Jordan Journal of Dentistry, Volume **3**, No. **1**, **2026**
DOI: https://doi.org/10.14525/JJD.v3i1.03

Jordan Journal of Dentistry

https://jjd.just.edu.jo

Quantification of *Haemophilus parainfluenzae* in Oral Squamous Cell Carcinoma Tissues among a Cohort of Male Patients: Preliminary Findings from a Multi-center Study

Manosha Perera [1], Irosha Perera [2], Aruni Tilakaratne [3], Chamara Athukorala [4]

1 Alumnus, School of Dentistry and Oral Health, Griffith University, Gold Coast, Qld, Australia.
2 Preventive Oral Health Unit, National Dental Hospital (Teaching) Sri Lanka, Ward Place, Colombo 7, Sri Lanka.
3 Department of Restorative Dentistry, Faculty of Dentistry, University of Malaya, Kuala Lampur,50603, Malaysia.
4 Oral & Maxillofacial Unit, Teaching Hospital, Badulla, Sri Lanka.

ARTICLE INFO

Article History:
Received: 26/2/2025
Accepted: 30/6/2025

Correspondence:
Irosha Perera
Preventive Oral Health Unit, National Dental Hospital (Teaching) Sri Lanka, Ward Place, Colombo 7, Sri Lanka.
irosha666rukmali@gmail.com, irosha_rukmali@yahoo.com

ABSTRACT

Objectives: This study aimed to quantify *Haemophilus parainfluenzae* (*H. parainfluenzae*) by percent average relative abundance, in oral squamous cell carcinoma (OSCC) tissues among a cohort of male patients. Comparisons were also made on the occurrence of *H. parainfluenzae* by selected host factors, such as socio-demographic characteristics, life-style-related risk habits, site of the lesion and periodontal disease indicators.

Materials and Methods: The sample comprised a 25 histologically confirmed OSCC tissues of male patients who attended selected Oral & Maxillofacial (OMF) Units of public hospitals across Sri Lanka. The V1 to V3 region of the 16S r- RNA gene was amplified for sequencing using the degenerate primers. Sequencing, data processing and taxonomy assignment up to species level were accomplished by using standard methodologies. Comparisons were made on occurrence of *H. parainfluenzae* by age group, educational attainment, frequency of betel quid chewing, smoking, alcohol consumption, site of the lesion, oral hygiene status and periodontal disease status using Fisher's exact test.

Results: The percent average relative abundance of *H. parainfluenzae* of OSCC tissues was 2.99% and the occurrence of *H. parainfluenzae* in 80% of the sample was less than 2.99%. There were no statistically significant differences in the occurrence of *H. parainfluenzae* by socio-demographic factors, frequency of life-style risk habits, site of the OSCC and periodontal disease indicators ($p>0.05$).

Conclusions: Our preliminary findings demonstrated the occurrence of *H. parainfluenzae*, a significant bacterial species increasingly linked with dysbiosis of oral cancers in the OSCC tumour micro-environment of male patients. Within the limitations of the present study, further research is warranted to generate more conclusive evidence on the quantification of *H. parainfluenzae* in OSCC tissues and to determine related host factors in comparison with matched healthy controls powered by larger sample sizes.

Keywords: Oral squamous cell carcinoma, *Haemophilua parainfluenzae*, Microbiome, Opportunistic pathogen, NGS technology, Relative abundance.

1. Introduction

Haemophilus parainfluenzae (*H.parainfluenzae*) is a Gram-negative, facultative anaerobic and a pleomorphic coccobacillus with fastidious growth requirements, such as enriched media, usually containing blood (e.g. chocolate agar). It can be differentiated from other *Haemophilus* spp. by the requirement for V factor (i.e., NAD: nicotinamide adenine dinucleotide) for growth

(1,2). It is a normal inhabitant of respiratory, oral, and genitourinary flora of humans (1,2).

Haemophilus parainfluenzae gained recognition as a highly abundant commensal microbe in oral cavities of healthy individuals correlating with good oral health, thus playing a role of healthy host status. However, it could cause opportunistic infections outside of the oral cavity (3). Accordingly, this opportunistic pathogen constitutes part of the HACEK group (*Haemophilus parainfluenzae, Aggregatibacter actinomycetemcomitans, Aggregatibacter aphrophilus, Aggregatibacter paraphrophilus, Cardiobacterium spp., Eikenella corrodens* and *Kingella spp.*), the group of bacteria which are implicated in bacteremia and infective endocarditis (1,2,4).

H. parainfluenzae has been implicated for other various infections with its pathogenic attributes (4). Other disease conditions related to *H. parainfluenzae* include cellulitis, myositis (5), biliary tract infections (6), meningitis (7), septic arthritis (8,9), neonatal sepsis (10), peritonitis (11), and acute exacerbation of chronic obstructive pulmonary disease (12,13). It can cause bronchitis, sinusitis, otitis media, infections of prosthetic and native joints, brain abscesses, soft tissue infections, chorioamnionitis in women, hepatic infections, ophthalmic infections (13,14) and urinary tract infections through oro-genital transmission (15).

