygiene practices are recommended along with proper treatment.

Keywords: *Porphyromonas gingivalis*; Periodontitis; Diabetes; Smoking; India

Introduction

Porphyromonas is a Gram-negative, asaccharolytic, anaerobic, bacteria belonging to the Porphyromonadaceae family[1]. *Porphyromonas gingivalis*, is the species of the genus that has undergone the most in-depth study and is widely regarded as a significant pathogen connected to periodontal disease in humans[2]. The ability to survive and transmit the infection are highly dependent on its numerous virulence factors, which include structural (lipopolysaccharide, fimbriae, heat shock proteins, etc.) and secretory components (gingipains and outer membrane vesicles)[3]. The known risk factors for periodontitis are smoking and diabetes, which increase its prevalence and severity tenfold[4]. Diabetes mellitus (DM) increases the severity, prevalence, and progression of periodontal disease. Not only does it affect the host immune response, but also modifies the oral environment, which may result in a different periodontal bacteria community than in non-diabetic condition. Increased gingival crevicular fluid glucose levels, in diabetic patients may alter the source of nutrition, influencing the proliferation of particular bacterial species.[5–7].

Smokers have been linked to having deeper tooth pockets, higher attachment loss, more noticeable radiographic indications of engagement with the furcation, and increased alveolar bone loss[8]. Smoking is responsible for about 42% of periodontitis cases, and around 24% of the diabetics have been found to smoke, forming three high-risk categories for periodontal diseases: smokers, diabetics, and

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diabetic smokers[4,9]. Smoking causes peripheral vasoconstriction, reduced vascular permeability, hyperemia, decreased gingival crevicular fluid flow, and lower oxygen tension[4,10,11].

Thus, it is crucial to understand the function of *P. gingivalis* and its implications because DM and smoking are both large and critical risk factors for periodontitis[12]. Therefore, this study was conducted to determine the prevalence of *P. gingivalis* and its association with diabetes mellitus and smoking in cases of periodontitis.

Materials and Methods

This was a cross-sectional study conducted in the department of Microbiology, Central lab, Sharda Hospital, Greater Noida, over a period of 3 months from February to April 2022.

Only those patients who had come to the outpatient department with periodontal plaques without any other oral pathology were included in the study. Plaque samples were then collected and immediately inoculated into Robertson's Cooked Meat (RCM) medium, which was sealed with paraffin oil to maintain anaerobiasis. The inoculated RCM was incubated for one week at 37°C and was checked for the turbidity. Once the turbidity was visible, a loopful of RCM was taken and subcultured on blood agar enriched with hemin and vitamin K. To maintain anaerobic conditions, the culture plates were incubated at 37°C for 1 week in a Gas-pak (HiMedia, Mumbai) jar system along with culture plate inoculated with *Pseudomonas aeruginosa* serving as a negative control for anaerobic culture. The sachet in the Gas Pak jar contained sodium bicarbonate and sodium borohydride, which reacted chemically in the presence of water to form hydrogen and CO_2 gas, and hence eliminated oxygen effectively.

The plates were examined for bacterial growth after one week. The anaerobic conditions were found to be effective as the plates inoculated with *P. aeruginosa* did not show any growth. The colonies showing black colour on the culture media were screened as *Porphyromonas gingivalis*. Presumptive diagnosis was made on the basis of Gram's staining (Gram negative bacilli), catalase test (negative) and motility (non -motile) testing. Confirmation of the isolates was done by automated VITEK-2 Compact system.

The other anaerobic bacteria isolated during the study included *Peptostreptococcus* species and *Bacteroides* species, which were identified on the basis of Gram stain only. Considering these to be oral commensal flora, further identification by Vitek 2 Compact was not done.

Statistical Analysis

Statistical analysis was done using IBM SPSS 22 software. Samples positive for growth of *Porphyromonas gingivalis* were compared with those that were negative. Fischer exact test was used to calculate the p-value.

Results

A total of 38 plaque samples were cultured out of which 27 showed growth of *P. gingivalis* (Table 1). So, the prevalence of *P. gingivalis* in dental caries was found to be 71.05%.

