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Association of Oral Microbiome with Periodontal Disease Progression: A Longitudinal Study

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ABSTRACT

Periodontal disease, a prevalent oral health condition, is characterized by the inflammation and destruction of the supporting tissues around the teeth and poses significant challenges to global public health. **Objectives:** To examine the association between the oral microbiome and periodontal disease progression in a Pakistani population. **Methods:** A total of 350 patients aged ≥ 18 years, diagnosed with periodontal disease, were registered from August 2023 to February 2024. Participants were evaluated for periodontal health indicators, including probing depth and clinical attachment loss, and their oral microbiome profiles were analyzed using high-throughput sequencing of the 16S rRNA gene. Machine learning algorithms, including Random Forest and Support Vector Machines, were applied to predict disease progression based on microbial profiles. **Results:** Porphyromonas gingivalis and Tannerella forsythia were strongly associated with greater probing depths and clinical attachment loss ($\beta = 0.45$, $p < 0.01$), indicating their role in disease progression. Conversely, Streptococcus and Lactobacillus were linked to reduced disease severity ($\beta = -0.30$, $p < 0.05$). The oral microbiome exhibited high diversity, with Firmicutes (35%), Bacteroidetes (25%), Proteobacteria (20%), and Actinobacteria (15%) being the predominant species. The Random Forest model predicted disease progression with 85% accuracy (Area under the curve (AUC) = 0.87), emphasizing the predictive value of microbial profiles. **Conclusions:** It was concluded that the study confirms a strong link between specific oral microbiota and periodontal disease progression, emphasizing the importance of microbial analysis in predicting and managing periodontal health.

INTRODUCTION

Periodontal disease, characterized by inflammation and destruction of the supporting tissues around the teeth, is a significant public health concern worldwide. According to data from the Global Burden of Disease (GBD) Study in 2019, periodontitis is the seventh most prevalent disease worldwide, affecting 1.09 billion people (Institute for Health Metrics and Evaluation (IHME), 2020) [1]. Since 1990, the prevalence has increased significantly due to population growth and ageing [2]. The age-standardized prevalence of periodontitis was estimated at 11.2% in 2010 and rose to 13.1% in 2019 [2, 3]. These trends and variations in incidence and disease burden differ by sex, age, and geographical region [1-4]. Data specific to the French population are limited, but the GBD study 2019 estimated the age-standardized prevalence of severe periodontitis in France at 9.6% [2]. This is close to the 10.2% prevalence of periodontal pockets greater than 5 mm found in the 2002-2003 National Periodontal and Systemic Examination Survey, conducted on a stratified quota sample of 2,144 adults aged 35–64 years in France [5]. In Pakistan, the burden of periodontal disease is notably high, with recent studies indicating that around 40% of adults suffer from moderate to severe periodontitis [6]. This high prevalence underscores the urgent need for effective

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diagnostic and therapeutic strategies tailored to the Pakistani population. Despite the significant burden, there is limited research exploring the specific factors contributing to the progression of periodontal disease in this region [7]. Emerging evidence suggests that the oral microbiome, the complex microbial community inhabiting the oral cavity, plays a crucial role in the pathogenesis and progression of periodontal disease. The oral microbiota, part of the human microbiota, includes over 700 bacterial species, many of which are commensal and help maintain oral physiological balance [8]. Disturbances in this microbial balance, known as dysbiosis, can lead to periodontal diseases such as gingivitis and periodontitis. Furthermore, dysbiosis has been linked to systemic conditions, including the formation of oral cancer [9]. Previous studies have highlighted the association between the oral microbiome and periodontal disease progression. However, these studies often focused on specific bacterial species without considering the broader microbial community structure and its stability over time. For instance, research has shown that patients with periodontitis have a higher prevalence of pathogenic bacteria such as Porphyromonas gingivalis and Tannerella forsythia, yet these studies did not fully explore the dynamic interactions within the microbial community that contribute to disease progression [10]. Additionally, many studies did not account for confounding factors such as genetic predispositions, lifestyle factors, and systemic health conditions, which can significantly influence periodontal health [11].

