Correlation analysis of oral mucosal microbiome and diabetes mellitus using microbial DNA in elderly male subjects

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ABSTRACT

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With the revelation that oral microbiomes are associated with various systemic diseases, the need for a deeper understanding of oral microbiomes has garnered substantial research interest. Herein, the differences between the oral gingival microbiomes of individuals with and without diabetes were analyzed to improve disease evaluation and therapeutic development. Gingival samples were collected from 10 and 16 individuals with and without diabetes, respectively, during an implant uncovering procedure. Genomic DNA from the samples was used for 16S rRNA sequencing, and the compositions of the microbiomes were compared between the two groups. There were no significant differences in the alpha and beta diversities of the diabetes and non-diabetes groups. However, the non-diabetes group presented with a relatively higher abundance of the Corynebacteriaceae family (p = 0.0040) and the genus *Corynebacterium* (p = 0.019). Our findings serve as a basis for future studies on the association between diabetes and bacterial species in the abovementioned taxonomic groups. Divergence between our results and those of past studies indicate a need for a unified study design for sample collection and the methods utilized for statistical analysis when implementing a bioinformatics-based approach to assess oral microbiome sequencing data from gingival samples.

Key words : **16s rRNA gene sequecing; Diabetes mellitus; Oral microbiome**

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I. INTRODUCTION

Chronic infections caused by imbalances in oral bacterial communities lead to oral diseases, such as dental caries (Aas, Griffen et al. 2008) and periodontal disease (Tanner, Haffer et al. 1979), which, in turn, are associated with cardiovascular disease (Joshipura, Rimm et al. 1996, Beck and Offenbacher 2005), diabetes (Genco, Grossi et al. 2005), and even cancer (Tezal, Sullivan et al. 2009). Furthermore, numerous studies have investigated the association between the oral microbiome and oral diseases, such as dental caries (Strużycka 2014), periodontal disease (Costalonga and Herzberg 2014), and systemic diseases (Wade 2013).

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia, which is caused by the dysregulation of insulin which regulates blood glucose levels in the body.

Chronic hyperglycemia caused by diabetes results in microvascular complications, such as arteriosclerosis as well as cardiovascular and macrovascular diseases (Brown 2008, Fowler 2011), leading to organ damage and dysfunction (Papatheodorou, Papanas et al. 2016) and systemic inflammation (Pitsavos, Tampourlou et al. 2007). In particular, diabetes aggravates periodontitis by maintaining the oral cavity in an inflammatory state (Taylor, Burt et al. 1998). Similarly, periodontitis worsens diabetes by increasing inflammatory precursor activity, thereby enhancing insulin resistance in the body (Kim and Amar 2006). In addition, one study has reported a correlation between dental caries and DM (Latti, Kalburge et al. 2018).

Based on this, it could be concluded that the oral environment and diabetes status are related, and indeed, this bilateral relationship has been demonstrated in several studies (Page 1998, Kim and Amar 2006, Lamster, Lalla et al. 2008, Preshaw, Alba et al. 2012). Reportedly, the delayed wound healing in the oral mucosa of patients with diabetes is caused by delayed vascularization, attenuated immunity, and decreased levels of growth factors, including insulin-like growth factor, transforming growth factor, platelet-derived growth factor, and nerve growth factor (Abiko and Selimovic 2010). Furthermore, the healing of impaired oral wounds in mice with diabetes has been linked to increased fibroblast apoptosis leading to a decrease in fibroblast numbers (Desta, Li et al. 2010). These findings suggest that diabetic status and the oral environment are involved in a reciprocal relationship and are not independent of one another. Thus, comparing the oral microbiomes of individuals with and without diabetes may be helpful in assessing the risks as well as in diagnosing diabetes. Diabetes can affect gingival vasculature, inflammatory and immune responses, alterations in collagen synthesis, and genetic predisposition to diseases (Ervasti, Knuuttila et al. 1985, Murrah 1985, Oliver, Tervonen et al. 1993, Noack, Jachmann et al. 2000, Katz 2001, Kinane and Marshall 2001); thus, we expected that the characteristic oral mucosal microbiome could be discovered through the analysis of mucosal tissues.

Previous studies on the oral microbiome in individuals with diabetes have predominantly utilized saliva samples or plaque samples, with minimal attention given to oral mucosa as samples. However, bacteria can be pushed into the epithelium of the oral mucosa through various means, including masticatory activities or even routine traumas such as oral hygiene practices. (Ji, Choi et al. 2015) Furthermore, these microorganisms may persist within the connective tissue of the oral mucosa evading immediate elimination by host immune responses and maintain a state of normal microbial community, without manifesting pathological conditions. (Dahlen, Basic et al. 2019) Thus, it is evident that distinctive microbial communities exist within the oral mucosa of diabetes patients. In addition to, a previous study observed significant differences in the composition of the microbiome between saliva samples and oral mucosal tissue samples in healthy individuals. (Wang, Zhao et al. 2020) This suggests that the oral mucosal microbiome may exhibit distinctive microbial characteristics compared to other samples such as saliva and plaque that have been studied extensively in the past.

