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### **Impact of Smoking on Gingival Microbiome Composition**

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**Objectives** The oral microbiome plays a crucial role in maintaining oral health and can be influenced by various factors, including smoking habits. This study aimed to investigate the differences in the gingival microbiome among never smokers, current smokers, and previous smokers.

**Methods** Subgingival plaque samples were collected from 1287 participants born in 1950-1951 participating in Hordaland Oral Health survey (2020-2022) and in the parent Hordaland Health Study (third wave occurring during 2018-2020) in Western Norway. Samples were obtained from individuals with periodontal pockets. Shotgun metagenomics analysis was conducted to assess the composition of the gingival microbiome.

**Results** Significant differences were revealed in the abundance of bacterial genera and species among current smokers, previous smokers, and never smokers. Current smokers exhibited significantly higher abundances of several bacteria, including *Tannerella forsythia* ( $p < 0.001$ ), *Fretibacterium fastidiosum* ( $p < 0.001$ ) and *Prevotella intermedia* ( $p < 0.01$ ) compared to never smokers. Conversely, certain bacteria, such as *Lautropia mirabilis* ( $p < 0.001$ ), *Actinomyces naeslundii* ( $p < 0.001$ ), and *Rothia aeria* ( $p < 0.001$ ), showed lower abundances compared to never smokers. Specific bacterial species displayed differential abundances between current and previous smokers.

**Conclusions** The findings underscore the importance of considering smoking status when assessing the gingival microbiome and its implications for oral health. The extensive alterations in bacterial composition observed in current smokers highlight the need for targeted interventions to mitigate the adverse effects of smoking on periodontal health. Further research is warranted to elucidate the mechanistic links between smoking, the oral microbiome, and periodontal disease. Furthermore, the potential reversibility of smoking effects on the gingival microbiome upon smoking cessation underscores the need for further research in this area.