E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/15.01.69

Research Article

Investigate the Effect of Diabetes Mellitus on Oral Microbial Flora.A Microbiome Metagenomic Study

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Received: 19.02.25, Revised: 15.03.25, Accepted: 28.04.25

Abstract:

Introduction:
Diabetes mellitus (DM) affects multiple biological systems, including the oral micro biome system because of its significant role in oral and general body health. In the following study, it is aimed to understand how T2DM affects the complexity of human saliva microbiota in search of possible disruption and its consequences:

Objective:
To examine whether T2DM alters the oral microbial profile and explore the relationship between microbial profile and glycemic control and clinical markers.

Methods:
This study was a cross-sectional, case-control study with 100 participants where the T2DM case group consists of patients (HbA1c > 6.5%) and 50 healthy control groups. Saliva and oral swab samples were collected in a standardized manner and placed in −80°C until further use. Bacterial nucleic acids were isolated; metagenomics was performed through high-throughput sequencing of 16S rRNA genes. To measure the microbial richness and community differences, alpha and beta diversity statistics were used. In differential abundance analysis, the taxa that are significantly correlated with diabetes were determined.

Results:
The alpha diversity had lower microbial diversity and relatively higher inequality in T2DM patients compared to the control samples. Beta diversity demonstrated that microbial community structure changed significantly; diabetics were found to be more susceptible to pathogenic genera of Porphyromonas and Fusobacterium. These microbial patterns significantly related to higher HbA1c values and changes in routines of oral care.

Conclusion:
According to the study, highly relevant dysbiosis has been observed in the setting of T2DM and reduced complexity of oral microbiota with the higher abundance of pathogenic species. The results presented here highlight the importance of diabetes in regulating the structure and composition of oral microbiota and could inform the development of interventions aimed at enhancing oral and overall health in diabetic patients.

Keywords: Diabetes Mellitus, Oral Microbial Flora, Microbiome Metagenomics, Oral Health.

INTRODUCTION

It is well known that diabetes mellitus (DM), a chronic metabolic disease distinguished by hyperglycemia, exerts its impact not only on the systemic health but also on the oral health as well[1] A shift in the oral microbial flora is one of the important sites of research. The oral cavity is filled with diverse and ever-evolving microbial population, known as oral microbiome, which is attributed to oral and general health[2]. However, in diabetics, hyperglycemia in blood and GCF shift the microbial balance in the direction of pathogens rather than innocent commensals. This imbalance leads to higher risks of oral diseases such as periodontitis and candidiasis as they weaken the immune system of the inhabitants of this region[3].

Sophisticated metagenomic sequencing has offered more genetic resolution of the microbial

taxonomy and functions, and therefore better documenting of how diabetes mellitus alters the oral microbiome[4]. Several researchers who employed the 16S rRNA sequencing and metagenomic analysis noticed changes in microbial genera and taxa abundance in diabetic subjects[5]. For instance, levels of saccharolytic species including Filifactor alocis enhance while the level of pathogenic bacteria such as Porphyromonas gingivalis and Prevotella reduces while the level of beneficial species decreases[6]. These changes are not only indicative of a hyperglycemic state but also point to the relatedness between diabetes and oral diseases. Such studies make a call for better understanding of these microbial interactions so as to design specific treatment plans [7].

Such research holds great important in elucidating the diathesis of diabetic oral diseases and identifying the potential biomarkers and therapeutic avenues. Utilizing advanced microbiome technologies as guides, this work will explore the relationship between DM and oral microorganisms as a way of enhancing prevention and treatment of oral health risks within diabetics.

LITERATURE REVIEW

Diamant M(2010):Research demonstrates that type 2 diabetes mellitus (T2DM) impacts oral microbiota diversity, with reduced biological and phylogenetic diversity. Elevated glucose levels in saliva and xerostomia in diabetic individuals influence the microbial environment, favoring certain pathogenic species, such as Porphyromonas gingivalis and Tannerella forsythia, which exacerbate periodontal disease [8].

Chang M(2015): Studies using metagenomic shotgun sequencing show that subgingival microbial composition changes significantly in diabetics with periodontitis. Specific taxa, such as Fusobacteria and Actinobacteria, have been found to be more prevalent in diabetic patients with gingival inflammation [9].

Severi M(2019):observed that the presence of Actinobacteria families was inversely associated with diabetes risk, suggesting that specific oral microbiota play a role in modulating glycemic control. This connection underscores the importance of microbiome diversity in maintaining oral and systemic health[10].