Sound knowledge on the normal salivary flora in humans is essential to understand microbial dysbiosis and the overgrowth of pathobiont in opportunistic infections (1). Open-ended sequencing techniques, especially direct whole-genome shotgun metagenomic sequencing, have revealed that *Haemophilus* can be a component of the salivary microbiome. Furthermore, multiple biotypes of *H. influenzae* and *H. parainfluenzae* have been isolated from saliva samples of patients with lower respiratory tract infections (2).

Microbial dysbiosis due to chronic inflammation in tumour micro-environment has been implicated to play a role in the aetiopathogenesis of colon, gastric, esophageal, pancreatic, breast, and gall bladder carcinomas (16). Oral cancer poses a major public health threat to low and lower-middle-income countries, like Sri Lanka, ranking as the number one cancer among males and among the top ten cancers among females (17,18). With the advancement of omics studies: metagenomics, metatranscriptomic, and metaproteomic studies, it is well evident that the dysbiotic, inflammatory bacteriome is associated with Oral Squamous Cell Carcinoma (OSCC) tumor micro-environment with functional redundancy as the elevation of pathobiont due to compromised immune surveillance in tumor micro-environment (19,20). These pathobiont can manipulate host's defense mechanisms for them to thrive well in immune-compromised persons and facilitate the selection of antimicrobial resistant strains (20-22). Consequently, *H.parainfluenzae* has been identified as a pathogen that can cause life threatening infections, not only among immune-compromised, but also among healthy individuals (23).

H.parainfluenzae garnered recognition alongside *Porphyromonas gingivalis, Fusobacterium nucleatum, Capnocytophaga gingivalis, Prevotella melaninogenica, Rothia mucilaginosa, Veillonella parvula and Streptococcus mutans* as the most prevalent and abundant oral bacterial species connected to dysbiosis of oral cancer (24-25). Consequently, in addition to oral and oro-pharyngeal squamous cell carcinomas, *H.parainfluenzae* emerged as a potential non-invasive biomarker for detecting esophageal squamous cell carcinoma offering a more accessible and accurate alternative to current screening methods (26). Further, this bacterial species comprises the comprehensive oral microbiome profile during the cancer progression from early to late stages, thus demonstrating an inversely associated relative abundance with cancer progression (27). As *H. parainfluenzae* is categorized to be a health-related commensal in oral microbiome due to its potential role in OSCC, recent *in-vitro* investigations on cancer cell lines revealed differential anti proliferative effect on cancer lines *vs.* the normal control and down regulating CD36, a gene that plays an important role in tumor growth and metastasis. Complicating the picture, *H. parainfluenzae* also demonstrated a mix of pro- and anti-cancer responses, including activation of acute phase response, and pro-inflammatory interleukins signaling (28-29).

Oral microbiome composition is not static across life course and alters by socio-demographic factors, such as gender attributed to sex-specific microbe hormonal interactions (30). Further, age, ethnicity, and environmental factors, such as geographic region influence life styles, dietary habits and risk habits across different populations, consequently influencing oral micro-environment. Alterations of OSCC tumour micro-environment associated oral microbiome gained

much research interest (20,24,25). Nevertheless, host factors affecting oral relative abundance of *H. parainfluenzae* in immune-compromised patients, such as oral cancer patients, potentially having an elevated risk of opportunistic infections remain underexplored. Against this backdrop, this study aimed to quantify *H. parainfluenzae* in OSCC tissues among a cohort of male patients and to compare differences by selected socio-demographic characteristics, life-style-related risk habits, site and periodontal disease indicators.

2. Materials and Methods

2.1 Study Design, Sample Size Calculation and Study Population

This was a sub-set (n=25) of a multi-center study involving selected Oral and Maxillo-Facial (OMF) Units across Sri Lanka representing six provinces; namely, Western, Southern, Sabaragamuwa, North Western, Uva and Central. These numbers were based on a sample size calculation with two-sided significance level of 0.05, power of 90, proportion of controls with exposure = 0.4, proportion of cases with exposure = 0.6 and ratio of sample size =1 (31). The representative sub-set comprised 25 Sinhala males with histologically confirmed OSCC.

2.2 Ethical Approval

Ethical approval for the study was received from the Ethics Review Committee Committee, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka (FRC/FDS/UOP/E/2014/32) and Griffith University Human Research Ethics Committee, Australia (DOH/18/14/HREC). Written informed consent was obtained from each participant as previously described. Administrative approval was obtained from Chief Dental Officer, Ministry of Health, Sri Lanka, Directors of respective hospitals and Oral & Maxillofacial Surgeons of participating OMF units.

2.3 Inclusion Criteria

Males aged ≥ 40 years belonging to Sinhalese ethnicity with histologically confirmed OSCC arising from buccal mucosa or tongue and those who were not on antibiotics for the past two months from the day of data collection comprised the inclusion criteria. In past microbiome studies, patients who were not on antibiotics for past 1 month to 3 months were included to detect microbiome profiles (32). Thus, patients who were on antibiotics for > 2 months were included in this study, assuming that original flora was established after 2 months. This was assessed by recall and cross-checked with clinical records.

