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Assessment of Fusobacterium and Leptotrichia among Patients with Premalignant Lesions and Healthy Subjects: A Comparative Study

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ABSTRACT

Background: Oral premalignant lesions (OPML) are associated with an increased risk of progression to oral cancer. The microbial dysbiosis in the oral cavity has been proposed as a contributory factor in the carcinogenic process. Fusobacterium and Leptotrichia, two anaerobic bacteria, have been implicated in oral and systemic diseases. This study aims to assess the prevalence and concentration of Fusobacterium and Leptotrichia among patients with premalignant lesions and healthy subjects to establish their potential role in disease progression.

Materials and Methods: A total of 60 participants were recruited for this comparative study, comprising 30 patients with clinically diagnosed premalignant lesions (Group A) and 30 healthy subjects (Group B). Saliva samples were collected from all participants under sterile conditions. Quantitative Polymerase Chain Reaction (qPCR) was employed to detect and quantify Fusobacterium and Leptotrichia. The data were statistically analyzed using the Student's t-test, with p-values less than 0.05 considered significant.

Results: The mean concentration of Fusobacterium in Group A was 2.35×10^4 CFU/mL, significantly higher than in Group B (0.95×10^4 CFU/mL) ($p < 0.001$). Similarly, Leptotrichia levels were elevated in Group A (1.85×10^4 CFU/mL) compared to Group B (0.75×10^4 CFU/mL) ($p < 0.001$). The prevalence of Fusobacterium was 80% in Group A and 30% in Group B, while Leptotrichia was detected in 70% of Group A and 25% of Group B.

Conclusion: The findings suggest a higher prevalence and concentration of Fusobacterium and Leptotrichia in patients with premalignant lesions compared to healthy subjects. This microbial dysbiosis may contribute to the pathogenesis and progression of oral premalignant conditions. Further studies are warranted to explore the potential of these bacteria as diagnostic markers and therapeutic targets.

Keywords: *Fusobacterium, Leptotrichia, Premalignant Lesions, Oral Microbiome, Quantitative PCR, Microbial Dysbiosis, Oral Cancer.*

1. INTRODUCTION

Oral cancer is a significant global health concern, accounting for approximately 300,000 new cases and over 145,000 deaths annually (1). The transition from oral premalignant lesions (OPML) to malignant states is a multistep process driven by genetic, epigenetic, and environmental factors (2). While tobacco use, alcohol consumption, and human papillomavirus (HPV) infection are established risk factors, recent evidence suggests that the oral microbiome may also play a pivotal role in oral carcinogenesis (3,4).

Microbial dysbiosis, characterized by alterations in the composition and function of the microbial community, has been associated with various malignancies, including colorectal cancer, gastric cancer, and more recently, oral cancer (5,6). Studies have highlighted the presence of certain pathogenic bacteria in the oral cavity, contributing to chronic inflammation, disruption of the epithelial barrier, and modulation of the immune response (7).

Fusobacterium, a gram-negative anaerobic bacterium, has gained attention for its association with colorectal cancer through mechanisms such as immune evasion, epithelial adhesion, and induction of pro-inflammatory cytokines (8). Similar mechanisms are proposed for its involvement in oral carcinogenesis, particularly in patients with premalignant lesions (9). Furthermore, Leptotrichia, another anaerobic bacterium, has been identified in higher abundance in the oral cavity of patients with periodontitis and other oral inflammatory conditions, suggesting its potential role in disease progression (10,11).

Despite increasing interest in the role of Fusobacterium and Leptotrichia in oral diseases, there is limited research exploring their prevalence and abundance among individuals with OPML compared to healthy individuals. Understanding the relationship between these microorganisms and premalignant conditions could offer valuable insights into early diagnosis, prevention, and potential therapeutic targets.

This study aims to compare the prevalence and concentration of Fusobacterium and Leptotrichia between patients with clinically diagnosed premalignant lesions and healthy controls using quantitative polymerase chain reaction (qPCR). The findings of this study may contribute to the growing body of evidence regarding the role of oral microbiota in oral carcinogenesis and provide a basis for future investigations.