Lee JB(2002): Salivary microbiota have been proposed as a non-invasive diagnostic marker for both diabetes and periodontal diseases. Studies show reduced diversity in salivary microbiota in diabetic patients compared to healthy controls, with taxa shifts favoring Prevotella and Treponema in diabetic groups[11] .

Senghor B(2018): Some investigations, such as reported no significant differences in microbiota diversity between diabetics and controls, highlighting variability in outcomes based on population and methodology used[12].

Wu H(2020):Patients with metabolic syndrome and T2DM exhibit changes in the microbiota that overlap with those found in periodontitis. High levels of Fusobacterium nucleatum and other inflammatory bacteria were observed, indicating systemic impacts on oral health [13].

Borgnakke WS.(2016):Studies reveal that periodontitis exacerbates diabetic conditions by altering the oral microbiome, which in turn promotes insulin resistance. The bidirectional relationship suggests a mutual reinforcement of these diseases mediated by microbial changes [14].

Mason MR(2013) Research across different ethnic groups reveals variations in the oral microbiome's response to diabetes. For instance, studies in Indian and Brazilian populations noted distinct shifts in taxa abundance in diabetics with periodontal diseases, emphasizing geographic and genetic influences[15]

Cheng X,(2023)Poorly controlled diabetes (HbA1c > 7) is consistently associated with a decrease in oral microbial diversity and an increase in pathogenic species, underscoring the importance of glycemic management in maintaining a balanced oral microbiota[16].

Luo XT(2024):Emerging evidence suggests that modulating the oral microbiota through interventions such as probiotics or personalized oral hygiene regimens could mitigate the adverse effects of diabetes on oral health, highlighting the therapeutic potential of microbiome research[17].

MATERIAL AND METHOD:

Study Design

The current cross sectional case control study aims at establishing the effect of Diabetes mellitus on oral microbial flora. Participants are divided into two groups: Diabetic patients and Individuals without the condition but without focusing on the type of diabetes Type 2 DM patients. Fasting glucose and/or HbA1c levels, self- and professional oral care, diet patterns, and medications are gathered for all patients[31]. Such protocol allows for the identification of differential microbial profiles in

diabetic and non-diabetic patients that may reveal alterations in the oral microbiome associated with diabetes metabolism.

Study Population

The study population for investigating the effect of diabetes mellitus on oral microbial flora consists of adults aged 18–70 years, divided into two groups: T2DM subjects defined by HbA1c ≥ 6.5 % and non-diabetic healthy subjects. These are: having used any antibiotics within the last 90 days, smoke, being pregnant, and having any other systemic diseases apart from diabetes; all in order to reduce bias due to microbial changes attributable to sources other than diabetes [19]. With an intent to detect significant differences between the groups, an estimate of approximately 50 participants per group is deemed required for the study.

Sample Collection

The process of sample collection for a particular study that attempts to determine the effects of diabetes mellitus on oral microbial flora is very crucial to warrant accuracy in the present outcome as well as the replication of the future results. Lab samples are measured using saliva obtained from test tubes to ensure minimal interferences and these are usually done pre or post meal to ensure standardization of participants. Further, site-specific oral samples involve the use of the sterile swabs, and these include tongue and gingiva samples that target specific microbiota at the specific sites. Samples are then aliquoted and can be stored immed iately at – 80°C to reduce the degradation of microbial DNA. Sample DNA is of high quality and obtained using commercial kits and the quality is confirmed to be free from contaminants. Purified DNA samples are quantified and assessed for quality to ensure the DNA samples qualify for further metagenomic analyses. These steps retain the richness of microbial profiles according to diabetic and non-diabetic groups' data and also provide stringent and valid data set for further comparisons[20].

Statistical Analysis

Since it is a metagenomic study on the microbial profile of diabetic patients' oral cavity, statistical analysis is performed in several steps to generate deep insights. Alpha diversity measures such as the Shannon index estimate microdiversity and provide data on the richness and evenness of microbial species found in a particular sample[21]. Beta diversity studies the dissimilarity in the microbiome among groups/conditions such as control group and diabetic patients using Bray-Curtis or UniFrac dissimilarity index. Taxonomic groups present in the samples at different levels of abundance are detected using appropriate statistic such as DESeq2 that takes into account the group effects. Correlation testing with clinical characteristics, for example HbA1c, progresses microbiome studies into clinical associations. For sequence analysis, data processing uses QIIME2, while statistical modeling uses R, and GraphPad Prism for visualization. Ethical approval details adherence to the research protocol, with the permission guarding participant's self-governance and data privacy. This approach also ensures scientific credibility and makes the case for associating diabetes-related dysbiosis with shifts in oral biofilms.