Exclusion criteria included age less than 40 years, ethnicities other than Sinhalese, those who were on antibiotics within the past two months from the day of data collection and OSCC in sites other than buccal mucosa and tongue as well as with lack of histological confirmation.

2.4 Tissue Sampling

Deep tissue samples (~100 mg each) were dissected from each incisional biopsy of OSCC, avoiding contamination from the tumor surface. The samples were then stored at −80 °C (33).

2.4.1 DNA Extraction and 16S rRNA Sequencing

DNA was extracted using the Gentra Puregene Tissue kit (Qiagen) according to the manufacturer's protocol for solid tissue, with minor modifications. Those modifications included incubation in the lysis buffer performed overnight, an additional lysis step using 50 units of mutanolysin at 37 ^{0} C for 1.5 hours to digest cell wall of Gram positive bacteria prior to the addition of proteinase K.

The V1 to V3 region of the 16S r RNA gene was amplified for sequencing using the degenerate primers 27FYM (5′-AGAGTTTGATCMTGGCTCAG-3′) and 519R (5′-GWATTACCGCGGCKGCTG-3′). Library preparation, indexing, and sequencing were conducted at the Australian Centre for Eco-genomics (University of Queensland, Australia) using v3 2 × 300 bp chemistry on an Illumina MiSeq platform (33).

2.4.2 Sequencing, Data Processing and Taxonomy Assignment up to Species

Data was deposited at Sequence Read Archive under project no. PRJNA415963. Pre-processing of data-including primer trimming, merging, quality filtration, alignment, and chimera check were performed with the exception of using a less stringent Q-score average cut-off for the sliding 50-nucleotide window (30 instead of 35). The high-quality nonchimeric merged reads were classified with the prioritized species-level taxonomy assignment algorithm as implemented by Al-Hebshi et al. (34-35). Each read was BLASTN searched against 4 databases of 16S r RNA gene reference sequences at

alignment coverage and percentage identity ≥98% and then assigned species-level taxonomy of the hit with highest percentage identity and bit score, and belonging to the highest-priority reference set. Reads with no matches at the set cut-offs were subject to de novo operational taxonomic unit calling and assigned to the closest species.

2.5 Socio-demographic and Clinical Data Collection, Clinical Oral Examination and Statistical Analysis

An interviewer-administered questionnaire was developed for the main study and pre-tested to collect information on socio-demographic characteristics, life-style-related risk habits and oral hygiene practices. Socio-demographic characteristics include age, gender, residential area, educational attainment, occupation, and ethnicity, Information on risk habits included frequency and duration of betel quid chewing, smoking and alcohol consumption. Further, number of betel quids chewed per day, number of cigarettes and other types of smoked tobacco used and quantity of alcohol consumed per week in bottles were collected. Pre-testing allowed to re-phrase the questions to improve clarity and understanding. However, for this analysis restricted to 25 Sinhalese male OSCC patients, it was not possible to use all the collected data within the scope of the study.

A clinical oral examination was conducted by a Dental Public Health Specialist who was versed in such assessments. Oral hygiene status was assessed using Simplified Oral Hygiene Index (S-OHI) by (Green & Vermillion, 1964) and periodontal disease status was assessed by periodontal disease classification of (Page & Eke, 2007) (36). As a single examiner conducted clinical examinations, there was no inter-examiner variability and every 10th patient was re-examined to minimize inter-examiner variability.

Data entry and analysis were carried out using SPSS-26, a statistical software package. The percent average relative abundance of *H. parainfluenzae* in OSCC tissues was used to quantify the bacterial species. It was also used as the cut-off point to dichotomize the occurrence of *H. parainfluenzae* in OSCC tissues as < percent average relative abundance and ≥ percent average relative abundance. Occurrences were compared by age group, educational attainment, frequencies of betel quid chewing, smoking, and alcohol consumption, site of the lesion, oral hygiene status and periodontal disease status by Fisher's exact test of statistical significance.

3. Results

3.1 Distribution of OSCC Patients by OMF Units and Their Socio-demographic Profile

As this study was a multi-center study, the distribution of OSCC patients by their respective OMF Unit locations is demonstrated in Table 1. Notably, 24.0% of the cases were recruited from the Colombo National Dental Hospital (Teaching) in Sri Lanka. Additionally, 20% of OSCC cases were recruited from the District General Hospital in Kegalle. As shown in Table 2, OSCC patients were males belonging to Sinhalese ethnicity and low socio-economic status.

Table 1: Distribution of OSCC patients by OMF unit location

Variable	Cases n=25 N	%
OMF Unit location		
Colombo-National Dental Hospital (Teaching) Sri Lanka*	6	(24.0)
Base Hospital Panadura	3	(12.0)
District General Hospital Kalutara	1	(4.0)
District Hospital Kegalle	5	(20.0)
Teaching Hospital Ratnapura	3	(12.0)
Teaching Hospital Karapitiya	2	(8.0)
Teaching Hospital Badulla	4	(16.0)
Teaching Hospital Kandy	1	(4.0)
Total	**25**	**(100.0)**

* Formerly Dental Institute.