Table 1: Growth of *P. gingivalis* from dental plaque

No. of samples	Growth, n (%)	No Growth, n (%)
38	27 (71.05%)	11 (28.9%)

Table 2: Demographic characteristics of the patients

Demographic Characteristics	n (%)
Gender	
Male	26 (68.4%)
Female	12 (31.5%)
Age (years)	
15 – 30	20 (52.6%)
31 – 50	12 (31.5%)
> 50	6 (15.7%)
Smokers	24 (63.1%)
Non – smokers	14 (36.8%)
Diabetics	22 (57.8%)
Non – Diabetics	16 (42.1%)

Out of 38 samples, 26 (68.4%) were from males and 12 (31.5%) from females. Twenty (52.6%) samples were from patients in the age group between 15–30 years, 31.5% (12) between 31–50 years and 15.7% (6) were over >50 years of age. Smokers accounted for 63.1% (24), while non-smokers for 36.8% (14) of the cases. Diabetic patients comprised 57.8% (22) of the total samples, whereas non-diabetics were found to be 42.1% (16) as shown in Table 2.

Table 3: Association of *P. gingivalis* with demographic characteristics

Demographic Characteristics	Growth of *P. gingivalis* n=27 (%)	No Growth of *P. gingivalis* n=11 (%)	p value
Gender			
Male	19 (70%)	07 (63.6%)	0.714
Female	08 (29%)	04 (36.6%)	
Age (in years)			
15 - 30 (n = 20)	13 (48%)	07 (63.6%)	0.641
31 - 50 (n = 12)	09 (33%)	03 (27%)	
> 50 (n = 6)	05 (18.5%)	01 (9%)	
Smokers	19 (70%)	05 (45%)	0.265
Non-smokers	08 (29%)	06 (54%)	
Diabetics	19 (70%)	03 (27%)	**0.028**
Non-Diabetics	08 (29%)	08 (72.7%)	
Diabetic smokers	18 (66%)	1 (9%)	**0.003**
Diabetic Non-smokers	01 (3.7%)	02 (18%)	
Non-Diabetic smokers	01 (3.7%)	04 (36.6%)	
Non-Diabetic, Non-smokers	07 (25%)	04 (36.6%)	

*p value was statistically significant if <0.05

The association between *P. gingivalis* and the demographic characteristics of the patients has been depicted in Table 3. The growth was not found to be significantly associated with gender, age, and smoking. However, it was statistically significant with diabetics as well as with diabetic smokers.

Discussion

The present study was conducted to determine the prevalence of an important oral pathogen *Porphyromonas gingivalis* in dental caries and to determine its association with smoking and diabetes.

We discovered that the prevalence rate of *P. gingivalis* was 71% over the course of our investigation, which was concordant with the studies conducted by Ingalagi P et al. in 2022 in India[13] and Ardila Carlos M et al. in 2011 in Columbia[14], with prevalence rates of 89.5% and 67.1%, respectively. This suggests that *P. gingivalis* is a major periodontic pathogen found in dental plaques and may be the primary etiological agent in the development of periodontal disease.

Tobacco smoke boosts the production of the collagenase matrix metalloproteases, nicotine causes the degradation of periodontal collagen, which leads to the separation of the periodontal ligaments exposing the tooth to bacterial infections.[15] The prevalence of *P. gingivalis* in smokers was found to be 70% in our study, which is comparable with the study conducted by Abreu MGL et al. in 2019 in Brazil[16] showing 66.7% prevalence of the bacteria in smokers. The probable reason could be due to a weakened immune response and/or increased bacterial pathogenicity[8]. Although the prevalence of *P. gingivalis* in smokers was high, the two were not significantly associated ($p > 0.05$). Our findings are supported by a study done by Zambon and Kinane's[15,17] on the prevalence of *P. gingivalis* in smokers, which showed that there was no statistically significant difference between smokers and non-smokers.

Our study showed a prevalence of 70.3% for the growth of *P. gingivalis* in diabetics, which was comparable to the study done by Montevecchi M et al. in 2021 in Korea[18], having a positivity rate of 56% and to the study conducted by Mohamed HG et al. in 2016 in Norway[19] showing 59.3% of the prevalence in diabetic individuals. So, it can be concluded that low levels of defence cells and high glucose levels in DM patients promote the growth of periodontogenic flora. *P. gingivalis* being a predominant oral pathogen, predisposes diabetic patients to develop severe periodontal disease.[8]

Given that there have been no prior studies assessing the factors of both smoking and diabetes to ascertain the prevalence of *P. gingivalis* in patients with periodontitis, we found that diabetic smokers contributed a statistically significant ($p = 0.003$) growth of *P. gingivalis* i.e., 66.6%.