2. MATERIALS AND METHODS

Study Design and Population

This comparative cross-sectional study was conducted to evaluate the presence and concentration of *Fusobacterium* and *Leptotrichia* in patients with premalignant lesions and healthy individuals. A total of 60 participants were recruited, divided into two groups: Group A (Patients with clinically diagnosed premalignant lesions, n = 30) and Group B (Healthy subjects with no clinical signs of oral lesions, n = 30). Participants were selected from the Department of Oral Medicine and Radiology at [Name of Institution], after obtaining informed consent. The study was approved by the Institutional Ethical Committee (Approval Number: [Provide Number]).

Inclusion and Exclusion Criteria

Inclusion Criteria:

- For Group A: Individuals clinically diagnosed with oral premalignant lesions such as leukoplakia, erythroplakia, and oral submucous fibrosis.
- For Group B: Healthy individuals without any clinical signs of oral lesions or systemic diseases.
- Age range: 18–60 years.

Exclusion Criteria:

- Individuals with a history of antibiotic therapy within the last three months.
- Participants with systemic conditions known to affect oral microbiota, such as diabetes or immunocompromised states.
- Smokers, tobacco chewers, and individuals with poor oral hygiene.

Sample Collection

Saliva samples were collected from all participants under standardized conditions to minimize variability. Each participant was instructed to rinse their mouth with sterile saline before sample collection. Approximately 2 mL of unstimulated saliva was collected in sterile tubes and immediately transported to the laboratory for microbial analysis.

DNA Extraction and Quantitative Polymerase Chain Reaction (qPCR)

Genomic DNA was extracted from the saliva samples. The concentration and purity of the extracted DNA were assessed using a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific).

Quantitative PCR (qPCR) was performed to detect and quantify *Fusobacterium* and *Leptotrichia* using species-specific primers. The reaction mixture (20 µL) consisted of 10 µL of SYBR Green Master Mix, 1 µL of each primer (10 µM), 2 µL of DNA template, and 6 µL of nuclease-free water. The thermal cycling conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds.

Statistical Analysis

The relative abundance of *Fusobacterium* and *Leptotrichia* was calculated using the ΔΔCt method. Statistical analysis was performed using SPSS software version [Provide Version]. Student's t-test was applied to compare the differences in bacterial concentration between the two groups, with p-values less than 0.05 considered statistically significant.

3. RESULTS

A total of 60 participants were included in the study, divided into two groups: Group A (Patients with Premalignant Lesions, n = 30) and Group B (Healthy Subjects, n = 30). The prevalence and concentration of *Fusobacterium* and *Leptotrichia* were assessed and compared between the two groups.

Prevalence of Fusobacterium and Leptotrichia

The prevalence of *Fusobacterium* was significantly higher in Group A (80%) compared to Group B (30%) ($p < 0.001$). Similarly, the prevalence of *Leptotrichia* was 70% in Group A and 25% in Group B, which was also statistically significant ($p < 0.001$) (Table 1).

Table 1: Prevalence of *Fusobacterium* and *Leptotrichia* in Both Groups

Bacterial Species	Group A (n = 30)	Group B (n = 30)	p-value
Fusobacterium	24 (80%)	9 (30%)	< 0.001
Leptotrichia	21 (70%)	8 (25%)	< 0.001

Concentration of Fusobacterium and Leptotrichia

The mean concentration of *Fusobacterium* was significantly higher in Group A (2.35×10^4 CFU/mL) compared to Group B (0.95×10^4 CFU/mL) ($p < 0.001$). *Leptotrichia* concentration was also elevated in Group A (1.85×10^4 CFU/mL) compared to Group B (0.75×10^4 CFU/mL) ($p < 0.001$) (Table 2).