Table 2: Socio-demographic profile of OSCC patients

Variable	
Age mean ± SD in years	61.00±9.50
Age range	40-83 years
Gender	N %
Male	25 (100.0)
Ethnicity	
Sinhalese	25 (100.0)
Level of Education	N %
No Schooling	4 (16.0)
Primary Education	9 (36.0)
Secondary Education	5 (20.0)
Above Secondary Education	7 (28.0)
Total	**25 (100.0)**
Occupation	N %
Farmer	13 (52.0)
Skilled/unskilled manual categories	10 (40.0)
Clerical/Professional	2 (8.0)
Total	**25 (100.0)**

3.2 Average Relative Abundance of *H. parainfluenzae* in OSCC Tissues

As depicted in Figure 1, the percentage of average relative abundance of *H. parainfluenzae* in OSCC tissues was 2.99% and when the sample was dichotomized as < percentage average relative abundance and ≥percentage average relative abundance, the occurrence of *H. parainfluenzae* in OSCC tissues was 80.0% and 20.0%, respectively.

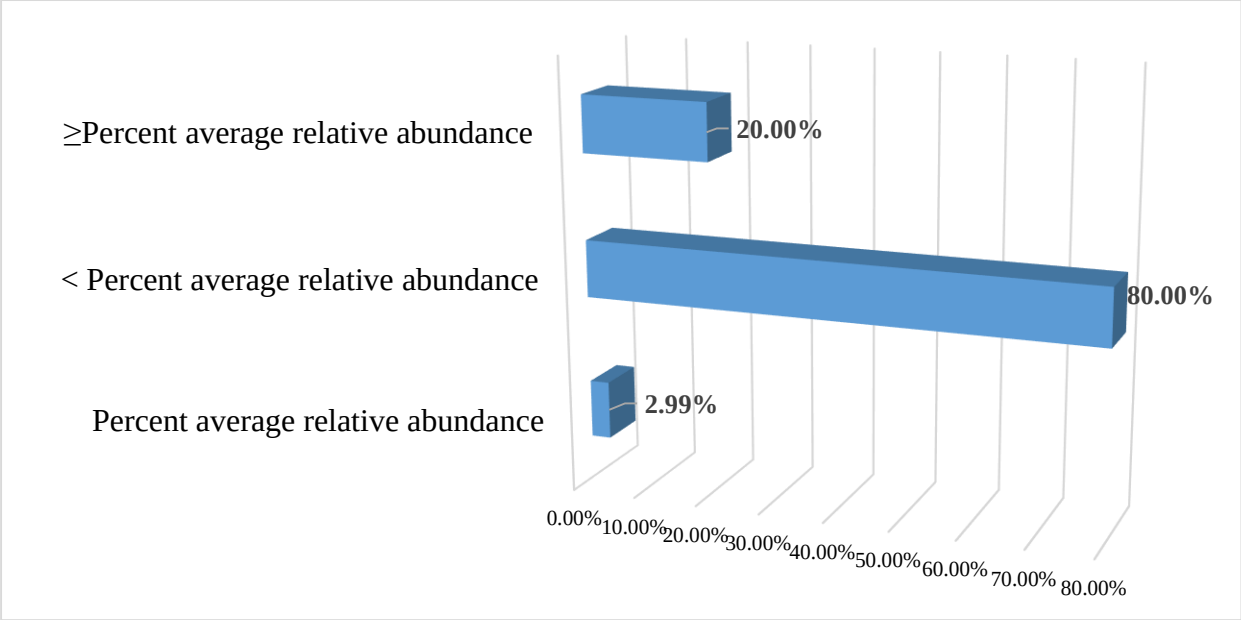

Figure 1: *H. parainfluenzae* quantification by percent average relative abundance and assessment of its occurrence in OSCC tissues of male patients

3.3 Comparison of Occurrence of *H. parainfluenzae* in OSCC Tissues by Selected Socio-demographic Factors. Frequency of Life Style Risk Habits, Site of the Lesion and Periodontal Disease Indicators

Table 3 illustrates a comparison of occurrence of *H. parainfluenzae* in OSCC tissues by age group, educational attainment, frequenting betel quid chewing, smoking and alcohol consumption of male patients. There were no statistically significant differences in the occurrence of *H. parainfluenzae* categorized as < percent average relative abundance and ≥percent average relative abundance by any of those factors (p>0.05).

Table 3: Occurrence of *H. parainfluenzae* by selected socio-demographic factors and frequency of risk habits among OSCC patients

Variable	**<2.99%**		**≥ 2.99%**		**p-value***
Age group	**N**	**%**	**N**	**%**	0.62
40-59 years	9	(45.0)	1	(20.0)	
60-83 years	11	(55.0)	4	(80.0)	
Total	**20**	**(100.0)**	**5**	**(100.0)**	
Educational attainment					0.28
Up to secondary education	13	(65.0)	5	(100.0)	
Above secondary education	7	(35.0)	0	(0.0)	
Total	**20**	**(100.0)**	**5**	**(100.0)**	
Frequency of betel quid chewing					0.14
Past	5	(15.0)	0	(0.0)	
Sometimes	0	(0.0)	1	(20.0)	
Daily	15	(75.0)	4	(80.0)	
Total	**20**	**(100.0)**	**5**	**(100.0)**	