Therefore, we conducted this study assuming that oral mucosal tissues would provide a unique characteristic microbiome in diabetes patients. Currently, it is unclear as to whether the oral mucosal microbiome of patients with diabetes has characteristics different from that of individuals without diabetes. We hypothesized that we would find dominant or inferior strains in patients with diabetes or that there would be differences in the diversity of the microbiome, compared to that of subjects without diabetes. The aim of this study was to evaluate the effect of DM on the composition and diversity of the microbiomes in the oral mucosal tissue from patients with diabetes compared to patients without diabetes using 16S ribosomal RNA (rRNA) sequencing, a form of next-generation sequencing. We also investigated whether the oral mucosal tissue obtained during dental implant surgery can act as a representative sample of the microbiome more accurately than other sample types.

II. MATERIALS AND METHODS

1. Sampling procedure

Twenty-six subjects with and without type 2 diabetes (24 men and 2 women; mean age: 71.3 ± 4.79), who were void of systemic diseases but had undergone an uncovering procedure following implant placement at the Dept. of Periodontology, Veterans Health Service Medical Center, were enrolled from July 2018 to December 2018. Patients who had received or required periodontal or dental caries treatment within 30 days, with uncontrolled systemic diseases, who had taken antibiotics within 30 days, who did not have either the will or the ability to make an informed consent, and female patients who were either pregnant or possibly pregnant were excluded. All patients were requested to avoid food intake after 11 p.m. the night before the uncovering procedure, and tooth-brushing on the day of sample collection. Sixteen subjects without and ten patients with diabetes were investigated. One subject was excluded because the sample size was not large enough for analysis, and two female subjects were also excluded because the presence of such a small number of women could cause statistical bias. (Fig. 1) The demographics of the subjects is described in Table 1. Diagnoses of diabetes were made according to the recommendations of the American Diabetes Association (Chamberlain, Rhinehart et al. 2016). This study was approved by the Institutional Review Board of VHS Medical Center (BOHUN IRB No. 2018-05-009), and all participants provided written informed consent. Furthermore, this study was conducted in accordance with the tenets of the Helsinki Declaration of 1975 and its later revisions. Oral mucosal tissues removed during the second implant surgery were collected using a 15c blade. The samples were then placed in a sterile microtube and stored at −80°C.

2. DNA extraction and gene sequencing

DNA was extracted from samples using a MoBio DNeasy PowerSoil Kit (Cat. No. 12888-100, Qiagen, Germantown, MD, USA) according to the manufacturer's protocol. Each sequenced sample was prepared according to Illumina 16S Metagenomic Sequencing Library protocols. Briefly, 16S amplicon PCR primers targeting 16S rRNA were used to produce PCR amplicon libraries. V4 hypervariable regions of 16S rRNA genes were PCR-amplified using specific 16S V3-V4 primers. The primer sequences are as follows:

16S Amplicon PCR Forward Primer

5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3'

16S Amplicon PCR Reverse Primer

5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'

Subsequently, a limited-cycle amplification step was performed to add multiplexing indices and Illumina sequencing adapters. Each 25 μL PCR mixture contained 2.5 μL of template DNA, 5 μL of amplicon for PCR primer (5 μM), 12.5 μL KAPA's HiFi HotStart ReadyMix (2×), and 5 μL of distilled water. The amplicon PCR protocol used was as follows: 3 min at 95°C; 25 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C; and 5 min at 72°C. The index PCR protocol was as follows: 3 min at 95°C for denaturation; eight cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C; and 5 min at 72°C.

Quantification and quality evaluation of DNA was performed using PicoGreen (Invitrogen, Carlsbad, CA, USA) and a Nanodrop spectrophotometer (Thermo Scientific™, Waltham, MA, USA). The final products were normalized and pooled using PicoGreen,

and the library sizes were verified using a TapeStation DNA screentape D1000 (Agilent); the microbiomes of the specimens were analyzed using 16S rRNA sequencing on an Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA; Fig. 2). Negative extraction blank controls were included for each batch of sample extraction to detect contaminants (Eisenhofer, Minich et al. 2019).