This study aimed to address these gaps by conducting a longitudinal analysis of the association between the oral microbiome and periodontal disease progression in the Pakistani population. This research will contribute to the development of more effective diagnostic tools and therapeutic strategies tailored to the needs of individuals suffering from periodontal disease in Pakistan.

METHODS

This longitudinal cohort study was conducted at De'Montmorency College of Dentistry Lahore from August 2023 to February 2024, involving 350 patients suffering from periodontal disease. The study was approved by the Institutional Review Board, De'Montmorency College of Dentistry Lahore (3115/DCD). The sample size for this study was calculated based on the prevalence of periodontal disease and the expected effect size of the association between the oral microbiome and periodontal disease progression. Using a prevalence rate of periodontal disease in Pakistan of approximately 40%, a confidence level of 95%, and a margin of error of 5%, the sample size was determined using the formula for sample size calculation for proportions:

$$n = \frac{Z^2 \cdot p \cdot (1-p)}{e^2}$$

Where n is the required sample size, Z is the Z-value (1.96 for a 95% confidence level), p is the estimated prevalence of the condition (0.40), and e is the margin of error (0.05). This resulted in a sample size of approximately 369.6, rounded to 350 patients to account for potential dropouts and non-response rates. Participants aged 18 years or older, diagnosed with varying degrees of periodontal disease including gingivitis and periodontitis, and willing to provide informed consent were included [12]. Those excluded were individuals who had recent periodontal treatment (within the last 3 months), received antibiotics, used antimicrobial rinse therapy, or had poor oral hygiene, as these factors could significantly affect the oral microbiome and periodontal health. Survey questionnaires were developed to collect comprehensive demographic information, medical history, and oral health habits. These self-administered questionnaires were pretested in a small group to ensure clarity, relevance, and reliability. Pretesting involved feedback on the questions' understandability and completion time, leading to necessary revisions before the actual data collection. Probing depth (PD) was measured in millimetres using a periodontal probe, indicating the distance from the gingival margin to the bottom of the periodontal pocket, with deeper depths suggesting more severe disease. Clinical attachment loss (CAL) was also measured in millimetres, indicating the distance from the cementoenamel junction to the bottom of the pocket, reflecting the extent of periodontal tissue destruction. Bleeding on probing was recorded as an inflammation indicator. Samples were collected from subgingival and supragingival regions of different quadrants of the mouth to ensure a comprehensive assessment of the oral microbiome. Plaque samples were placed in sterile Eppendorf tubes containing a suitable transport medium, thioglycolate broth, while saliva samples were collected in sterile polypropylene containers. All samples were labelled, kept on ice, and transported to the laboratory within two hours to maintain microbial viability. To determine the relative abundance of different bacteria, microbial DNA was extracted from the samples and subjected to Polymerase Chain Reaction (PCR) amplification of the 16S rRNA gene. The PCR products were then sequenced using high-throughput sequencing technologies. The resulting sequences were processed

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and aligned against reference databases to identify bacterial taxa. The relative abundance of each bacterial species was calculated by dividing the number of sequences for each taxon by the total number of sequences in the sample and expressed as a percentage. To ensure high reliability and accuracy, data collection protocols included standardized training for researchers and clinical staff, periodic calibration sessions, double data entry, and regular monitoring and audits. Data were analyzed using SPSS version 27.0, and multivariate regression analysis and machine learning algorithms were implemented to determine the significance of relationships between microbial profiles and periodontal disease parameters. This comprehensive approach aimed to elucidate the factors influencing periodontal disease progression in the study population.

RESULTS

The study involved 350 patients with periodontal disease, with a mean age of 45.01 ± 8.23 years. The probing depth, an important measure of periodontal health, averaged 4.2 ± 1.0 mm, while clinical attachment loss averaged 3.5 ± 0.8 mm. Bleeding on probing was observed in 30 ± 10% of participants (Table 1).

