Comparative Assessment of *P. gingivalis* Level in Periodontitis Patients with and without Diabetes Mellitus- A PCR based Study

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

**Introduction:** Periodontal diseases if left untreated can lead to tooth loss with the main cause being bacterial plaque. Among the subgingival plaque bacterial species, *Porphyromonas gingivalis* has been implicated as a major etiological agent causing tooth loss. Diabetic patients are at high risk for periodontal disease. Our aim was to compare the involvement of *P. gingivalis* in diabetes mellitus (DM) patients associated with periodontitis and to compare them with periodontitis patients having no other systemic pathologies.

**Materials and Methods:** Subgingival plaque samples from a total of 8 patients were collected. DNA was isolated from the collected samples and was quantified using RT-PCR for standardizing the polymerase chain reaction. Paired t test was performed using the statistical software Graphpad prism (Version 7.0).

**Results:** There was a statistically significant level of *P. gingivalis* seen in periodontitis patients having DM (p=0.0053), whereas the least score was seen in periodontitis patients without DM.

**Conclusion:** Poor glycemic control, as indicated by HbA1c≥7%, is associated with increased levels and frequencies of periodontal pathogens in the subgingival biofilm of subjects with DM.
Keywords: Diabetes mellitus; innovative technology; periodontitis; porphyromonasgingivalis; real-time polymerase chain reaction.

1. INTRODUCTION

Periodontal disease is one of the most common diseases of the oral cavity and is the major cause of tooth loss in adults [1]. Recently, there have been studies correlating the relationship of periodontal disease to important systemic diseases, such as cardiovascular disease and complications in pregnancy [2]. There are two main categories of periodontal disease in which loss of supporting structures around the tooth occurs: periodontitis and aggressive periodontitis [3]. Periodontitis is the result of a polymicrobial infection with variable microbial patterns. periodontitis is an inflammatory disease of the supporting tissues of the teeth associated with bacteria. It results in either localized or generalized destruction of the supporting tissues of the teeth; the periodontal ligament, bone, and soft tissues. In contrast, aggressive periodontitis involves rapid attachment loss and bone destruction, and the destruction seen is usually not commensurate with the amount of microbial deposits [4].

Bacteria are the primary etiologic factor of periodontal diseases, however, recent evidence also lists yeast and herpesviruses as putative pathogens responsible for periodontitis [5,6]. Porphyromonasgingivalis (P. gingivalis) being Gram-negative non spore forming, non-motile, obligate anaerobe, rod-shaped and highly virulent organism has been implicated as a major pathogen in destructive periodontal disease since it has the ability to adhere and invade oral epithelium. These pathogens gain entry into circulation through the ulcerated epithelium and exposed capillaries during periodontal inflammation and may induce systemic symptoms. It is also implicated to be involved in the development of systemic diseases due to systemic inflammation with increased circulating cytokines and mediators, direct infection and cross-reactivity/molecular mimicry between bacterial antigens and self-antigens [7–10].

Diabetes Mellitus has been undoubtedly confirmed as a major risk factor for periodontitis [11]. In the early 1990s periodontitis was sometimes referred to as the ‘sixth complication of diabetes’ [12], and in 2003 the ADA acknowledged that periodontal disease is often found in people with diabetes [13]. There has recently been much emphasis on the ‘two-way’ relationship between diabetes and periodontitis. That is, not only is diabetes a risk factor for periodontitis, but periodontitis could have a negative effect on glycaemic control [14]. In addition, various studies have reported that the prevalence and severity of non-oral diabetes-related complications, including retinopathy, diabetic neuropathy, proteinuria and cardiovascular complications, are correlated with the severity of periodontitis [15,16]. However there is no clear cut idea about the bacterial species most commonly involved in causing periodontitis in diabetes and also if there is any increased involvement of the pathogens. Although being able to determine which subjects are at greater risk of future periodontal breakdown is undoubtedly beneficial to the patient and clinician, determination of increased risk of disease most commonly by a specific bacteria within a patient would be the ideal in periodontal diagnosis. There is also a lack of prospective longitudinal data regarding the association of levels of pathogenic species in subgingival plaque with the progress. In this way this study fulfills the lacunae created by the previous studies and sheds light for the dentists and general population. With this background in mind, the current study aims to quantify and compare the P. gingivalis levels in periodontitis patients with/ without diabetes.

Our team has extensive knowledge and research experience that has translate into high quality publications [17–29,30–34,35,36].