3. Data collection and statistical analyses

After sequencing, the MiSeq raw data were used to extract high-quality sequences of approximately 440–465 bp in length with a 120–160-bp overlap using FLASH (Magoč and Salzberg 2011). The sequences obtained were clustered with sequences showing more than 97% sequence similarity using CD-HIT-out (Li, Fu et al. 2012), a CD-HIT-EST based operational taxonomic unit (OTU) analysis program that removes low quality sequences, ambiguous sequences, and chimeric sequences, considered to be sequencing errors, to form species-level OTUs. The representative sequence of each OTU was taxonomically assigned by selecting subjects showing highest similarities in terms of organism information via BLASTN (Zhang, Schwartz et al. 2000), in accordance with the NCBI 16S Microbial reference database (DB). If the query coverage of a best hit that matched the DB was less than 85%, and the identity of the matched area was less than 85%, its taxonomy was considered as undefined. Using the OTU-related data, a comparative analysis of various microbial communities was performed using QIIME (Caporaso, Kuczynski et al. 2010).

Alpha diversity was assessed using the following indices: Chao1, which represents the richness estimate for a defined OUT; Shannon, which considers the number and evenness of species; and inverse Simpson, which represents the probability that two randomly selected individuals in a habitat belong to the same species. Beta diversity between samples was assessed using the weighted UniFrac distance, and flexible relationships between samples were visualized via a principal coordinate analysis (PCoA).

Data analyses of the relative abundance of OTUs were performed using the R Statistical Package, Version 4.0.1 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set at $P < 0.05$. Student's t-tests were performed to test for differences in alpha diversity between patients with and without diabetes. OTUs with low counts across all patients were excluded prior to commencing differential expression analyses. OTUs involving counts per million (cpm) of 100, or greater, in at least two samples were used for differential expression analyses and retained for alpha and beta diversity analyses. Differentially abundant OTUs that were significantly associated with disease states were identified via the Robinson and Smyth approach (Robinson and Smyth 2007), and later incorporated into a generalized linear model framework (GLM). A negative binomial GLM was fitted to the count data following which likelihood ratio tests were performed to compare abundance levels between groups. For multiple comparison tests, the false discovery rate (FDR) was controlled using the Benjamini-Hochberg step-up procedure.

III. RESULTS

1. Sequencing results and alpha diversity indices

We compared the oral microbiota of 10 patients with and 16 individuals without diabetes. The average number of analyzed sequences was 149,114 (max: 173,275, min: 112,220) for the 10 specimens from patients with diabetes and 139,598 (max: 191,755, min: 1,038,963) for the 16 specimens from individuals without diabetes. The P-values for OTU, as well as Chao1, inverse Simpson, and Shannon indices were 0.717, 0.728, 0.904, and 0.802 respectively (Table 2). There was no significant difference in alpha-diversities between the two groups (Student's t-test; Fig. 3).

2. Comparison of beta diversity between the groups

To compare community structure differences, a PCoA based on a weighted UniFrac matrix was used (Fig. 4). Evident grouping of subjects with diabetes and those without diabetes was not observed.

3. Taxonomy-based comparisons of oral microbiota between the groups

We compared the relative abundance of taxa to investigate possible differences between specific bacterial taxa in the oral microbiota of subjects with and without diabetes.

A likelihood ratio test was performed for each OUT detected depending on the diabetes status variable. When the FDR value was maintained at 0.05, a significant difference between the diabetes and non-diabetes groups was observed for only one family (Corynebacteriaceae) and one genus (Corynebacterium) (Table 3). These two showed a

relatively higher abundance in the non-diabetes group. No significant differences were found at any other phylum, class, order, or species level.

Bar plots showing taxonomic profiles of diabetes patients and non-diabetes subjects at the phylum (Fig. 5A) and genus (Fig. 5B) levels are presented. Only those with a relative abundance > 1% are listed.

IV. DISCUSSION

Microbial dysbiosis reportedly affects inflammatory as well as chronic metabolic disease states (Seymour, Ford et al. 2007, Gao, Xu et al. 2018), as demonstrated by the correlation between gut microbiomes and inflammatory bowel diseases (Fava and Danese 2011). Similarly, microbial changes in the oral cavity might increase the severity of secondary chronic diseases, such as diabetes. This may be due to the oral microbiota playing a regulatory role in the initiation of systemic inflammatory conditions (Han and Wang 2013). Accordingly, several studies have investigated the association between diabetes and oral cavity microbiomes.