Table 1: Demographic Data, Medical History and Oral Health Habits of the Patients

Parameter	Value
Demographic Information	
Mean Age (years)	45.01 ± 8.23
Gender	55 %Male, 45% Female
Medical History	
History of Diabetes	30%
Cardiovascular Conditions	20%
Smoking	15%
Probing Depth (mm)	4.2 ± 1.0
Clinical Attachment Loss (mm)	3.5 ± 0.8
Bleeding on Probing (%)	30 ± 10
Oral Health Habits	
Brushing Frequency (Twice Daily)	60%
Regular Mouthwash Use	30%
Regular Dental Check-ups	50% Irregular, 50% Regular

The relative abundance of bacterial species in the oral microbiome showed Firmicutes as the predominant phylum, constituting 35% of the microbiome, followed by Bacteroidetes (25%), Proteobacteria (20%), Actinobacteria (15%), and others (5%). The major bacterial phyla in subgingival plaque samples were highlighted (Table 2).

Table 2: Major Phyla in Subgingival Plaque Samples

Phylum	Value
Firmicutes	35
Bacteroidetes	25
Proteobacteria	20
Actinobacteria	15
Others	5

Analysis of specific bacterial taxa revealed that Porphyromonas gingivalis and Tannerella forsythia was associated with greater probing depths and clinical attachment loss. The mean probing depths for P. gingivalis and T. forsythia were 5.0 ± 1.2 mm and 4.8 ± 1.0 mm, respectively, with corresponding clinical attachment losses of 4.5 ± 1.0 mm and 4.3 ± 0.9 mm. In contrast, Streptococcus and Lactobacillus exhibited lower mean probing depths and clinical attachment loss, with probing depths of 3.8 ± 0.9 mm and 3.5 ± 0.8 mm, and clinical attachment losses of 3.2 ± 0.7 mm and 2.8 ± 0.6 mm, respectively (Table 3).

Table 3: Association between Microbial Taxa and Periodontal Disease

Microbial Taxa	Probing Depth (mm)	Clinical Attachment Loss (mm)
Porphyromonas Gingivalis	5.0 ± 1.2	4.5 ± 1.0
Tannerella Forsythia	4.8 ± 1.0	4.3 ± 0.9
Streptococcus	3.8 ± 0.9	3.2 ± 0.7
Lactobacillus	3.5 ± 0.8	2.8 ± 0.6

The microbial community exhibited a Shannon Diversity index of 3.2, indicating a high level of species diversity. The Simpson Diversity index was 0.9, suggesting high species evenness and dominance within the community. The evenness value was 0.8, reflecting a balanced distribution of species abundance (Table 4).

Table 4: Diversity Indices for Oral Microbiome

Diversity Index	Value
Shannon Diversity	3.2
Simpson Diversity	0.9
Evenness	0.8
Richness	150

The multivariate regression analysis identified that increased relative abundances of P. gingivalis and T. forsythia were significantly associated with greater probing depths ($\beta = 0.45$, $p < 0.01$) and clinical attachment loss ($\beta = 0.40$, $p < 0.01$). Conversely, higher levels of Streptococcus and Lactobacillus were associated with lower probing depths ($\beta = -0.30$, $p < 0.05$) and clinical attachment loss ($\beta = -0.25$, $p < 0.05$). The model explained approximately 55% of the variance in periodontal health indicators ($R^2 = 0.55$) (Table 5).

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Table 5: Multivariate Regression Analysis Results

Microbial Taxon	Probing Depth (mm) β	Clinical Attachment Loss (mm) β	p-value
Porphyromonas Gingivalis	0.45	0.40	<0.01
Tannerella Forsythia	0.45	0.40	<0.01
Streptococcus	-0.30	-0.25	<0.05
Lactobacillus	-0.30	-0.25	<0.05

Machine learning algorithms, including Random Forest and Support Vector Machine (SVM), were employed to predict periodontal disease progression based on microbial profiles. The Random Forest model achieved an accuracy of 85% and an area under the curve (AUC) of 0.87, indicating strong predictive performance. The SVM model also demonstrated robust performance with an accuracy of 82% and an AUC of 0.84. These models identified P. gingivalis, T. forsythia, and Streptococcus as key predictors of disease severity, highlighting their importance in disease progression (Table 6).

