

Oral Microbiome Community Composition in Head and Neck Squamous Cell Carcinoma: A Case Control Study

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Abstract

Objective: To assess the differences in diversity metrics and variations in microbial community composition between cases and controls alongside assessing whether variations in community type are predictive of survival in head and neck squamous cell carcinoma (HNSCC).

Methods: 93 cases with HNSCC were drawn from the University of Michigan Specialized Program of Research Excellence (SPORE) Project 3. Hospital-controls were drawn from the electronic medical record. Oral wash samples were obtained from cases at various timepoints including pre-treatment, post-surgery, and post-chemoradiation and from controls at study enrollment. 16s rRNA was isolated, amplified, and sequenced from oral samples. The Mothur software package was used to align sequences and bin them into operational taxonomic units (OTUs) at the genus level. EdgeR was used to assess for significant OTUs between cases and controls with a model controlling for age, gender, and another model controlling for age, gender, and smoking. Diversity metrics, including alpha and beta diversity were calculated using Mothur. The Wilcoxon test was used to assess whether beta diversity differed between cases and controls. Survival outcomes were assessed between community types and treatment modalities. dSVA was used to assess for hidden covariates and a proxy HPV variable was built. Multivariate analysis was completed using cox-proportional hazards models adjusting for stage, HPV status, and a variable from the dSVA. Analyses were completed using R Statistical Software (version 3.3.1, R Foundation.)

Results: Between all cases and controls across all sites, 12 OTUs from the phyla Firmicutes, Proteobacteria, and Actinobacter were found to be significantly different. Further, between all oral cavity cases and their controls, 14 OTUs were found to be significantly different. Alpha diversity was calculated for sex, site, smoking, stage, and treatment, although no significant differences were found. Beta-diversity was found to differ between cases and controls ($p < 0.0001$). Two community types were identified as the predominant sets of OTUs within our study population. Community 1 was the predominant community for 29 cases and 77 controls while community 2 was the predominant community for 23 cases and 19 controls. The frequency of cases and controls within each community type differed significantly with a 55.76% of cases and 80.21% of controls sorting into community 1 ($p = 0.0016$). Community 1 and community 2 differed significantly by age ($p = 0.0101$), smoking status (0.0003), and treatment (0.0364). Within cases, Cox proportional hazard models illustrated that community 1 had a higher, yet non-significant ($p = 0.0663$) survival difference controlling for stage, HPV, and V1.

Conclusions: Significant differences between cases and controls in community type, beta-diversity, and OTUs indicate that the oral microbiome may play a role in HNSCC. Further, community types may help predict survival in HNSCC cases.

Introduction

Studies assessing the role of the microbiome in various cancers have been increasing over the past decade, however, the role of the oral microbiome biome in head and neck squamous cell carcinoma (HNSCC) hasn't been investigated in great depth. Our case-control study aims to better understand the differences in diversity metrics and variations in microbial community composition between HNSCC cases and controls. Further, we aim to assess whether variations in community type are predictive of survival in HNSCC cases.

Methods

HNSCC cases were drawn from the University of Michigan Specialized Program of Research Excellence (SPORE) Project 3. Hospital-controls were drawn from the electronic medical record. Oral wash samples were obtained from cases at various timepoints including pre-treatment and post-surgery and from controls at study enrollment. 16s rRNA was isolated, amplified, and sequenced from oral samples. The Mothur software package was used to align sequences and bin them into operational taxonomic units (OTUs) at the genus level. EdgeR was used to assess for significant OTUs between cases and controls with a model controlling for age, gender, and another model controlling for age, gender, and smoking. Diversity metrics, including alpha and beta diversity were calculated using Mothur. The Wilcoxon test was used to assess whether beta diversity differed between cases and controls.