This study only showed a significant difference in the Corynebacteriaceae family, and the genus Corynebacterium. These results are in contrast to those of other studies. A previous study analyzed the microbiomes of 29 morbidly obese individuals, including 13 patients with diabetes, and reported that the genus Bifidobacterium had a significantly lower abundance in patients with diabetes (Shillitoe, Weinstock et al. 2012). Another study, which investigated 20 patients with diabetes and 11 control subjects, indicated that streptococci and lactobacilli were more abundant in the former than in the latter (Kampoo, Teanpaisan et al. 2014). These studies and ours had a relatively small sample size compared to others; however, unlike in this study, only certain types of bacteria were assessed. A study that evaluated the association between oral microbiomes and diabetes risk without the above limitations reported that the relative abundance of phylum Actinobacteria was associated with a reduced risk for diabetes, while the relative abundance of genus Actinomyces was strongly associated with an increased risk (Long, Cai et al. 2017). In contrast, a study comparing a diabetes group with a high-risk group (fasting glucose levels >7 mmol/L) and a low-risk group (fasting glucose levels <7 mmol/L) reported that certain genera such as Leptotrichia, Staphylococcus, Catonella,

and Bulleidia were relatively enriched in the high-risk group (Wang, Xu et al. 2019). These differences highlight the variation in oral microbiome depending on the study design and the parameters of the cohort.

Here, we have analyzed microbial diversity pertaining to diabetes status within each group (alpha diversity) as well as between groups (beta diversity). In a previous study that analyzed the relationship between diabetes and the saliva microbiome, patients with diabetes showed significant decreases in alpha diversity (Observed, Chao1, ACE, and Shannon indices) and beta diversity compared to individuals without diabetes. However, the differences reported were prominent at the genera level, but not at the species level (Sabharwal, Ganley et al. 2019). In contrast, in an uncontrolled diabetic status study, some OTUs were either significantly more or significantly less abundant in samples from individuals with diabetes than in those without diabetes (Casarin, Barbagallo et al. 2013). Interestingly, the results of the present study did not show a significant difference between alpha or beta diversities of either group, with the groups diverging only in the relative abundance of the Corynebacteriaceae family and the genus Corynebacterium in individuals without diabetes. A previous study, wherein more families and genes were correlated between the oral microbiome and diabetes, as determined using a mouth rinse sample, reported similar results with respect to the relative abundance of the Corynebacteriaceae family and the genus Corynebacterium among subjects without diabetes. (Sabharwal et al. 2019) This may serve as a basis for the association with diabetes for these two bacterial taxa in the future.

Corynebacterium has not been extensively studied in the past, but it has recently been identified as a cause of serious infections. In particular, it is recognized that it can cause opportunistic infections in patients with prosthetic devices, or in patients with compromised immune systems due to systemic diseases such as diabetes. (Bernard 2012) In a study comparing chronic wounds from diabetic ulcers with intact skin, more Corynebacterium were detected in the wound group (Dowd, Wolcott et al. 2008,

Gontcharova, Youn et al. 2010). This suggests that Corynebacterium is a significant opportunistic contributor in chronic skin infections. However, in this study, Corynebacterium were detected at higher levels in the mucosal samples from subjects without diabetes than in diabetes patients, which is contradictory with the results of previous studies. This difference is presumed to be due to the difference between the role of Corynebacterium as an opportunistic infection in the skin of diabetics and the role of healed gingiva in the oral cavity of diabetics. This suggests that elucidating the oral role of Corynebacterium is important to characterize the oral microbiome of diabetes patients. Therefore, additional research is needed to explore the role of these pathogens in the oral mucosal tissues of diabetes. Corynebacterium attenuates the virulence of Staphylococcus aureus by regulating a specific pathway which decreases the transcription of virulence genes (Ramsey, Freire et al. 2016). The risk of S. aureus bacteremia is substantially increased with diabetes, thus potentially aggravating disease risk and mortality (Smit, Søgaard et al. 2016, Hansen, Gotland et al. 2017). Therefore, a decrease in Corynebacterium in patients with diabetes might lead to an increase in the pathogenesis of S. aureus, which might affect the diabetic status of the patients and present complications such as diabetic foot ulcers. A future study is required to uncover such a relationship.

Notably, previous investigations have predominantly used supra- or subgingival plaque, or saliva samples, and only a few microbiome studies have used gingiva as sample. Therefore, it is difficult to make an accurate comparison between our results and those of the other studies. Further discrepancies could be explained by differences between the sample collection methods used by our study and those used by the other studies. Saliva has previously proven to be a useful specimen which reflects oral health (Barnes, Kennedy et al. 2014) and has been used in several microbiome studies due to the ease and non-invasiveness of its collection. Plaque and biofilms might also be similarly useful as specimens for bacterial analysis (Taba, Kinney et al. 2005). However, to our

knowledge, few studies have used the gingiva samples for the purpose of microbiome analysis as we elected to do here. Since our results did not agree with those of previous studies using different sampling methods, we presume that oral mucosal tissue from the edentulous region might not be as representative of the intraoral microbiome as other samples.