Frequency of smoking					0.3
Never	3	(15.0)	1	(20.0)	
Past	4	(20.0)	3	(60.0)	
Sometimes	4	(20.0)	0	(0.0)	
Daily	9	(45.0)	1	(20.0)	
Total	**20**	**(100.0)**	**5**	**(100.0)**	
Frequency of alcohol consumption					
Never	1	(5.0)	0	(0.0)	0.72
Past	4	(20.0)	2	(40.0)	
Sometimes	3	(15.0)	1	(20.0)	
Daily	12	(60.0)	2	(40.0)	
Total	**20**	**(100.0)**	**5**	**(100.0)**	

** Fisher's exact test.*

Table 4 depicts a comparison of occurrence of *H. parainfluenzae* in OSCC tissues by site of the lesion, oral hygiene status and periodontal disease status. Statistically significant differences in the occurrence of *H.parainfluenzae* categorized as < percent average relative abundance and ≥percent average relative abundance were not evident by any of those factors ($p>0.05$).

Table 4: Occurrence of *H. parainfluenzae* by site of the lesion, oral hygiene status and periodontal disease status and frequency of risk habits among OSCC patients

Variable	**< 2.99%**		**≥ 2.99%**		**p-value***
Site of the lesion	**N**	**%**	**N**	**%**	1.00
Buccal mucosa	13	(65.0)	4	(80.0)	
Tongue	7	(35.0)	1	(20.0)	
Total	**20**	**(100.0)**	**5**	**(100.0)**	
**Oral hygiene status					0.73
Good	2	(10.0)	0	(0.0)	
Fair	14	(70.0)	3	(60.0)	
Poor	4	(20.0)	2	(40.0)	
Total	**20**	**(100.0)**	**5**	**(100.0)**	
***Periodontal disease status					0.10
Mild	3	(15.0)	1	(20.0)	
Moderate	7	(35.0)	4	(80.0)	
Severe	10	(50.0)	0	(0.0)	
Total	**20**	**(100.0)**	**5**	**(100.0)**	

* Fisher's exact test.
** Greene JC, Vermillion JR. The simplified oral hygiene index. J Am Dent Assoc.1964;68:7-13.
*** Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. J Periodontol.2007;78:1387-1399.

4. Discussion

To the best of the knowledge of the authors, this study marks the first study to unravel the average relative abundance of *Haemophilus parainfluenzae* in OSCC tissues with the comparison of its occurrence by selected host factors, such as socio-demographic profile, risk habits, site of the lesion, oral hygiene status and periodontal disease status. Oral cancer dominated by OSCC poses a significant public health challenge to Sri Lanka as the number one cancer among males disproportionately affecting low socio-economic groups engaging in life-style related habits, such as betel quid chewing, smoking and alcohol consumption (17,18). Our findings corroborated the typical profile of oral cancer patients in Sri Lanka (17,18). By histological types, 48% were well differentiated squamous cell carcinomas and the rest belonged to moderately differentiated squamous cell carcinomas.

H. parainfluenzae as a generalist in the oral cavity, thus occupying multiple niches in high abundance, such

as supra gingival plaque, dorsum of the tongue, keratinized gingivae and saliva (3), gained recognition as a health related bacterial species in OSCC. Nevertheless, it demonstrates a mix of pro-and anti-cancer responses (29,30). Therefore, further *in-vitro* studies are much warranted to unveil the pathogenic mechanisms of this oral commensal with the potential to be converted into an opportunistic pathogen in healthy persons, especially among immune-compromised patients, like patients with advanced oral cancers undergoing treatment modalities, such as radiotherapy and chemo-therapy.

Our findings demonstrated a percentage relative abundance of 2.99% of *H. parainfluenzae* in OSCC tissues involving buccal mucosa and tongue of male patients. Further, the occurrence of this bacterial species in 80% of OSCC samples was less than 2.99%. Hence, it is prudent to argue that this finding is an indication of possible dysbiosis related reduction in relative abundance. Given the absence of comparative healthy control group in our study, it is not possible to compare the relative abundances and occurrences of *H. parainfluenzae* in OSCC tissues of those patients. Moreover, methodological variations among studies with regard to sample type, site, 16S RNA region and quantification methods could make the comparisons between studies quite difficult.

As this study included frozen biopsy tissues, comparing the findings with those that have used other types of samples, such as saliva, oral swabs and oral rinses, may not be optimal. Despite such concerns, our findings corroborated the findings of similar studies conducted elsewhere. For example, an Indian study assessed bacterial communities in saliva of eight oro-pharyngeal and seven hypo-pharyngeal cancer patients as compared to healthy controls using 16S r RNA gene V3–V4 region sequencing. As emerged from the findings, *H. parainfluenzae* was among bacterial species that demonstrated a higher abundance in oro- and hypo-pharyngeal cancer patients compared to healthy controls, thus gaining recognition to be a non-invasive diagnostic bio-marker for oro- and hypo-pharyngeal cancer patients (37). Another study was conducted among HPV + ve and –ve Head & Neck Cancer (HNC) patients and healthy controls in France. Saliva samples and swabs of buccal mucosa, supragingival plaque, and tongue were collected from HNC patients (N = 23 patients, n = 92 samples) prior to cancer treatment. Next-generation sequencing (16S-r RNA gene V3–V4 region) was used to determine bacterial taxa relative abundance. Findings reported a higher abundance of *H. parainfluenzae* in both groups (38).