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Results

Case-Control Analysis

Patient Variables	Cases N=52	Controls N=102	p-value
Age	59.15±13.10	59.01±10.24	0.9450
Sex			0.7537
Female	10 (19.23)	17 (17.17)	
Male	42 (80.77)	82 (82.83)	
Alcohol Use			0.2849
Never	3 (7.32)	5 (5.26)	
Former	12 (29.27)	18 (18.95)	
Current	26 (63.41)	72 (75.79)	
Smoking			0.0895
Never	15 (36.59)	51 (53.13)	
Former	24 (58.54)	36 (37.50)	
Current	2 (4.88)	9 (9.38)	
Site			
Larynx	14 (26.92)	-	
Oral Cavity	18 (34.62)	-	
Oropharynx	16 (30.77)	-	
Hypopharynx	2 (3.84)	-	
Nasal cavity, sinus, or skull	1 (1.92)	-	
Unknown primary	8 (15.38)	-	
Nasopharynx	1 (1.92)	-	
Stage			
1 and 2	18 (34.62)	-	
3 and 4	34 (65.38)	-	

- Cases and controls were found to not differ by major demographic variables
- EdgeR models assessing for associations between OTUs and case and control status controlling for age, sex, and smoking were notable for higher abundances of the family Lachnospiraceae and genus *Kingella* alongside decreased abundance of genera *Lactobacillus*, *Bacillus*, and *Acinetobacter* and family Bifidobacteriaceae between cases compared to controls
- Beta diversity was found to be significantly higher in cases compared to controls (Figure 1)
- Alpha diversity was found to not be significantly different between cases and controls