Furthermore, the previous all had different study designs. The diabetic status of subjects at the time of sample collection was slightly different for each study and hence the statistical methods used to compare the relative abundance of OTUs were different. Some studies have overlooked the high probability of statistical error that arises when multiple OTUs are compared, by neglecting the concept that statistical error increases as the number of comparisons is increased. This may have led to results that have compromised the reproducibility and reliability of microbiome experiments (Benjamini, Drai et al. 2001, Hawinkel, Mattiello et al. 2019). Therefore, further studies with improved coordination between sampling methods and statistical analyses might be needed.

Since dental caries or periodontitis, which exist independently of diabetes, can affect the composition of the oral microbiome (Schulz, Porsch et al. 2019, Uchida-Fukuhara, Ekuni et al. 2020), which in turn, can also affect the gingival supernatant, and since systemic diseases other than diabetes can also affect the oral microbiome (Zarco, Vess et al. 2012, He, Li et al. 2015, Kleinstein, Nelson et al. 2020), the above inclusion criteria were established to exclude these effects. However, as a limitation, since diabetes can be correlated with oral disease (Al-Maskari, Al-Maskari et al. 2011, Bascones-Martinez, Gonzalez-Febles et al. 2014) as well as other systemic diseases, future studies also need to consider subjects with oral and systemic diseases related to diabetes.

Some studies have suggested that gender has an effect on microbiome composition (Kenyon 2020), while others have not (Chen and Jiang 2014, Demmitt, Corley et al.

2017). Due to the nature of this hospital, only a limited number of women were enrolled initially (two), and therefore, the gender distribution was not even. In order to eliminate the slight bias that may occur due to this gender imbalance only data that were derived from male patients was used for the statistical analysis. However, it should be noted that although the inclusion of the data from the two women did not affect the statistical results, the effect of differences in subject gender distribution should be evaluated in future studies.

In addition, this study was conducted on crestal gingiva obtained during implant-uncovering surgery. However, a previous study has reported that the bacterial composition can be affected by the position of the tooth or the tooth surface from which the sample was taken (Simon-Soro, Tomás et al. 2013). Unlike the case for previous studies, our study is different due to the fact that it targeted the gingiva, but the limitation of this study is that the microbiome composition may be affected depending on the sampling site.

Although widespread changes in community diversity could be captured by the amplicon sequence of the 16S rRNA gene, its low sensitivity and limited resolution are needed the device of whole-community shotgun metagenome in the future study.(Poretsky, Rodriguez-R et al. 2014, Jovel, Patterson et al. 2016)

To the best of our knowledge, this is the first preliminary study to analyze the correlation between the oral mucosal microbiome and DM using 16S rRNA gene amplicon sequencing. Our result can serve as a basis for future studies investigating the relationship between systemic diseases and the oral cavity. Future research should focus on larger sample sizes and broader inclusion criteria to increase the generalizability of findings and account for potential confounding factors, and statistical analysis methods need to be carefully chosen and adjusted for the number of comparisons. This will ensure the reliability and reproducibility of microbiome research.

V. CONCLUSION

Our findings revealed no significant differences in alpha and beta diversity between individuals with and without diabetes, indicating that the overall microbial community structure in oral mucosal tissues remains relatively stable regardless of diabetes status. However, we observed a noteworthy distinction in the relative abundance of the Corynebacteriaceae family and the genus Corynebacterium, with these taxa being more prevalent in the oral mucosal microbiome of individuals without diabetes.

This study challenges previous research that has primarily focused on saliva, plaque, or biofilm samples, as we specifically analyzed oral mucosal tissues obtained during dental implant surgery. The unique composition of the oral mucosal microbiome in individuals without diabetes, characterized by the dominance of Corynebacteriaceae and Corynebacterium, prompts further exploration into the role of these bacteria in oral health and their potential implications for systemic diseases.

Further studies with larger and diverse cohorts, incorporating standardized methodologies, will be needed to enhance our understanding of the intricate relationship between systemic diseases, such as diabetes, and the oral microbiome.

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TABLES

Table 1. Demographics of subjects included in study

	Diabetes group (n = 10)	**Non-diabetes group (n = 13)**
Age, years	73.9±4.39	71.5±2.10
BMI	25.00±1.39	24.61 ± 2.22
HbA1c, %	8.07±0.007	N/A

Variables are presented as the mean ± SD.