2. MATERIALS AND METHODS

Group I periodontitis patients without diabetes mellitus and Group II periodontitis patients with diabetes mellitus were selected by assessing the periodontal status, HbA1c and blood glucose levels. The enrollment criteria for the study are as follows

Inclusion criteria:

1. For periodontitis included individuals with not >2 teeth missing in each quadrant; ≥ 30% of periodontal sites with PD ≥ 4 mm; ≥20% of periodontal sites with interproximal clinical AL >2 mm; ≥30% of sites show- ing BOP; and radiographic evidence of bone loss visible in posterior vertical bitewing films.
2. For Diabetes mellitus patients included RBS >200 mg/dl
Fasting > 110 mg/ dl
HbA1C >7

Exclusion criteria: Individuals with pregnancy, previous or current smokers, menopause, cardiovascular disorders, thyroid disorders, use of antioxidant supplements, long-term steroid medications, patient who had taken anti-inflammatory or antibiotics within previous 3 months or underwent periodontal treatment in the past 6 months were excluded from the investigation.

Sample collection: Supragingival and subgingival plaque samples were collected from 20 patients (10 patients in each group) with the help of curette and transported to phosphate buffered saline and stored at -80 degree celsius for further analysis.

Isolation and Quantification of DNA: Genomic DNA was extracted from subgingival plaque of both the patients group using a QIA amp DNA Mini kit (QIAGEN Inc., USA, 9300 Germantown Road, Germantown, MD 20874). The DNA concentration and purity was maintained using Nano-Drop Spectrophotometer (NanoDrop™ 2000/2000c Spectrophotometer, 168 Third Avenue, Waltham, MA USA 02451) with a multi-wavelength programme (260/280 nm).

Quantification of *P. gingivalis* by Real Time-PCR: Quantitative RT-PCR was performed with the CFX 96 Real Time system (Bio-Rad, USA) with SYBR Premix Ex Taq (Takara, Japan) in triplicates using the primers listed. The double standard DNA-binding dye SYBR Green I (KAPA SYBR FAST q-PCR Kit) using species-specific primers used for *P. gingivalis* (Forward: 5’-AGG CAG CTT GCC ATA CTG CG-3’ Reverse:5’-ACT GTT AGC AAC TAC CGA TGT-3’), and the reaction efficiency was optimized as follows: Enzyme activation (PCR initial activation): 95°C for 30 min; denaturation 95°C for 55 sec; Annealing: 60°C & 57°C for 45 sec and extension: 72°C for 30 sec with 40 cycles. All the reactions were performed in triplicate along with no template control (NTC). Melt curve analysis was performed using the thermal cycling programmed at 59°C - 95°C for each sample to determine the presence of multiple amplicons, non-specific products, and contaminants. Random samples (PCR products) from control and treated groups were resolved on a 2% agarose gel electrophoresis along with 100 bp molecular marker DNA and visualized with the use of ethidium bromide as a quality control. Agarose gel was compared with melting curves for the presence of the appropriate sized amplicon as well as the presence of a single PCR product. The relative amount of genes was calculated by using the comparative CT method.

Statistical analysis: The data was tabulated in Microsoft Excel sheet. Data analysis was performed using SPSS software version 23. Paired t test was performed for comparison of *P. gingivalis* expression among the diabetic and non-diabetic groups.

3. RESULTS AND DISCUSSION

As periodontal diseases are polymicrobial infections with various etiologic factors, accurate quantitation of the number of cells of individual bacterial species in dental plaque samples is needed for understanding the bacterial etiology of periodontitis. Hence, in our study, we intend to quantify the *P. gingivalis* count using real-time polymerase chain reaction (RT-PCR), since it is more efficient and sensitive compared to other methods and provides precise counts through direct monitoring of the increasing amount of PCR product throughout the enzymatic assay [37]. Also, in our study subgingival plaque samples were chosen over other samples such as saliva and gingival crevicular fluid for better yield and to understand bacterial etiology of periodontitis.

The results of the study revealed that the *P. gingivalis* are of statistically significant level (p=0.0053) in the periodontitis patients with diabetes mellitus (Table 1); Fig. 1 reveals the three fold increase of *P. gingivalis* level in periodontitis patients with diabetes mellitus than patients without diabetes.

| Group               | Periodontitis | Periodontitis +DM | P value   |
|---------------------|---------------|-------------------|-----------|
| *P. gingivalis* mRNA (Fold change over control) | 1±0.000       | 1.350±0.02887     | p<0.0067  |
Fig. 1. Amplification plots showing the mRNA levels of *P. gingivalis* in periodontitis without diabetes mellitus using gene specific primers. Each bar represents mean ± SD (n=20). Significance at P <0.05, **- Significantly different from the control group (Periodontitis).