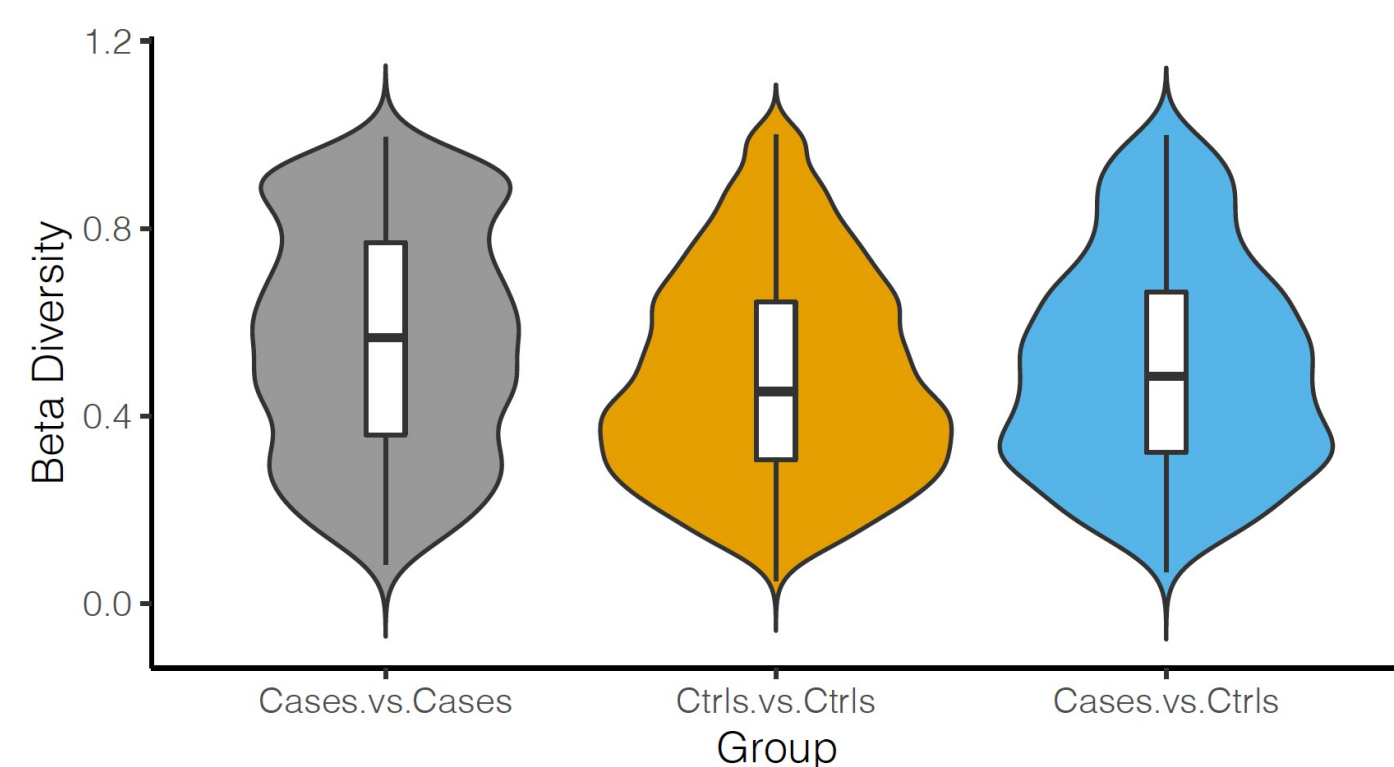


Figure 1: Violin Plots of beta-diversity between cases vs. cases, controls vs. controls, and cases vs. controls.

Group 1	Group 2	Median 1	Median 2	P-adj (Holm)
Cases vs. Cases	Controls vs. Controls	0.57	0.45	2.6E-26
Cases vs. Cases	Cases vs. Controls	0.57	0.49	4.0E-9
Control vs. Controls	Cases vs. Controls	0.45	0.49	1.3E-3

Table 2: Kruskal-Wallace Rank Sum Test comparing the median Beta-diversity between cases vs. cases, controls vs. controls, and cases vs. controls.

Community Type Analysis

Patient Variables	Com. 1 N=109	Com. 2 N=45	p-value
Age	57.57±11.18	62.67±10.68	0.0100
Sex			0.5372
Female	18 (16.67)	9 (20.93)	
Male	90 (83.33)	34 (79.07)	
Alcohol Use			0.0770
Never	5 (5.15)	3 (7.69)	
Former	17 (17.53)	13 (33.33)	
Current	75 (77.32)	23 (58.97)	
Smoking			0.0062
Never	55 (56.12)	11 (28.21)	
Former	38 (38.78)	22 (56.41)	
Current	5 (5.10)	6 (15.38)	
Case-Control Status			0.0048
Case	29 (26.61)	23 (51.11)	
Control	80 (73.39)	22 (48.89)	
Site			0.2040
Larynx	8 (27.59)	6 (26.09)	
Oral Cavity	7 (24.14)	11 (47.83)	
Oropharynx	11 (37.93)	5 (21.74)	
Hypopharynx	0 (0.00)	1 (4.35)	
Nasal cavity, sinus, or skull	1 (3.45)	0 (0.00)	
Unknown primary	2 (6.90)	0 (0.00)	
Stage			0.0822
1 and 2	13 (44.83)	5 (21.74)	
3 and 4	16 (55.17)	18 (78.26)	

