Oral Microbiome Analysis in Patients with Dental Caries: A Comparative Study of Different Caries Risk Levels

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Abstract

Dental caries is a prevalent chronic condition influenced by various factors, including the oral microbiome. This study aimed to compare the oral microbiome composition between individuals with high and low caries risk in a tertiary hospital setting. A cross-sectional analysis was conducted involving 100 participants, with 16S rRNA sequencing used to determine microbial profiles. Results showed that high caries risk individuals exhibited significantly reduced microbial diversity and an increased abundance of cariogenic bacteria, such as *Streptococcus*, *Lactobacillus*, and *Veillonella*. The findings suggest that microbial profiling could serve as a predictive tool for caries susceptibility, supporting the ecological plaque hypothesis. Future research should focus on longitudinal studies to establish causal relationships and on exploring the functional aspects of the oral microbiome.

Keywords: Dental Caries, Oral Microbiome, Microbial Diversity, Cariogenic Bacteria, Caries Risk, Microbial Profiling.

Introduction

Dental caries, a major public health concern globally, is one of the most prevalent chronic conditions, affecting individuals of all age groups. The pathogenesis of dental caries is complex, involving multiple factors, including diet, host susceptibility, oral hygiene practices, and most importantly, the oral microbiome. The oral microbiome consists of diverse microbial communities that interact dynamically within the oral cavity, influencing oral health and disease (Marsh, 2003). Studies have shown that shifts in the balance of these microbial communities can contribute significantly to the development of dental caries (Aas et al., 2005).

The relationship between the composition of the oral microbiome and caries risk has garnered considerable attention in recent years. Traditional caries research focused on specific pathogens such as *Streptococcus mutans*, which has long been recognized as a primary cariogenic bacterium. However, recent advances in molecular and genomic analysis have highlighted the importance of the entire microbial community in caries development, rather than a single pathogen (Gross et al., 2012). Changes in microbial diversity and abundance within the oral cavity are associated with the onset and progression of caries, suggesting that caries risk is determined by an interplay between multiple species rather than a singular pathogenic factor.

Comparative analysis of microbial profiles from individuals with different levels of caries risk can provide insights into the factors driving the cariogenic shift in the oral microbiome. Understanding these variations is crucial for developing more effective preventive and therapeutic approaches, tailored to an individual's microbial risk profile. This study aims to compare the oral microbiome composition between individuals with high and low caries risk, thereby identifying specific microbial patterns that may be predictive of caries susceptibility.

Literature Review

The oral microbiome plays a central role in maintaining oral health and is implicated in the pathogenesis of various oral diseases, including dental caries. Traditional research on dental caries primarily focused on the role of *Streptococcus mutans* as the principal pathogen responsible for caries development (Loesche, 1986). However, contemporary studies have demonstrated that dental caries is a polymicrobial disease, involving a complex community of bacteria that interact with each other and the host environment (Takahashi &Nyvad, 2011). This shift in understanding has been facilitated by advances in molecular techniques, such as 16S rRNA sequencing, which allow for a more comprehensive analysis of the oral microbiome.

Recent studies have identified several bacterial species that are associated with dental caries beyond *S. mutans*. For instance, *Lactobacillus* species and *Bifidobacterium* have been found to play significant roles in the progression of caries, particularly in advanced stages (Becker et al., 2002). Additionally, *Veillonella* species have been shown to contribute to caries development by metabolizing lactate produced by primary colonizers, thereby creating an acidic environment that promotes demineralization of tooth enamel (Klein et al., 2015). The synergistic interactions among these bacteria highlight the importance of considering the entire microbial community when assessing caries risk.

The concept of microbial dysbiosis has emerged as a key factor in the development of dental caries. Dysbiosis refers to an imbalance in the microbial community that leads to a shift from a healthy to a disease-associated state. Marsh (2006) proposed the ecological plaque hypothesis, which suggests that environmental changes, such as increased sugar intake, can lead to a shift in the balance of the oral microbiome, favoring the proliferation of acidogenic and aciduric bacteria. This shift results in an acidic environment that demineralizes tooth enamel and promotes caries formation. Studies have shown that individuals with high caries risk tend to have lower microbial diversity and an increased abundance of acidogenic bacteria compared to those with low caries risk (Yang et al., 2012).

In addition to bacterial composition, the functional potential of the oral microbiome has also been linked to caries risk. Metagenomic analyses have revealed that the oral microbiome of individuals with caries is enriched in genes related to carbohydrate metabolism and acid production (Klassert et al., 2022). These functional capabilities enable the microbial community to efficiently metabolize dietary sugars, leading to the production of organic acids that contribute to enamel demineralization. Therefore, both the taxonomic composition and the functional potential of the oral microbiome are important determinants of caries risk.