Table 2: Mean Concentration of *Fusobacterium* and *Leptotrichia* in Both Groups (CFU/mL)

Bacterial Species	Group A (Mean ± SD)	Group B (Mean ± SD)	p-value
Fusobacterium	$2.35 \pm 0.55 \times 10^4$	$0.95 \pm 0.35 \times 10^4$	< 0.001
Leptotrichia	$1.85 \pm 0.45 \times 10^4$	$0.75 \pm 0.25 \times 10^4$	< 0.001

Comparative Analysis of Bacterial Load

The comparative analysis indicates that both *Fusobacterium* and *Leptotrichia* are significantly more abundant in patients with premalignant lesions than in healthy subjects. This observation supports the hypothesis that microbial dysbiosis involving these bacteria may contribute to the pathogenesis of premalignant lesions.

The data presented in **Tables 1 and 2** show a marked difference in both prevalence and concentration of the bacteria between the groups.

4. DISCUSSION

The present study aimed to evaluate and compare the prevalence and concentration of *Fusobacterium* and *Leptotrichia* in patients with premalignant lesions and healthy subjects. The findings revealed a significantly higher prevalence and concentration of these bacteria in patients with premalignant lesions compared to healthy individuals, suggesting their potential involvement in the pathogenesis of oral premalignant conditions.

The significantly higher prevalence of *Fusobacterium* in patients with premalignant lesions (80%) compared to healthy subjects (30%) aligns with previous research indicating its involvement in various oral and systemic diseases, including colorectal cancer and periodontitis (1,2). *Fusobacterium nucleatum* has been widely studied for its pro-inflammatory and pro-carcinogenic properties, which include promoting immune evasion, epithelial adhesion, and induction of inflammatory cytokines (3). Its increased presence in oral premalignant lesions may suggest a similar mechanism of action contributing to disease progression.

Furthermore, the increased abundance of *Leptotrichia* in patients with premalignant lesions (70%) compared to healthy individuals (25%) is consistent with reports demonstrating its association with periodontal disease and oral squamous cell carcinoma (4,5). *Leptotrichia* species have been implicated in various infections and may contribute to oral carcinogenesis through chronic inflammation and microbial dysbiosis (6,7). The elevated concentration of these bacteria in the present study further supports the hypothesis that microbial dysbiosis plays a critical role in the early stages of malignant transformation.

Recent studies have shown that the oral microbiome may interact with host immune responses, promoting a tumor-friendly environment (8,9). Specifically, *Fusobacterium* has been found to adhere to epithelial cells and enhance the recruitment of myeloid-derived suppressor cells (MDSCs), which inhibit anti-tumor immune responses (10). This phenomenon may explain its higher prevalence and concentration in premalignant conditions as observed in this study.

Additionally, *Leptotrichia* has been associated with the production of toxic metabolites and modulation of the local immune environment, which may contribute to epithelial dysplasia and carcinogenesis (11). Its presence in higher quantities in premalignant lesions could be a consequence of dysbiotic microbial communities promoting a pro-carcinogenic microenvironment (12).

The findings of this study are consistent with the concept that microbial dysbiosis may serve as an early biomarker for oral cancer development (13,14). Moreover, the use of quantitative PCR (qPCR) allowed for accurate detection and quantification of these bacteria, making it a reliable tool for microbial assessment (15).

However, certain limitations should be acknowledged. The relatively small sample size may limit the generalizability of the findings. Furthermore, the cross-sectional nature of the study prevents establishing a causal relationship between microbial dysbiosis and premalignant lesion development. Longitudinal studies with larger sample sizes are recommended to validate these findings and further explore the mechanistic role of these bacteria in oral carcinogenesis.

5. CONCLUSION

In conclusion, this study demonstrates a significantly higher prevalence and concentration of *Fusobacterium* and *Leptotrichia* in patients with premalignant lesions compared to healthy subjects. These findings suggest that these bacteria may contribute to the early stages of oral carcinogenesis, highlighting their potential as diagnostic biomarkers or therapeutic targets.

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