Fig. 2. Assessment of mRNA expression of *P. gingivalis*. The mRNA expression was assessed by Real Time-PCR using gene specific primers. Each bar represents mean ± SD (n=20). Significance at P <0.05, **- Significantly different from the control group (Periodontitis).

The reason for this increased level of *P. gingivalis* is the fact that diabetes increases the glucose concentration in the gingival crevicular fluid and decreases the salivary levels of epidermal growth factor which plays an important role in wound healing. These modifications in GCF affect plaque composition which is supported by an increased amount of plaque and increased numbers of Gram-negative anaerobes. So in addition to diabetes, if the patient suffers from periodontitis, it will impair cellular functions, impair host defense, vascular alterations, prolonged inflammation, impair bone formation or repair ultimately resulting in tooth mobility and premature loss of teeth [38].

Since there is a two-way relationship between DM and periodontitis, reduction of bacterial
burden by periodontal therapy may show a greater impact in the prevention of periodontal disease progression, which in turn can lead to the reduction in the glycemic control in diabetic patients. There have been many recent studies with good evidence to support this hypothesis. Grossi and others have suggested that effective control of periodontal infection in diabetic patients reduces the level of AGEs in the serum [39]. The level of glycemic control seems to be the key factor. Tervonen and Karjalainen followed diabetic patients and nondiabetic controls for 3 years; They found that the level of periodontal health in diabetic patients with good or moderate control of their condition was similar to that in the nondiabetic controls. Those with poor control had more attachment loss and were more likely to exhibit recurrent disease [40]. This phenomenon has been pointed out by several other researchers also. From this, we can conclude that prevention and control of periodontal disease must be considered an integral part of diabetes control. Hence it is suggested that periodontal therapy should be included as a part of prevention programs in systemic diseases such as diabetes [41–43].

The only limitation of this study is smaller sample size. Hence, further studies need to be done with a large sample size to get even more statistically significant results. Larger studies of this kind can throw light on a variety of etiological microorganisms and also will confirm the findings of our study.

4. CONCLUSION

In our study, periodontitis individuals with diabetes mellitus harbor increased P. gingivalis bacteria (p= 0.0053) than patients without diabetes. This states the strong association of periodontitis and diabetes mellitus. Hence, reduction of bacterial burden by periodontal therapy may show a greater impact in the prevention of periodontal disease progression, especially in diabetic individuals. Therefore it is suggested that periodontal therapy should be included as an integral part of prevention programs in systemic diseases such as diabetes.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

As per international standard or university standard, patients’ written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fox CH. New considerations in the prevalence of periodontal disease. CurrOpin Dent. 1992 Mar;2:5–11.
2. de Pablo P, Chapple ILC, Buckley CD, Dietrich T. Periodontitis in systemic rheumatic diseases. Nat Rev Rheumatol. 2009 Apr;5(4):218–24.
3. Yilmaz Ö. The ises of Porphyromonas gingivalis: the microbium, the human oral epithelium and their interplay [Internet]. Microbiology. 2008;154:2897–903. Available:http://dx.doi.org/10.1099/mic.0.2 008/021220-0
4. Armitage GC. Development of a classification system for periodontal
diseases and conditions [Internet]. Annals of Periodontology. 1999;4:1–6. Available: http://dx.doi.org/10.1902/annals.1999.4.1.1

5. Pihlstrom BL. Periodontal risk assessment, diagnosis and treatment planning [Internet]. Periodontology. 2001;25:37–58. Available: http://dx.doi.org/10.1034/j.1600-0757.2001.22250104.x

6. Contreras A, Slots J. Herpesviruses in human periodontal disease. J Periodontal Res. 2000 Feb;35(1):3–16.

7. Slots J, Chen C. The oral microflora and human periodontal disease [Internet]. Medical Importance of the Normal Microflora. 1999;101–27. Available: http://dx.doi.org/10.1007/978-1-4757-3021-0_5

8. Maresz KJ, Hellvard A, Sroka A, Adamowicz K, Bielecka E, Koziel J, et al. Porphyromonas gingivalis facilitates the development and progression of destructive arthritis through its Unique Bacterial Peptidylarginine Deiminase (PAD) [Internet]. PLoS Pathogens. 2013;9:e1003627. Available: http://dx.doi.org/10.1371/journal.ppat.1003627

9. Mantri CK, Chen C, Dong X, Goodwin JS, Pratap S, Paromov V, et al. Fimbriae-mediated outer membrane vesicle production and invasion of Porphyromonas gingivalis [Internet]. MicrobiologyOpen. 2015;4:53–65. Available: http://dx.doi.org/10.1002/mbo3.21