Table 2. Comparison of richness and diversity estimates between diabetes and non-diabetes groups

Variable	Diabetes group	Non-diabetes group	*p*-value
OTUs	153.70 ± 77.40	165.54 ± 82.51	0.73
Chao1	154.67 ± 77.82	166.20 ± 82.63	0.737
Inverse Simpson	0.93 ± 0.06	0.91 ± 0.10	0.749
Shannon	5.35 ± 1.13	5.40 ± 1.21	0.919

Variables are presented as the mean ± SD. OTU, operational taxonomic unit.

Table 3. Relative abundances* of OTUs in the diabetes group compared to the non-diabetes group

OTU	*p*-value	FDR adjusted *p*-value	log_2 fold-change (logFC)	Average log_2 counts per million (logCPM)
Corynebacteriaceae (family level)	< 0.001	0.008	-4.298	17.01
Corynebacterium (genus level)	<0.001	0.008	-4.545	17.06

*Only significant results are shown. The degree of differential abundance is represented by a log_2 fold-change (logFC), which indicates a positive or negative interaction (logFC > 0 or < 0) of the specified operational taxonomic unit (OTU). log_2 fold-change (logFC) was calculated for the non-diabetes group as the denominator and the diabetes group as the numerator.

FIGURES

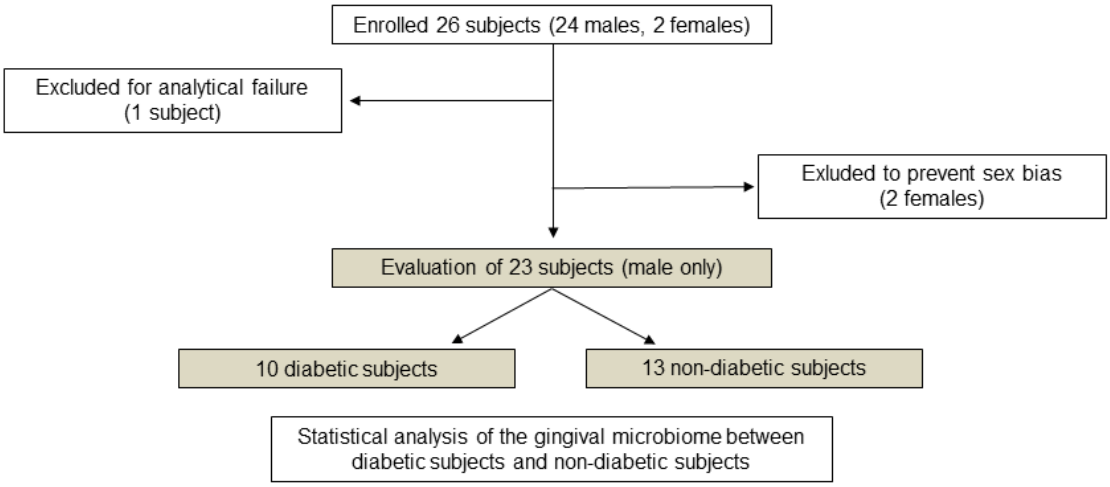

Figure 1. Study flow.

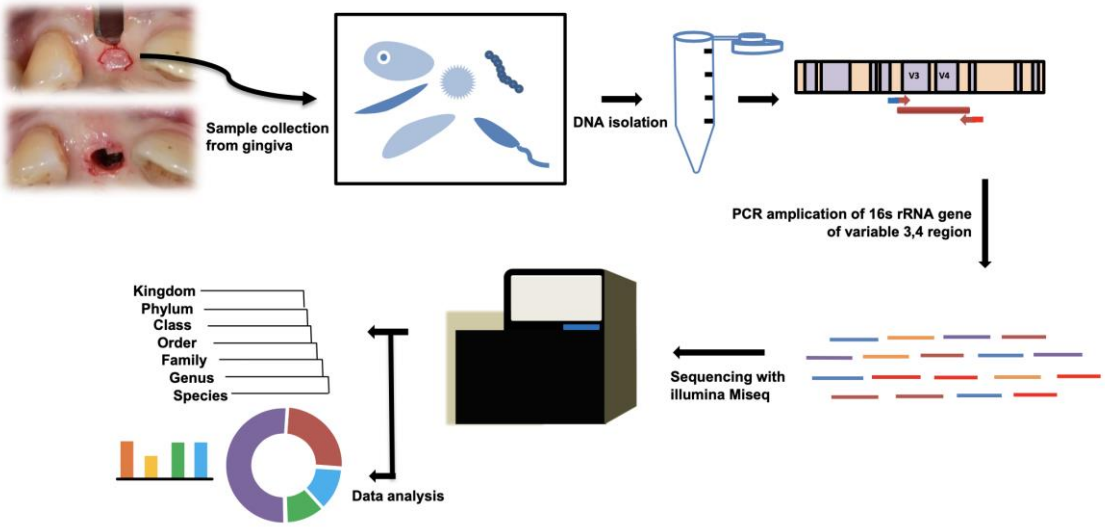

Figure 2. Flow diagram showing the study design.

Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA).

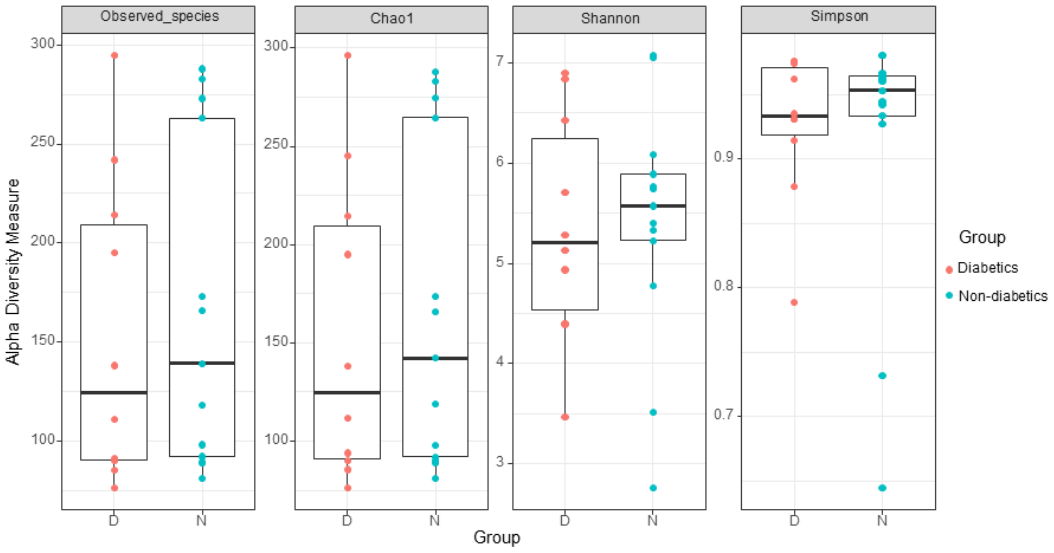

Figure 3. Comparison of alpha diversity

Observed, Chao1, Shannon and Inverse Simpson indices were used to compare betwe en the diabetes group (diabetes) and the control group (non-diabetes).The Student's t-t est was used.

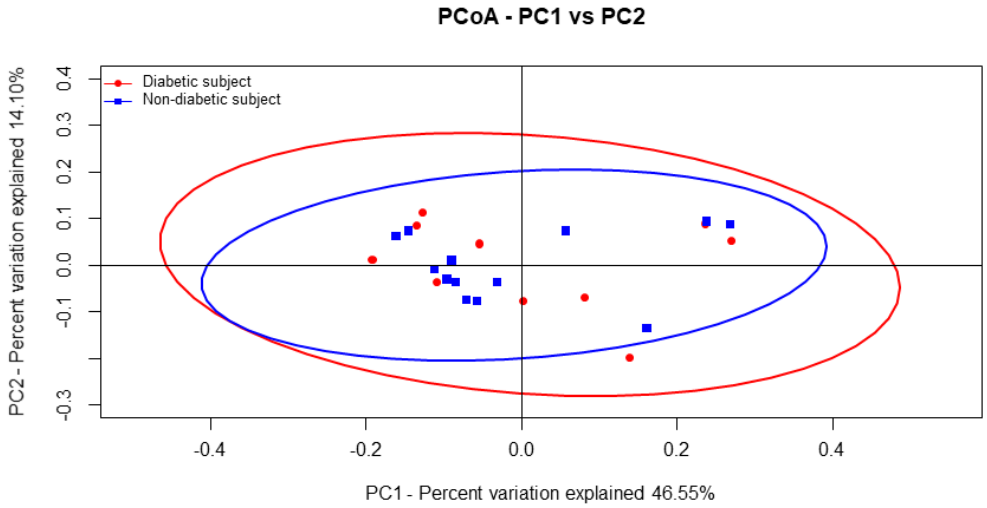

Figure 4. Comparison of beta diversity.

There was no significant difference between groups (p = 0.753). Principal Coordin ate Analysis (PCoA) plot using weighted UniFrac of diabetic patients (red circles) and non-diabetic subjects (blue squares). The ellipses represent 95% confidence int ervals for each group.