In contrast, another study found higher abundance of *H. parainfluenzae* among healthy controls compared to OSCC patients using real time q PCR assay of oral rinse samples (39). Corroborating those findings, a study conducted in Taiwan employing oral rinse from 51 healthy individuals and 197 OSCC patients at different stages by 16S r RNA gene V3-V4 amplicon sequencing revealed an inverse association of *H. parainfluenzae* with OSCC progression (27).

OSCC patients in Sri Lankan context typically present older males from lower educational attainment, practicing life-style related risk habits whilst carrying a high burden of periodontal disease compounded by poor oral hygiene. In the light of combined evidence on basic tumour immunology as well as innate and adoptive defense mechanism, it is reasonable to speculate that OSCC tumour micro-environment is a terrorizing war front whilst providing a sanctuary for opportunistic pathogens or pathobiont, like *H. parainfluenzae* (20). *H. parainfluenzae* seems the origin and reservoir for the dissemination of β-lactamase-carrying plasmids to other bacterial species (23). Nevertheless, the molecular mechanisms of anti-microbial resistance to several commonly prescribed antibiotics have not been fully disclosed. It has been demonstrated that transferable resistant determinants harbored by this bacterium can be spread to more clinically significant bacteria (40). Extensively drug resistant urethral colonization of *H. parainfluenzae* has been reported (41). Our findings suggest the possible threat of selective proliferation of *Haemophilus parainfluenzae* in OSCC patients which might increase the risk of emergence of potential multidrug-resistant community-acquired and healthcare-associated infections. Anti-microbial resistance (AMR) poses a major public health threat and an economic burden around the world (22-23). Emergence of multi-drug resistant strains of *H. parainfluenzae* can cause life-threatening conditions in both immune-compromised and healthy individuals (2, 3, 23). Therefore, our preliminary findings having important clinical and epidemiological implications urge for further research. Further, as *H. parainfluenzae* has garnered recognition as a non-invasive diagnostic and prognostic biomarker of OSCC, extensive

investigations in temporal changes in its relative abundance assessed by cohort studies on OSCC progression could be recommended.

Whilst many studies have quantified *H. parainfluenzae* in OSCC patients compared to healthy controls, a notable research gap remains in host factors associated with average relative abundance of this bacterium. A meta genomic study on microbiome alterations among betel quid chewers based on cotton swabs of hard and soft palate, buccal mucosa, tongue and sub-gingival region V3-V4 region of 16s r RNA gene revealed 11.0% percent average relative abundance of *H. parainfluenzae* among past or occasional betel quid chewers (42). Another study suggested a strain-specific pathogenic role of *H. parainfluenzae* in intestinal inflammation, thus highlighting the potential effect of periodontal disease on intestinal colonization by pathogenic *H. parainfluenzae* strains in patients with Crohn's Disease with periodontitis (43). As oral cavity is the entry point of the gastro-intestinal tract, the impact of high burden of periodontitis on possible intestinal colonization of pathogenic strains of this bacterium among OSCC patients remains under-explored.

Age and educational attainment were selected as socio-demographic variables which are related to duration of risk habits, oral hygiene practices and cumulative burden of periodontal disease which might impact relative abundance of *H. parainfluenzae*. Dorsum of the tongue is known to be an oral site of high abundance of this bacterium (3). Therefore, this analysis attempted to explore possible associations of selected host factors: socio-demographic profile, site of the lesion, life-style-related risk habits and periodontal indicators that provided a rationale with *H.parainfluenzae* relative abundance. Nevertheless, the small sample size did not allow the detection of such associations. Hence, further research with large sample sizes is much warranted to generate more conclusive evidence.

This study has the strength of being a multi-center study that included 9 OMF units across 6 out of 9 provinces in Sri Lanka. However, geography could have affected oral micro-biome, but exploring this aspect is beyond the scope of this study. Small sample size and lack of a matched comparison group could be identified as the main limitations of the present study. Further, as this study included incisional biopsies and there was no completed patient data on staging of OSCC, it was not possible to make comparisons by stage of the OSCC. Nevertheless, our preliminary findings garnered new insights into conducting further research with methodological rigour to explore host factors associated with average relative abundance of *H. parainfluenzae* among OSCC patients compared to matched healthy controls.

5. Conclusions

Within the limitations of this study, the percent average relative abundance of *H. parainfluenzae* in OSCC tissues of Sri Lankan male patients was 2.99%. The occurrence of this bacterial species linked to oral cancer dysbiosis in the overwhelming majority of samples was less than 2.99% (percent average relative abundance). Further, the occurrence of *H. parainfluenzae* was not significantly differed by age group, educational attainment, frequencies of betel quid chewing, smoking, and alcohol consumption, site of the lesion, oral hygiene status and periodontal disease status of the OSCC patients. Given the risk of serious extra oral infection and selective potential of emergence of multi-drug-resistant strains attributed to *H. parainfluenzae* among OSCC patients having impaired immune status, further studies are warranted in this regard. Larger sample sizes and matched comparisons with healthy controls would enhance methodological rigour to generate more conclusive evidence on host factors associated with relative abundance of *H. parainfluenzae*.