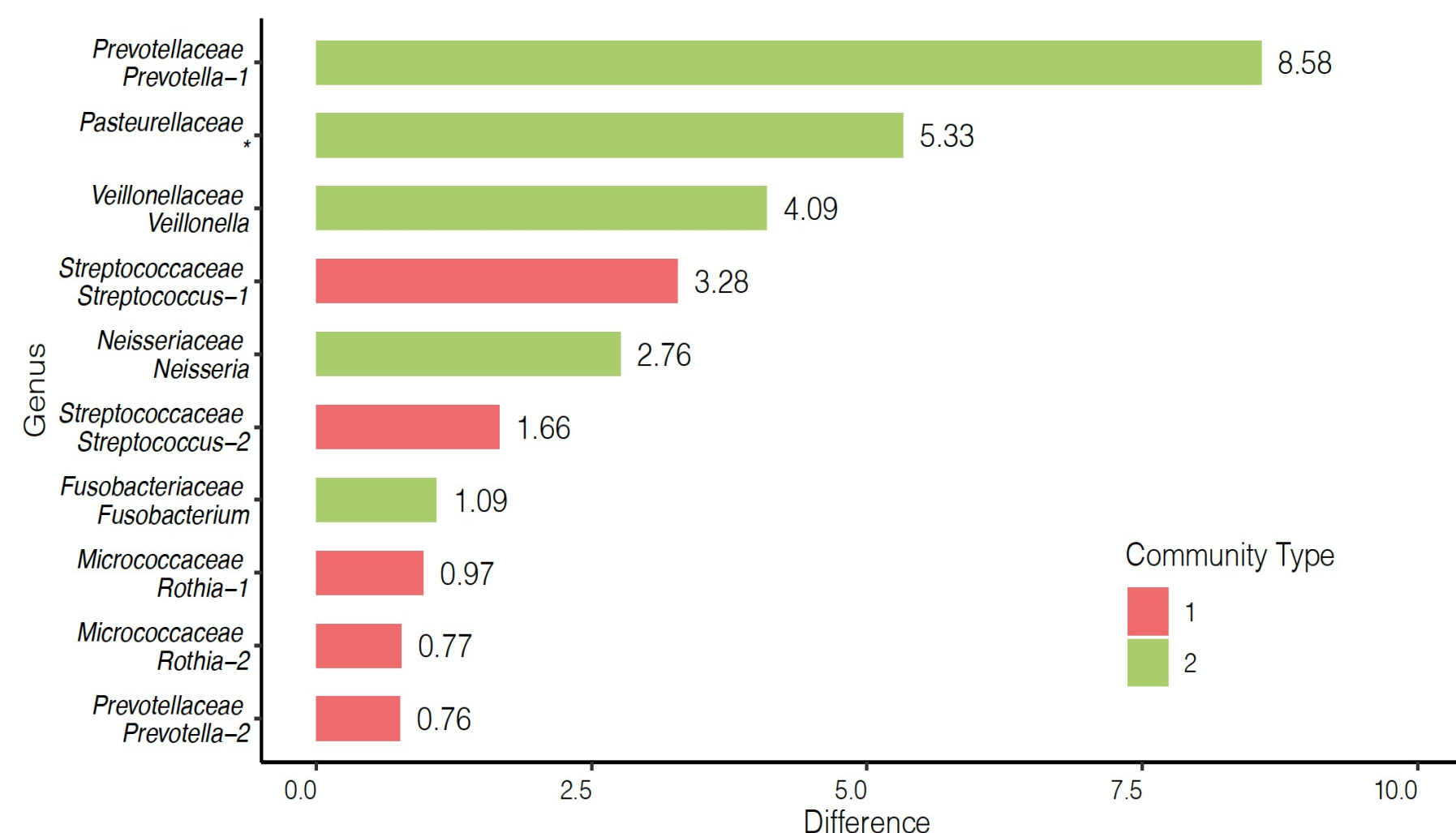


Figure 2: Abundances of OTUs among community 1 and community 2.