10. Löhr G, Beikler T, Podbielski A, Standar K, Redanz S, Hensel A. Polyphenols from Myrothamnus flabellifolia Welw. Inhibit in vitro adhesion of Porphyromonas gingivalis and exert anti-inflammatory cytotoxic effects in KB cells [Internet]. Journal of Clinical Periodontology. 2011;38:457–69. Available: http://dx.doi.org/10.1111/j.1600-051x.2010.01654.x

11. Thomas E. Oral Health and Medicine. 2019:221.

12. Loe H. Periodontal disease: The sixth complication of diabetes mellitus [Internet]. Diabetes Care. 1993;16:329–34. Available: http://dx.doi.org/10.2337/diacare.16.1.329

13. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [Internet]. Diabetes Care. 2003;26:S5–20.

14. Taylor BA, Toftler GH, Carey HMR, Morel-Kopp M-C, Philcox S, Carter TR, et al. Full-mouth tooth extraction lowers systemic inflammatory and thrombotic markers of cardiovascular risk [Internet]. Journal of Dental Research. 2006;85:74–8. Available: http://dx.doi.org/10.1177/154405910608500113

15. Thorstensson H, Kuylenstierna J, Hugoson A. Medical status and complications in relation to periodontal disease experience in insulin-dependent diabetics [Internet]. Journal of Clinical Periodontology. 1996;23:194–202. Available: http://dx.doi.org/10.1111/j.1600-051x.1996.tb02076.x

16. Karjalainen KM, Knuttila MLE, von Dickhoff KJ. Association of the severity of periodontal disease with organ complications in type 1 diabetic patients [Internet]. Journal of Periodontology. 1994;65:1067–72. Available: http://dx.doi.org/10.1902/jop.1994.65.11.1067

17. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between periodontitis and healthy patients - A case-control study. J Periodontol. 2018 Oct;89(10):1241–8.

18. Paramasivam A, Priyadharsini JV, Raghunandhakumar S, Elumalai P. A novel COVID-19 and its effects on cardiovascular disease. Hypertens Res. 2020 Jul;43(7):729–30.

19. Gokila S, Gomathi T, Vijayalakshmi K, Faleh AA, Sukumaran A, Sudha PN. Development of 3D scaffolds using nanochitosan/silk-fibroin/hyaluronic acid biomaterials for tissue engineering applications. Int J Biol Macromol. 2018 Nov 1;1241;11:1067-85.

20. Del Fabbro M, Karanxha L, Panda S, Bucchi C, NadathurDoraswamy J, Sankari M, et al. Autologous platelet concentrates for treating periodontal infrabony defects. Cochrane Database Syst Rev. 2018 Nov 26;11:CD011423.

21. Paramasivam A, Vijayashree Priyadharsini J. MitomiRs: New emerging microRNAs in mitochondrial dysfunction and cardiovascular disease. Hypertens Res. 2020 Aug;43(8):851–3.