The role of host factors, such as saliva flow rate, pH, and immune response, also cannot be overlooked in the development of dental caries. Saliva plays a critical role in maintaining oral health by buffering acids, providing antimicrobial proteins, and facilitating the clearance of bacteria (Humphrey & Williamson, 2001). Reduced salivary flow or alterations in salivary composition can create conditions that favor the growth of cariogenic bacteria, thereby increasing the risk of caries. Moreover, the host immune response, including the

production of specific antibodies against cariogenic bacteria, can influence the composition and activity of the oral microbiome (Smith & Taubman, 1997).

Overall, the literature suggests that dental caries is a multifactorial disease influenced by complex interactions between the oral microbiome, host factors, and environmental conditions. Understanding the microbial profiles and functional characteristics associated with different levels of caries risk is essential for developing targeted preventive and therapeutic strategies. This study aims to build on existing knowledge by comparing the oral microbiome composition of individuals with high and low caries risk, with the goal of identifying microbial patterns that may serve as predictive markers for caries susceptibility.

Methodology

This study was conducted at a tertiary hospital with the aim of comparing the oral microbiome composition between individuals with high and low caries risk. The study design was observational and cross-sectional, involving participants recruited from the dental outpatient department of the hospital. Ethical approval was obtained from the ethics committee, and informed consent was obtained from all participants prior to their enrollment in the study.

Participants

A total of 100 participants were recruited for the study, comprising 50 individuals with high caries risk and 50 individuals with low caries risk. Caries risk was assessed based on participants' dental history, oral examination, and established risk assessment criteria, including diet, oral hygiene practices, and previous caries experience. Participants were aged between 18 and 65 years, and individuals with systemic conditions affecting the oral cavity or those on antibiotics within the past three months were excluded from the study.

Sample Collection

Saliva samples were collected from each participant using a standardized protocol. Participants were instructed to refrain from eating, drinking, or oral hygiene activities for at least one hour prior to sample collection. Unstimulated saliva was collected in sterile containers, immediately placed on ice, and transported to the hospital's microbiology laboratory for further processing. In addition, supragingival plaque samples were collected from caries-active sites in high-risk participants and from randomly selected healthy sites in low-risk participants.

Microbial Analysis

DNA was extracted from the saliva and plaque samples using a commercially available DNA extraction kit, following the manufacturer's instructions. The extracted DNA was then subjected to 16S rRNA gene sequencing to determine the composition of the oral microbiome. Sequencing was performed using the Illumina MiSeq platform, and the resulting sequences were analyzed using QIIME 2 software to identify bacterial taxa present in each sample.

Data Analysis

Microbial diversity was assessed by calculating alpha and beta diversity metrics. Alpha diversity, representing within-sample diversity, was calculated using metrics such as Shannon and Simpson indices, while beta diversity, representing between-sample diversity, was assessed using Bray-Curtis dissimilarity. Differences in microbial composition between the high and low caries risk groups were analyzed using permutational multivariate analysis of variance (PERMANOVA). In addition, differential abundance

analysis was conducted to identify specific bacterial taxa that were significantly associated with either high or low caries risk.

Statistical Analysis

Statistical analyses were performed using R software. The differences in microbial diversity and abundance between groups were assessed using appropriate statistical tests, including the Wilcoxon rank-sum test for alpha diversity and PERMANOVA for beta diversity. A p-value of less than 0.05 was considered statistically significant. The relationship between microbial composition and caries risk factors, such as diet and oral hygiene practices, was also explored using correlation analysis.

Findings

The findings from this study revealed significant differences in the oral microbiome composition between individuals with high and low caries risk. The microbial diversity, as measured by alpha diversity indices (Shannon and Simpson), was significantly lower in the high caries risk group compared to the low caries risk group ($p < 0.01$). Table 1 shows the alpha diversity metrics for both groups.

Group	Shannon Index (Mean ±SD)	Simpson Index (Mean ±SD)
High Caries Risk	2.45 ±0.30	0.74 ±0.05
Low Caries Risk	3.12 ±0.25	0.82 ±0.04

Beta diversity analysis, assessed using Bray-Curtis dissimilarity, showed distinct clustering of microbial communities based on caries risk, indicating significant differences in microbial composition between the high and low caries risk groups (PERMANOVA, $p < 0.001$). Figure 1 illustrates the principal coordinates analysis (PCoA) plot of beta diversity, showing clear separation between the two groups.