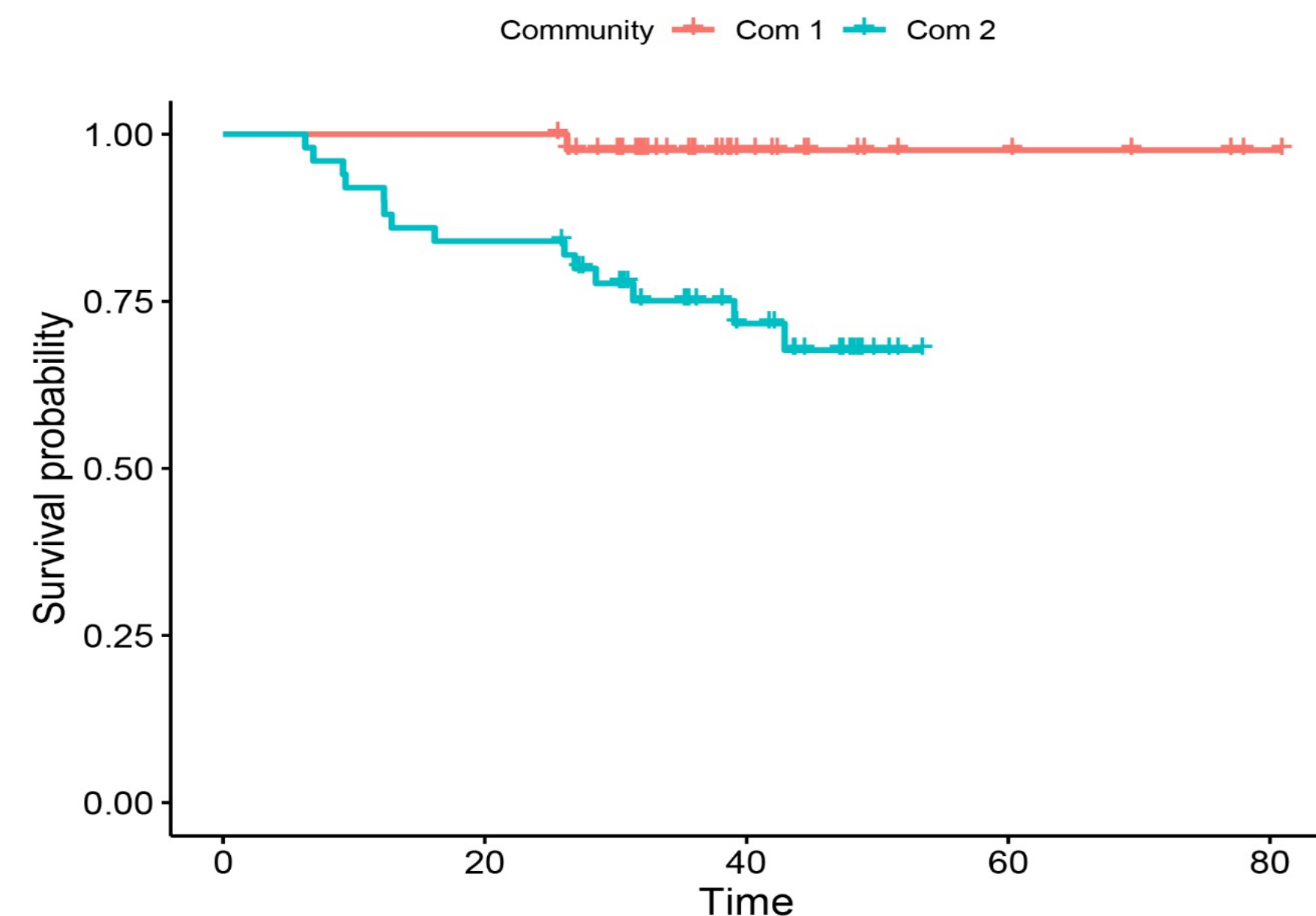


Figure 3: Case only (n=52) Kaplan-Meier survival analysis comparing 80-month survival among community 1 and community 2. In Cox-Proportional Hazard Models adjusting for stage, HPV, and V1 (from dSVA) community 1 was noted to not significantly associated with survival, albeit the p-value was close to our alpha cut-off ($p = 0.0667$).

- Community type was found to significantly differ by age, smoking, and case-control status.
- Community 1 was found to contain a high abundance of *Streptococcus*, *Rothia*, and *Prevotella*
- Community 2 was found to contain a high abundance of *Prevotella*, *Veillonella*, *Neisseria*, and *Fusobacterium* (Figure 2)
- In Cox-Proportional Hazards models adjusting for HPV, V1 (produced by dSVA), and stage were notable for a near significant survival difference between community 1 and community 2 ($p = 0.0667$) (Figure 3)

Conclusions

- Significant differences between cases and controls pertaining to beta diversity, community type, and OTUs indicate that the oral microbiome may play a role in HNSCC
- Cox-proportional models may indicate that community type analysis may be able to predict survival with an appropriate sample size
- Further investigation should be taken to better understand the role of the oral microbiome in the underlying pathogenesis, natural history, and progression of HNSCC

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