Differential Microbial Enrichment is Associated with Oral Cancer and Perineural Invasion

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Background: Advanced oral cavity squamous cell carcinoma (OSCC) is an aggressive disease, with 5-year overall survival rates below 50%. While smoking and alcohol are the most established risk factors, there is growing evidence implicating the role of the oral microbiome in promoting immunosuppressive states in OSCC. The objective of this study was to evaluate the taxonomic profile of oral bacteria in advanced OSCC patients and correlate with clinicopathologic features.

Methods: The Institutional Review Board approved this prospective study of patients diagnosed with OSCC treated surgically from 2016-2020. Samples were taken from tumor and paired adjacent normal tissues at the time of surgery and snap frozen. Demographics, clinical and pathologic information was collected. DNA from 51 samples was isolated and 16s rRNA gene sequencing was performed after PCR amplification of the V1-V3 regions of the 16s rRNA gene for paired samples. 16S rRNA sequences were processed using DADA2, implemented in QHIME2 package, and taxonomic assignments were made using RDA classifier using the Human Oral Microbiome Database V15.22 as the reference database. Sequencing reads processing, denoising, dereplicating, chimeras filtering, and amplicon sequence variants (ASVs) generation was done using DADA2. The association between clinicopathological factors and bacterial profiles was evaluated using R Software.

Results: In our cohort, the most common OSCC subtypes were oral tongue (47%) and floor of mouth (25.5%), with 83.4% of patients at advanced stage. The median age was 68 years old, and 84% were either current or former smokers. 16S rRNA gene sequencing and analysis revealed 9 ASVs that were significantly enriched in cancer samples and 22 enriched in normal samples using LEfSe (Linear discriminant analysis effect size). On the genus level, Fusobacterium (a known pathobiont), Lactobacillus, and Bacteroides were enriched in tumor samples, while Rothia and Streptococcus were enriched in normal tissue samples. Beta diversity was significantly different in cancer vs. normal tissue samples (p=0.03, PERMANOVA). Normal tissue had significantly higher alpha diversity than tumor tissue (p=0.003, Chao1 GLS). Interestingly, decreased alpha diversity was associated with more aggressive perineural invasion (p=0.03, Chao1 GLS).

Conclusions: Among patients with OSCC, the bacterial diversity of primary tumor tissues was found to be significantly reduced compared to normal tissue in the same patients. For patients with decreased alpha diversity, there was increased likelihood of tumor perineural invasion, but no other associations found with aggressive pathologic factors. Additional studies are needed to further elucidate the role of microbes in oral cancers.

REFERENCES