Differential abundance analysis identified several bacterial taxa that were significantly associated with either high or low caries risk. Table 2 lists the top five bacterial genera that were differentially abundant between the groups.

Bacterial Genus	High Caries Risk (Mean Relative Abundance %)	Low Caries Risk (Mean Relative Abundance %)	p-value
Streptococcus	28.5	12.3	<0.001
Lactobacillus	15.2	5.8	<0.001
Veillonella	10.7	3.5	<0.001
Prevotella	8.9	2.1	<0.001
Bifidobacterium	6.3	1.9	<0.01

Correlation analysis revealed that the abundance of cariogenic bacteria, such as *Streptococcus* and *Lactobacillus*, was positively correlated with high sugar intake and poor oral hygiene practices ($r = 0.68$, $p < 0.01$). In contrast, bacterial diversity was positively correlated with good oral hygiene practices and a balanced diet ($r = 0.72$, $p < 0.01$).

Discussion

The results of this study demonstrate significant differences in the oral microbiome composition between individuals with high and low caries risk, supporting the concept that dental caries is a multifactorial disease

driven by microbial dysbiosis. The reduced microbial diversity observed in individuals with high caries risk is consistent with previous research indicating that a diverse oral microbiome is crucial for maintaining oral health (Yang et al., 2012). Reduced diversity may result in an imbalance that favors the proliferation of cariogenic bacteria, ultimately leading to the demineralization of tooth enamel and the progression of caries.

The differential abundance analysis identified several bacterial taxa, including *Streptococcus*, *Lactobacillus*, *Veillonella*, *Prevotella*, and *Bifidobacterium*, that were significantly more abundant in individuals with high caries risk. These findings are in line with the literature, which has highlighted the role of these bacteria in the caries process. For example, *Streptococcus* and *Lactobacillus* are well-known acidogenic and aciduric species that contribute to the acidic environment necessary for enamel demineralization (Loesche, 1986; Becker et al., 2002). Additionally, *Veillonella* species have been shown to metabolize lactate produced by other bacteria, further contributing to acid production and creating conditions conducive to caries development (Klein et al., 2015).

The positive correlation between cariogenic bacteria abundance and high sugar intake or poor oral hygiene practices underscores the importance of lifestyle factors in modulating caries risk. Diets rich in fermentable carbohydrates provide a substrate for acidogenic bacteria, promoting their growth and acid production, while inadequate oral hygiene allows for the accumulation of biofilms that facilitate microbial dysbiosis (Takahashi &Nyvad, 2011). Conversely, good oral hygiene practices and a balanced diet were found to be positively correlated with microbial diversity, suggesting that preventive measures targeting these factors may help maintain a healthy oral microbiome and reduce caries risk.

Beta diversity analysis showed clear separation between the high and low caries risk groups, indicating distinct microbial community structures associated with different levels of caries susceptibility. This finding suggests that individuals with high caries risk harbor a unique microbiome composition that predisposes them to disease. The identification of specific microbial patterns associated with caries risk highlights the potential for developing microbial profiling tools that could be used in clinical practice to predict caries susceptibility and guide personalized preventive and therapeutic interventions.

The ecological plaque hypothesis proposed by Marsh (2006) provides a useful framework for interpreting the findings of this study. According to this hypothesis, environmental changes such as increased sugar intake can lead to a shift in the balance of the oral microbiome, favoring the growth of acidogenic and aciduric bacteria. The results of this study align with this hypothesis, as individuals with high caries risk exhibited an increased abundance of acidogenic bacteria and reduced microbial diversity. This supports the notion that caries prevention should focus not only on reducing specific pathogens but also on maintaining a balanced and diverse oral microbiome.

Despite the insights provided by this study, several limitations should be acknowledged. The cross-sectional design precludes causal inferences regarding the relationship between microbiome composition and caries development. Longitudinal studies are needed to establish causal links and to determine whether changes in the oral microbiome precede the onset of caries. Additionally, this study relied on 16S rRNA gene sequencing, which provides information on bacterial taxonomy but not on functional potential. Metagenomic or metatranscriptomic analyses could provide deeper insights into the functional capabilities of the oral microbiome and their role in caries progression.

In conclusion, this study demonstrates that individuals with high caries risk exhibit reduced microbial diversity and an increased abundance of cariogenic bacteria. The identification of specific bacterial taxa associated with caries risk highlights the potential for using microbial profiling as a predictive tool for caries susceptibility. Future research should focus on longitudinal studies to establish causal relationships and on exploring the functional aspects of the oral microbiome to develop more targeted preventive and therapeutic strategies.

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