It has been suggested that smoking impairs subcutaneous blood flow and decreases insulin absorption from subcutaneous tissues. Additionally, it promotes the development of pathogenic microorganisms which reduces the immunological host response and increases inflammatory mediator release.[20] Those

inflammatory mediators that are linked to type 2 diabetes can be increased by *P. gingivalis*. Bacterial endotoxin or lipopolysaccharide (LPS) from infected periodontal areas enters the circulatory system easily and causes endotoxemia. Thus, bacterial endotoxins may drive inflammatory responses in distant tissues, resulting in elevated levels of systemic inflammatory mediators, which increase insulin resistance and promote diabetes development.[21,22]

According to research by Gupta et al.[10] and Orbak R et al.[20], smoking and diabetes are the main risk factors for periodontitis. However, there is currently no existing study that establishes a connection between *P. gingivalis* and both of these factors together. Therefore, further research is needed to investigate this relationship.

Conclusion

To the best of our knowledge, this is the first study to look at the prevalence of *P. gingivalis* in periodontitis patients in relation to both diabetes and smoking in Indian community. According to our study, *P. gingivalis* is one of the major pathogens responsible for periodontitis, taking into account smoking and diabetes as the key risk factors for periodontitis. Periodontal problems are more common among diabetic smokers. Hence, such people, besides having frequent monitoring of their blood sugar levels, should also be motivated to stop smoking, and pay frequent visits to their dentists so as to avoid oral and dental complications.

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Conflict of Interest

The Author(s) declare(s) that there is no conflict of interest.

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References

1. Bostanci N, Belibasakis GN. Porphyromonas gingivalis: an invasive and evasive opportunistic oral pathogen. FEMS Microbiology Letters. 2012;333(1):1–9. Available from: https://doi.org/10.1111/j.1574-6968.2012.02579.x.
2. Bennett JE, Dolin R, Blaser MJ. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 9th ed. Philadelphia. Elsevier. 2020. Available from: https://www.us.elsevierhealth.com/mandell-douglas-and-bennetts-principles-and-practice-of-infectious-diseases-9780323482554.html.
3. Aleksijević LH, Aleksijević M, Škrlec I, Šram M, Šram M, Talapko J. Porphyromonas gingivalis Virulence Factors and Clinical Significance in Periodontal Disease and Coronary Artery Diseases. Pathogens. 2022;11(10):1–19. Available from: https://doi.org/10.3390/pathogens11101173.
4. Ganesan SM, Joshi V, Fellows M, Dabdoub SM, Nagaraja HN, O'Donnell B, et al. A tale of two risks: smoking, diabetes and the subgingival microbiome. ISME Journal. 2017;11(9):2075–2089. Available from: https://doi.org/10.1038/ismej.2017.73.
5. Zhou M, Rong R, Munro D, Zhu C, Gao X, Zhang Q, et al. Investigation of the Effect of Type 2 Diabetes Mellitus on Subgingival Plaque Microbiota by High-Throughput 16S rDNA Pyrosequencing. PLOS ONE. 2013;8(4):1–8. Available from: https://doi.org/10.1371/journal.pone.0061516.
6. Ohlrich EJ, Cullinan MP, Leichter JW. Diabetes, periodontitis, and the subgingival microbiota. Journal of Oral Microbiology. 2010;2(1):1–8. Available from: https://doi.org/10.3402/jom.v2i0.5818.
7. Zhang Z, Liu D, Liu S, Zhang S, Pan Y. The Role of Porphyromonas gingivalis Outer Membrane Vesicles in Periodontal Disease and Related Systemic Diseases. Frontiers in Cellular and Infection Microbiology. 2020;10:1–12. Available from: https://doi.org/10.3389/fcimb.2020.585917.
8. Padmalatha GV, Bavle RM, Satyakiran GV, Paremala K, Sudhakara M, Makarla S. Quantification of Porphyromonas gingivalis in chronic periodontitis patients associated with diabetes mellitus using real-time polymerase chain reaction. Journal of Oral and Maxillofacial Pathology . 2016;20(3):1–6. Available from: https://doi.org/10.4103/0973-029x.190933.
9. National diabetes statistics report: estimates of diabetes and its burden in the United States. 2014. Available from: https://stacks.cdc.gov/view/cdc/23442/cdc_23442_DS1.pdf.
10. Gupta S, Maharjan A, Dhami B, Amgain P, Katwal S, Adhikari B, et al. Status of Tobacco Smoking and Diabetes with Periodontal Disease. Journal of Nepal Medical Association (JNMA). 2018;56(213):818–824. Available from: https://doi.org/10.31729/jnma.3610.
11. Taylor JJ, Preshaw PM, Lalla E. A review of the evidence for pathogenic mechanisms that may link periodontitis and diabetes. Journal of Clinical Periodontology. 2013;40(Suppl. 14):S113–S134. Available from: https://doi.org/10.1111/jcpe.12059.
12. Baek O, Zhu W, Kim HC, Lee SW. Effects of nicotine on the growth and protein expression of Porphyromonas