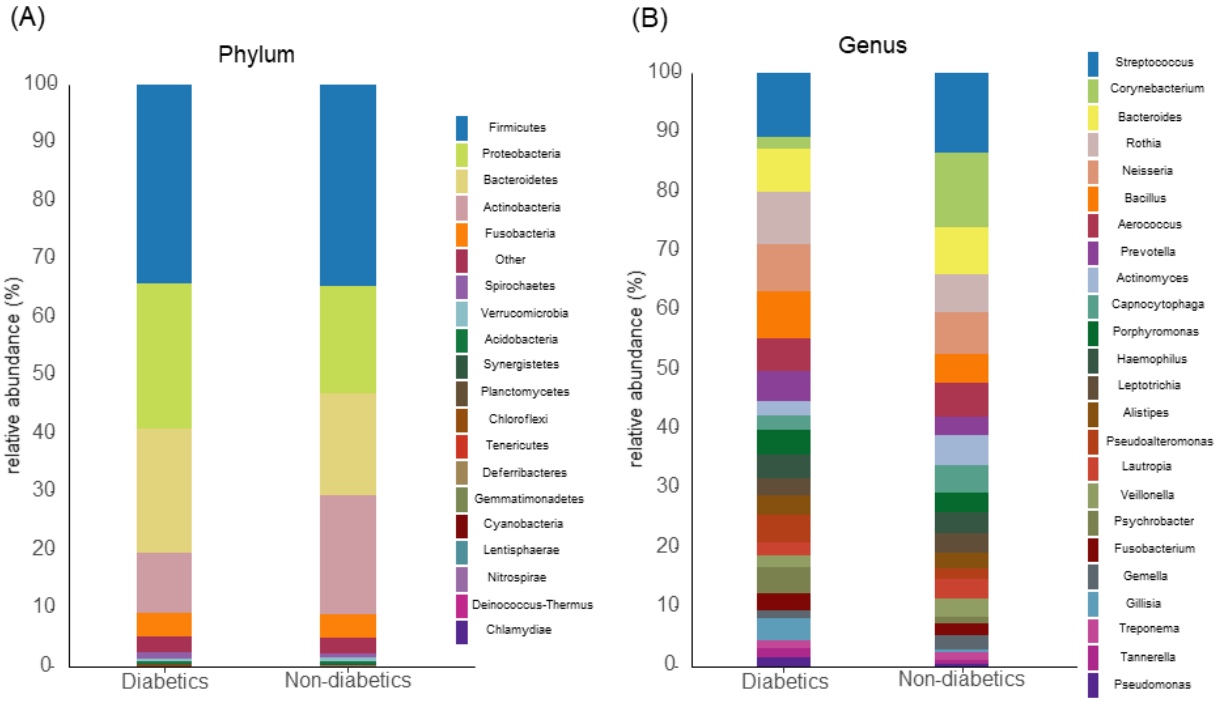

Figure 5. Oral microbial community distribution.

(A) Bar plots showing the taxonomic profiles of diabetes patients and subjects without diabetes at the phylum level, (B) Bar plots showing taxonomic profiles of diabetes patients and subjects without diabetes at the genus level.

Abstract in Korean

남성 노인 환자에게서 차세대염기서열 분석을 통한 당뇨와 구강점막마이크로바이옴의 상관성 분석

세균, 바이러스, 효모 및 진균 등 인체에 존재하는 다양한 미생물은 인간과 체내 항상성을 유지하고 공생관계를 유지하며 마이크로마이옴을 구성하고 있는데, 만성전신질환은 이들의 분포와 수에 영향을 미칠 수 있다. 구강과 같이 병원균이 처음으로 발견되는 진입점에서, 점막 표면은 숙주 면역을 지속적으로 형성하는 박테리아 공생균 층을 이루고 있으나, 구강 마이크로바이옴 샘플들 중 점막에 관한 연구는 그간 활발하지 않았다. 따라서 구강점막을 이용한 구강마이크로바이옴과 당뇨의 연관성을 파악하여 구강 내 미생물의 변화와 당뇨의 역학관계를 밝히기 위한 기초자료를 제공하고 이를 활용한 진단이나 치료법 개발의 토대를 제공하는 것이 본 연구의 목적이다.

임플란트 이차수술을 위해 치주과에 내원한 환자들의 구강점막을 채취해서 구강 마이크로바이옴 분석을 실시했다. 알파 및 베타 다양성측면에선 당뇨군과 대조군사이에 통계학적으로 유의미한 차이가 없었으나, 미생물군의 상대적 분포 비교에서 당뇨군에서 Corynebacteriaceae (family level)와 Corynebacterium (genus level) 미생물들이 더 적은 분포로 나타나는 경향을 보였다. 향후 더 많은 표본과 표준화된 실험을 통해 이 세균들의 생물학적 특성과 관계, 그리고 구강 혹은 체내에서의 역할 등을 밝히는 것이 필요해 보인다.

핵심되는 말 : 구강마이크로바이옴, 당뇨, 16s RNA 시퀀싱