Acknowledgements

The authors wish to sincerely acknowledge the late Emeritus Professor Newell W. Johnson from the School of Dentistry and Oral Health, Menzies Health Institute Queensland, Griffith University, Griffith, Queensland, Australia, for his valuable inputs for this study.

Conflict of Interests

There is no actual or potential conflict of interests to be declared by the authors regarding the present manuscript.

Funding Information

There was no funding source for the present manuscript.

References

1. Hasan NA, Young BA, Minard-Smith AT, Saeed K, Li H, et al. Microbial community profiling of human saliva using shotgun metagenomic sequencing. PLoS One. 2014;9:e97699.
2. Janda WM. Update on the HACEK group of fastidious gram-negative bacilli-Part I. Clin Microbiol Newsl. 2013;35:87-92.
3. de Palma TH, Powers C, McPartland MJ, Mark Welch J, Ramsey M. Essential genes for *Haemophilus parainfluenzae* survival and bio-film growth. mSystems. 2024;9:e0067424.
4. Sen Yew H, Chambers ST, Roberts SA, Holland DJ, Julian KA, et al. Association between HACEK bacteraemia and endocarditis. J Med Microbiol. 2014;63:892-895.
5. Owensby JE, Elliott S, Tu K, Hernandez JE. Cellulitis and myositis caused by Agrobacterium radiobacter and *Haemophilus parainfluenzae* after influenza virus vaccination. South Med J. 1997;90:752-754.
6. Frankard J, Rodriguez-Villalobos' H, Struelens MJ, Jacobs F. *Haemophilus parainfluenzae*: An under-diagnosed pathogen of biliary tract infections? Eur J Clin Microbiol Infect Dis. 2004;23:46-8.
7. Cardines R, Giufrè M, Ciofi degli Atti ML, Accogli M. *Haemophilus parainfluenzae* meningitis in an adult associated with acute otitis media. New Microbiol. 2009;32:213-215.
8. Carey TW, Jackson K, Roure R, Abell BE. Acromioclavicular septic arthritis: A case report of a novel pathogen. Am J Orthop. 2010;39:134-136.
9. Mora A, Marimón I, Mesquida J, Pérez A. *Haemophilus parainfluenzae* septic arthritis: Report of a case and review of the literature. Enferm Infecc Microbiol Clin. 2011; 29:472-473.
10. Govind B, Veeraraghavan B, Anandan S, Thomas N. *Haemophilus parainfluenzae*: Report of an unusual cause of neonatal sepsis and a literature review. J Infect Dev Ctries. 2012; 6:748-750.
11. Gardenier JC, Sawyer RG, Sifri CD, Brayman K, Wispelway B, et al. Peritonitis caused by *Haemophilus parainfluenzae*, leifsonia aquatica, and gordonia spp. in a patient undergoing continuous ambulatory peritoneal dialysis. Surg Infect. 2012;13:409-412.
12. Ye F, He LX, Cai BQ, Wen FQ, Chen BY, et al. Spectrum and anti-microbial resistance of common pathogenic bacteria isolated from patients with acute exacerbation of chronic obstructive pulmonary disease in mainland of China. Chin Med J (Engl.). 2013; 126:2207-2214.
13. Kosikowska U, Biernasiuk A, Rybojad P, Łoś R, Malm A. *Haemophilus parainfluenzae* as a marker of the upper respiratory tract microbiota changes under the influence of pre-operative prophylaxis with or without postoperative treatment in patients with lung cancer. BMC Microbiol. 2016;16:62.
14. Khauv P, Turner P, Soeng C, Soeng S, Moore CE, et al. Ophthalmic infections in children presenting to Angkor Hospital for Children, Siem Reap, Cambodia. BMC Res Notes. 2014;7:784.
15. Saxena A, Dawar R, Bora U. *Haemophilus parainfluenzae* urethritis through orogenital transmission. Indian J Sex Transm Dis AIDS. 2018;39:127-129.
16. Sheflin AM, Whitney AK, Weir TL. Cancer-promoting effects of microbial dysbiosis. Curr Oncol Rep. 2014;16:406.
17. Cancer Incidence & Motality Data Sri Lanka 2021. National Cancer Control Programme, Cancer Incidence and Mortality in Sri Lanka 2021.Pdf (Health.Gov.Lk).
18. Perera I, Amarasinghe H, Jayasinghe RD, Udayamalee I, Jayasuriya N, et al. An overview of the burden of oral cancer in Sri Lanka and its inequalities in the face of contemporary economic and social malaise. Community Dent Oral Epidemiol. 2023;51:680-696.
19. Perera M, Al-Hebshi NN, Speicher DJ, Perera I, Johnson NW. Emerging role of bacteria in oral carcinogenesis: A review with special reference to perio-pathogenic bacteria. J Oral Microbiol. 2016;8:32762.
20. Perera M., Perera I., Tilakaratne WM. Oral micro-biome and oral cancer. Immunology for Dentistry. 2020:79-99.
21. Bank, W. Drug-resistant infections: A threat to our economic future. World Bank, Washington, DC, USA. March 2017.
22. Bartlett JG., Gilbert DN, Spellberg B. Seven ways to preserve the miracle of anti-biotics. Clin. Infect. Dis. 2013;56:1445-1450.
23. Abotsi RE, Govinden U, Essack SY. Mechanisms of antibiotic resistance in *Haemophilus parainfluenzae*. S Afr J Infect Dis. 2017;32:111-114.
24. Mager DL, Haffajee AD, Devlin PM, Norris CM, Posner MR, et al. The salivary microbiota as a diagnostic indicator of oral cancer: A descriptive, non-