gingivalis. Journal of Microbiology. 2012;50(1):143–148. Available from: https://doi.org/10.1007/s12275-012-1212-8.
13. Ingalagi P, Bhat KG, Kulkarni RD, Kotrashetti VS, Kumbar V, Kugaji M. Detection and comparison of prevalence of Porphyromonas gingivalis through culture and Real Time-polymerase chain reaction in subgingival plaque samples of chronic periodontitis and healthy individuals. Journal of Oral and Maxillofacial Pathology. 2022;26(2):1–7. Available from: https://doi.org/10.4103/jomfp.jomfp_163_21.
14. Ardila CM, Guzmán IC. Association of Porphyromonas gingivalis with high levels of stress-induced hormone cortisol in chronic periodontitis patients. Journal of Investigative and Clinical Dentistry. 2016;7(4):361–367. Available from: https://doi.org/10.1111/jicd.12175.
15. Lindhe J, Karring T, Lang NP, et al., editors. Clinical Periodontology and Implant Dentistry. 4th ed. Wiley–Blackwell. 2003.
16. Abreu M, Kawamoto D, Mayer MPA, et al. Frequency of Porphyromonas gingivalis fimA in smokers and nonsmokers after periodontal therapy. Journal of Applied Oral Science. 2019;27:1–8. Available from: https://doi.org/10.1590/1678-7757-2018-0205.
17. Zambon JJ. Periodontal diseases: Microbial factors. Annals of Periodontology. 1996;1(1):879–925. Available from: https://doi.org/10.1902/annals.1996.1.1.879.
18. Montevecchi M, Valeriani L, Gatto MR, D'Alessandro G, Piana G. Subgingival pathogens in chronic periodontitis patients affected by type 2 diabetes mellitus: a retrospective case-control study. Journal of Periodontal & Implant Science. 2021;51(6):409–421. Available from: https://doi.org/10.5051/jpis.2100180009.
19. Mohamed HG, Idris SB, Mustafa M, Ahmed MF, Åstrøm AN, Mustafa K, et al. Influence of Type 2 Diabetes on Prevalence of Key Periodontal Pathogens, Salivary Matrix Metalloproteinases, and Bone Remodeling Markers in Sudanese Adults with and without Chronic Periodontitis. International Journal of Dentistry. 2016;2016:1–9. Available from: https://dx.doi.org/10.1155/2016/6296854.
20. Orbak R, Tezel A, Çanakçi V, Demir T. The Influence of Smoking and Non-Insulin-Dependent Diabetes Mellitus on Periodontal Disease. Journal of International Medical Research. 2002;30(2):116–125. Available from: https://dx.doi.org/10.1177/147323000203000203.
21. Jia S, Li X, Du Q. Host insulin resistance caused by Porphyromonas gingivalis-review of recent progresses. Frontiers in Cellular and Infection Microbiology. 2023;13:1–16. Available from: https://doi.org/10.3389/fcimb.2023.1209381.
22. Martínez-García M, Hernández-Lemus E. Periodontal Inflammation and Systemic Diseases: An Overview. Frontiers in Physiology. 2021;12:1–26. Available from: https://dx.doi.org/10.3389/fphys.2021.709438.

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