22. Jayaseelan VP, Arumugam P. Dissecting the theranostic potential of exosomes in
autoimmune disorders. Cell Mol Immunol. 2019 Dec;16(12):935–6.
23. Vellappally S, Al Kheraif AA, Divakar DD, Basavarajappa S, Anil S, Fouad H. Tooth implant prosthesis using ultra low power and low cost crystalline carbon bio-tooth sensor with hybridized data acquisition algorithm. Comput Commun. 2019 Dec 15;148:176–84.
24. Vellappally S, Al Kheraif AA, Anil S, Assery MK, Kumar KA, Divakar DD. Analyzing relationship between patient and doctor in public dental health using particle Mément Multivariable Logistic Regression Analysis Approach (MLRA2). J Med Syst. 2018 Aug 29;42(10):183.
25. Varghese SS, Ramesh A, Veeraiyan DN. Blended module-based teaching in biostatistics and research methodology: A retrospective study with postgraduate dental students. J Dent Educ. 2019 Apr;83(4):445–50.
26. Venkatesan J, Singh SK, Anil S, Kim S-K, Shim MS. Preparation, characterization and biological applications of biosynthesized silver nanoparticles with chitosan-fucoidan coating. Molecules [Internet]. 2018 Jun 12;23(6). Available: http://dx.doi.org/10.3390/molecules23061429
27. Alsubait SA, Al Ajlan R, Mitwalli H, Aburaisi N, Mahmoud A, Muthurangan M, et al. Cytotoxicity of different concentrations of three root canal sealers on human mesenchymal stem cells. Biomolecules [Internet]. 2018 Aug 1;8(3). Available: http://dx.doi.org/10.3390/biom8030068
28. Venkatesan J, Rekha PD, Anil S, Bhatnagar I, Sudha PN, Dechsakulwatana C, et al. Hydroxyapatite from cuttlefish bone: Isolation, characterization, and applications. Biotechnol Bioprocess Eng. 2018 Aug 1;23(4):383–93.
29. Vellappally S, Al Kheraif AA, Anil S, Wahba AA. IoT medical tooth mounted sensor for monitoring teeth and food level using bacterial optimization along with adaptive deep learning neural network. Measurement. 2019 Mar 1;135:672–7.
30. Pradeep Kumar AR, Shemesh H, Niveditha MS, Hashir MMJ, Arockiam S, Uma Maheswari TN, et al. Diagnosis of vertical root fractures by cone-beam computed tomography in root-filled teeth with confirmation by direct visualization: A systematic review and meta-analysis. J Endod. 2021 Aug;47(8):1198–214.
31. Hannah R, Ramani P, Tilakaratne WM, Sukumaran G, Ramasubramanian A, Krishnan RP. Critical appraisal of different triggering pathways for the pathobiology of pemphigus vulgaris-A review. Oral Dis [Internet]; Jun 21, 2021. Available: http://dx.doi.org/10.1111/odi.13937
32. Ezhilarasan D, Lakshmi T, Subha M, Deepak Nallasamy V, Raghuandhakumar S. The ambiguous role of sirtuins in head and neck squamous cell carcinoma. Oral Dis [Internet]; Feb 11, 2021. Available: http://dx.doi.org/10.1111/odi.13798
33. Sarode SC, Gondivkar S, Sarode GS, Gadball A, Yuwanati M. Hybrid oral potentially malignant disorder: A neglected fact in oral submucous fibrosis. Oral Oncol. 2021 Jun 16;105390. Available: https://doi.org/10.1016/j.oraloncology.2021.105390
34. Kavarthapu A, Gurumoorthy K. Linking periodontitis and oral cancer: A review. Oral Oncol. 2021 Jun 14;105375.
35. Vellappally S, Abdullah Al-Kheraif A, Anil S, Basavarajappa S, Hassanain AS. Maintaining patient oral health by using a xeno-genetic spiking neural network. J Ambient Intell Humaniz Comput [Internet]; Dec 14, 2018. Available: https://doi.org/10.1007/s12652-018-1166-8
36. Aldhuwayhi S, Mallineni SK, Sakhamuri S, Thakare AA, Mallineni S, Sajja R, et al. Covid-19 knowledge and perceptions among dental specialists: A cross-sectional online questionnaire survey. Risk Manag Healthc Policy. 2021 Jul 7;14:2851–61.
37. Mahendra J, Suresh S, Kumar AP, Singh G, Jayaraman S, Paul R. Comparative analysis of subgingival red complex bacteria in obese and normal weight subjects with and without periodontitis [Internet]. Journal of Indian Society of Periodontology. 2017;21:186. Available: http://dx.doi.org/10.4103/jisp.jisp _241_17
38. Page RC. The pathobiology of periodontal diseases may affect systemic diseases: Inversion of a paradigm [Internet]. Annals of Periodontology. 1998;3:108–20. Available: http://dx.doi.org/10.1902/annals.1998.3.1.108
39. Grossi SG, Skrecpinski FB, DeCaro T, Robertson DC, Ho AW, Dunford RG, et al.
Treatment of periodontal disease in diabetics reduces glycated hemoglobin. J Clin Periodontol. 1997 Aug;68(8):713–9.

40. Tervonen T, Karjalainen K. Periodontal disease related to diabetic status. A pilot study of the response to periodontal therapy in type 1 diabetes. J Clin Periodontol. 1997 Jul;24(7):505–10.

41. Christgau M, Palitzsch KD, Schmalz G, Kreiner U, Frenzel S. Healing response to non-surgical periodontal therapy in patients with diabetes mellitus: Clinical, microbiological, and immunologic results. J Clin Periodontol. 1998 Feb;25(2):112–24.

42. Stewart JE, Wager KA, Friedlander AH, Zadeh HH. The effect of periodontal treatment on glycemic control in patients with type 2 diabetes mellitus. J Clin Periodontol. 2001 Apr;28(4):306–10.

43. Westfelt E, Rylander H, Blohmé G, Jonasson P, Lindhe J. The effect of periodontal therapy in diabetics. Results after 5 years. J Clin Periodontol. 1996 Feb;23(2):92–100.