randomized study of cancer-free and oral squamous cell carcinoma subjects. J Transl Med. 2005;3:27.

25. Zhao H, Chu M, Huang Z, Yang X, Ran S, et al. Variations in oral microbiota associated with oral cancer. Sci Rep. 2017;7:1-10.
26. Yu Y, Xia L, Wang Z, Zhu T, Zhao L, et al. A cross-cohort study that identifies potential oral microbial markers for esophageal squamous cell carcinoma. iScience. 2024;27:111453.
27. Yang CY, Yeh YM, Yu HY, Chin CY, Hsu CW, et al. Oral microbiota community dynamics associated with oral squamous cell carcinoma staging. Front Microbiol. 2018;9:862.
28. Baraniya D, Jain V, Lucarelli R, Tam V, Vanderveer L, et al. Screening of health-associated oral bacteria for anti-cancer properties *in vitro*. Front Cell Infect Microbiol. 2020;10:575656.
29. Baraniya D, Chitrala KN, Al-Hebshi NN. Global transcriptional response of oral squamous cell carcinoma cell lines to health-associated oral bacteria: An *in vitro* study. J Oral Microbiol. 2022;14:2073866.
30. Liu X, Tong X, Jie Z, Zhu J, Tian L, et al. Sex differences in the oral microbiome, host traits, and their causal relationships. iScience. 2022;26:105839.
31. Kelsey JL, Whitmore AS, Evans AS, Thompson WD. Methods in observational epidemiology. 2nd edition, 1996. Oxford University Press, USA.
32. Pushalkar S, Ji X, Li Y, Estilo C, Yegnanarayana R, et al. Comparison of oral microbiota in tumor and non-tumor tissues of patients with oral squamous cell carcinoma. BMC Microbiol. 2012;12:144.
33. Perera M, Al-Hebshi NN, Perera I, Ipe D, Ulett GC, et al. Inflammatory bacteriome and oral squamous cell carcinoma. J Dent Res. 2018;97:725-732.
34. Al-Hebshi NN, Nasher AT, Idris AM, Chen T. Robust species taxonomy assignment algorithm for 16s rRNA NGS reads: Application to oral carcinoma samples. J Oral Microbiol. 2015;7:28934.
35. Al-Hebshi NN, Nasher AT, Maryoud MY, Homeida HE, Chen T, et al. Inflammatory bacteriome featuring *Fusobacterium nucleatum* and *Pseudomonas aeruginosa* identified in association with oral squamous cell carcinoma. Sci Rep. 2017;7:1834.
36. Perera M, Al-Hebshi NN, Perera I, Ipe D, Ulett GC, et al. A dysbiotic mycobiome dominated by Candida albicans identified within oral squamous-cell carcinomas. J Oral Microbiol. 2017;9:1385369.
37. Panda M, Rai AK, Rahman T, Das A, Das R, et al. Alterations of salivary microbial community associated with oropharyngeal and hypopharyngeal squamous cell carcinoma patients. Arch Microbiol. 2020;202:785-805.
38. Mougeot J-LC, Beckman MF, Langdon HC, Lalla RV, Brennan MT et al. Haemophilus pittmaniae and Leptotrichia spp. constitute a multi-marker signature in a cohort of human papillomavirus-positive head and neck cancer patients. Front Microbiol. 2022;12:794546.
39. Sawant S, Dugad J, Parikh D, Singh H. Absolute quantitation of oral bacteria involved in oral cancer by real time PCR. Med Microecol. 2021;7:100034.
40. Belay WY, Getachew M, Tegegne BA, Teffera ZH, Dagne A, et al. Mechanism of anti-bacterial resistance, strategies and next-generation anti-microbials to contain anti-microbial resistance: A review. Front Pharmacol. 2024;15:1444781.
41. Tinguely R, Seiffert SN, Furrer H, Perreten V, Droz S, et al. Emergence of extensively drug-resistant *Haemophilus parainfluenzae* in Switzerland. Anti-microb Agents Chemother. 2013;57:2867-2869.
42. Bahuguna M, Hooda S, Mohan L, Gupta RK, Diwan P. Identifying oral microbiome alterations in adult betel quid chewing population of Delhi, India. PLoS One. 2023;18:e0278221.
43. Sohn J, Li L, Zhang L, Genco RJ, Falkner KL, et al. Periodontal disease associated with increased gut colonization of pathogenic *Haemophilus parainfluenzae* in patients with Crohn's disease. Cell Rep. 2023;42:112120.