Table 5: Multivariate Regression Analysis Results

Model	Accuracy (%)	Area Under the Curve (AUC)	Key Predictors
Random Forest	85	0.87	Porphyromonas gingivalis, Tannerella forsythia, Streptococcus
Support Vector Machine (SVM)	82	0.84	Porphyromonas gingivalis, Tannerella forsythia, Streptococcus

DISCUSSION

Current study was designed to explore the association between the oral microbiome and periodontal disease progression. Through the analysis of microbial profiles in patients with varying degrees of periodontal disease, significant associations between specific bacterial taxa and periodontal health indicators were identified. The findings of our study highlight the complexity of the oral microbiome and its role in periodontal disease progression. The microbial diversity of the oral cavity was measured using Shannon and Simpson diversity indices. The Shannon Diversity index of 3.2 and the Simpson Diversity index of 0.9 show a high level of species diversity and consistency, respectively, within the oral microbiome. This diversity is a key constituent in maintaining oral health, as a balanced microbial community can help prevent the overgrowth of pathogenic bacteria that contribute to periodontal disease [13]. Current findings are consistent with previous research highlighting the significance of microbial diversity in periodontal health. Relvas *et al.*, (2021) state that a diverse microbial community is associated with healthier periodontal status, while reduced diversity is linked to disease progression [14]. Porphyromonas gingivalis and Tannerella forsythia were found to be significantly associated with greater probing depths and clinical attachment loss. These findings are justified by previous studies that have recognized these species as key pathogens in periodontitis. P. gingivalis and T. forsythia are known for their virulence that contribute to tissue destruction and immune evasion [15, 16]. The multivariate regression analysis in our study supports these links, showing a significant positive correlation between the relative abundance of these taxa and periodontal disease severity ($\beta = 0.45$, $p < 0.01$ for both taxa). These results are similar with the results of Ardila *et al.*, (2020), who reported that elevated levels of P. gingivalis and T. forsythia are predictive of disease progression [17]. Conversely, Streptococcus and Lactobacillus were associated with lower probing depths and clinical attachment loss, indicating a potential protective role in periodontal health. These genera are often considered beneficial members of the oral microbiome due to their involvement in maintaining ecological balance and inhibiting the growth of pathogenic species [18]. The inverse relationship between the abundance of Streptococcus and Lactobacillus and periodontal disease indicators ($\beta = -0.30$, $p < 0.05$) suggests their importance in supporting periodontal health. Similar results were described by scholars, who found that these genera are related to improved periodontal outcomes [19]. The use of machine learning algorithms, such as Random Forest and Support Vector Machine (SVM), provided valuable insights into the predictive potential of microbial profiles for periodontal disease progression. Both models demonstrated strong predictive performance, with accuracies of 85% and 82% and AUCs of 0.87 and 0.84, respectively. The identification of P. gingivalis, T. forsythia, and Streptococcus as key predictors of disease severity underscores their relevance in clinical assessments of periodontal disease [20]. These results are similar to the published literature, which highlighted the utility of machine learning in predicting periodontal outcomes based on microbiome data. Our findings have significant suggestions for the diagnosis and management of periodontal disease. The identification of specific bacterial taxa linked with disease severity highlights the potential for targeted therapeutic involvements aimed at modulating the oral microbiome.

CONCLUSIONS

It was concluded that there is an association between the oral microbiome and periodontal disease progression. The findings highlight the significance of microbial dysbiosis in periodontal pathogenesis and highlight specific microbial taxa implicated in disease severity. These results contribute to our understanding of the complex interplay between microbial communities and periodontal health, paving the way for the development of novel diagnostic and therapeutic approaches to combat periodontal disease.

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Authors Contribution

Conceptualization: RJ, MR, MFI, IE, SR, BZ

Methodology: DC

Formal analysis: CR, DC DM

Writing-review and editing: ME

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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