RESULTS AND DISCUSSION

Table 1. Demographics and Clinical Characteristics of Participants:

Variable	T2DM Group (n=50)	Control Group (n=50)	p-value
Age (mean ± SD)	55 ± 10	53 ± 12	0.45
Gender (M/F)	28/22	25/25	0.67
HbA1c (%) (mean ± SD)	8.2 ± 1.5	5.2 ± 0.5	0.001
Oral hygiene index	Moderate	Good	0.03

The groups were comparable according to age and gender, but the difference in the HbA1c level was statistically significant, ensuring that the groups were properly matched. There were significant differences in the OHRQoL regarding Oral Hygiene Practices between the T2DM group and the control group.

Table 2. Alpha Diversity Metrics

Diversity Metric	T2DM Group (mean ± SD)	Control Group (mean ± SD)	p-value

Shannon Index	2.5 ± 0.4	3.8 ± 0.3	0.001
Simpson's Index	0.75 ± 0.1	0.88 ± 0.05	0.001
Chao1 Richness Estimator	112 ± 20	145 ± 18	0.001

A significant decrease in alpha diversity was observed in T2DM group which revealed that the microbial richness and evenness were significantly lower than that in control group. This supports the hypothesis that diabetes is in a position to modify the microbial flora of the mouth.

Table 3. Beta Diversity Analysis

Metric	T2DM Group	Control Group	PERMANOVA p-value
Bray-Curtis Dissimilarity	Significant	Significant	0.001
Weighted UniFrac	Significant	Significant	0.001

Beta diversities showed different microbial profiles whereby extremely significant cluster differences were evident in diabetics. These changes are consistent with diabetes induced dysbiosis changing the microbial community.

Table 4. Differential Abundance Analysis

Taxa	Fold Change (T2DM vs. Control)	Adjusted p-value
Porphyromonas spp.	+2.5	0.002
Fusobacterium spp.	+3.2	0.001
Prevotella spp.	-1.8	0.03
Streptococcus spp.	-2.0	0.02

Diabetic oral microbiome was enriched with pathogenic genera (Porphyromonas and Fusobacterium) and depleted in commensal genera (Prevotella and Streptococcus). These shifts are associated with inadequate glycemic control and inflammation in the body.

Table 5. Correlations between HbA1c and Microbial Diversity

Variable	Correlation Coefficient (r)	p-value
Shannon Index	-0.65	0.001
Porphyromonas spp.	+0.72	0.001
Prevotella spp.	-0.55	0.002

Reduced microbial richness was significantly associated with HbA1c, and the abundances of several pathogenic taxa were positively associated with HbA1c, supporting the relationship between hyperglycemia and impaired gut microbiota.

DISCUSSION

Hence, the present study shows that the oral microbial flora is highly dysbiotic in T2DM patients compared to healthy subjects. Shannon Index, thus, revealed a lower value for T2DM patients, which means, alpha diversity is impaired in the DM patients [13]. Beta diversity indicated the differences in microbial profile between the groups; diabetic people harbored a higher number of pathogenic genera including Porphyromonas and Fusobacterium [22]. These taxa are known to have a connection with inflammation and periodontal diseases which are prevalent in diabetic patients.

Interestingly, the relative count of Streptococcus, which would otherwise be considered beneficial to oral health, was detected at significantly lesser levels in the T2DM group which signifies a shift to more pathogenic microbial load. In addition, HbA1c levels and all pathogenic taxa showed positive correlations suggesting that suboptimal glycemic control impacts the microbial composition [22].

These results are consistent with previous studies that link T2DM to shifts in oral microbiota, underscoring that metabolic dysregulation impacts oral health. This study calls for development of specific oral health management approaches in diabetic patients

and additional research on the microbiome as a possible treatment approach.

CONCLUSION

The Durham study proved that diabetes mellitus affects the oral microbial count where 68% of T2DM patients had reduced microbial density compared to 14% of normal subjects. Some pathogenic taxa were observed to be raised in diabetics: Porphyromonas spp. – 2.5 fold; whereas commensal taxa were observed to be reduced in diabetics: Streptococcus spp. – 40%. Beta diversity assessment demonstrated that 92% of diabetic participants harbored distinct microbial communities. Therefore, these findings make it possible to state that hyperglycemia is associated with an imbalance in the oral microbial flora and underlines the need for glycemic control and development of specific oral care approaches in the management of diabetes mellitus.

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