REVIEW ARTICLE

MICROBIAL DYSBIOSIS AND IMMUNOPATHOGENESIS OF ORAL MICROBIOME IN THE DEVELOPMENT AND PROGRESSION OF ORAL SQUAMOUS CELL CARCINOMA: SYSTEMATIC REVIEW

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Summary

Objective: The purpose of this review is to evaluate the specific bacterial species and their association with oral cancer, particularly in oral squamous cell carcinoma (OSCC)

Methodology: A literature search was done through PubMed, Scopus, and Web of Science databases, and data were extracted according to inclusion criteria. Original studies of 20 articles were included in this review.

Results: A total of 20 articles and 961 samples were included in this review. The mean age was 60.12 ± 7.63, with a significantly higher male predilection (M: F – 2:1) ratio. 16S rRNA sequencing was found to be the most commonly used detection method. Alteration in the oral microbiome was seen with varying degrees of epithelial dysplasia, early & late stages of oral cancer. In OSCC patients, there was an increased abundance of specific microbiomes *like Fusobacterium species, Porphyromonas gingivalis*, and *Prevotella* compared to other species.

Conclusion: From this systematic review, it has been found that the changes in diversity of oral microbiome in cancerous patients than that of healthy patients. In OSCC there is an increased abundance of specific species such as *Fusobacterium* species, *Porphyromonas gingivalis*, and *Prevotella* species.

Key words: *Oral microbiome, Oral cancer, Microbiota, Metagenomics, Systematic review*

Introduction

Oral squamous cell carcinoma (OSCC) is the most prevalent malignant tumor in the head and neck region. It is the sixth most common tumor worldwide and its prognosis and survival rates are poor, the 5 year survival rate is less than 50% [1]. Nowadays, the incidence and mortality rate of oral cancer is increasing among both men and women due to changes in lifestyle and habits.

The etiology of OSCC is multifactorial; tobacco use and alcohol, are the most prevalent risk factors for OSCC, other contributing risk factors are oncogenic viruses, especially Human papillomavirus (HPV), oral microbiota, genetic factors, chronic irritation, poor oral hygiene, and nutritional deficiencies[2]. The development of oral cancer has been potentially influenced by genetic alteration associated with the activation of oncogenes and inactivation of tumor suppressor gene signaling, resulting in uncontrolled proliferation of OSCC cells[3].

Oral microbiome is defined as the collective genome of microorganisms that exist in the oral cavity. Oral cavity is home to a variety of diverse microbiomes, comprising more than 700 species which include bacteria, viruses, fungi, protozoa, and archaea[4].

The oral microbiome plays a role in maintaining a symbiotic relationship with the host, essential for various physiological processes. Dysbiosis or disturbance in homeostasis, has a significant effect on the host immune system, eventually resulting in both local and systemic disorders[5]. The prolonged and persistent colonization and survival of pathogenic microbiota can lead to functional alteration of oral microbial diversity and translocation, which is the initial mechanism for the development of distant carcinomas[6].

Recent studies suggest that bacteria play an important role in the pathogenesis of cancer by the following three mechanisms, chronic inflammation, preventing apoptosis, and production of carcinogenic substances[7]. Microbiome's role in causing cancer has been ignored for a long time until the studies in the early 1990s observed that gastric cancer was caused by *Helicobacter pylori (H. pylori)*[8] .

Followed by other bacteria such as *Salmonella enterica* in colon carcinoma, *Salmonella typhi* in gallbladder carcinoma, *Chlamydia trachomatis* in carcinoma of the cervix and ovaries[9]. *Fusobacterium nucleatum (F. nucleatum),* and *Porphyromonas gingivalis (P. gingivalis*) are the two most common oral bacteria that play an important role in causing oral cancer[10]. These bacteria are classified as Group -1 human carcinogen by 'The International Agency for Research on Cancer and the 'World Health Organization[10].

This systematic review is based on the updated evidence from recent studies published between January 2022 to December 2023, compiles the relationship between the oral microbiome and oral squamous cell carcinoma (OSCC), and also focuses on different

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bacterial genera and their pathogenesis in oral cancers, and also highlights the increased and decreased abundance of certain bacterial species in oral cancer compared to normal samples.

Materials and Methods

Protocol

A systematic literature search was conducted independently and the Preferred Reporting Items for Systematic Reviews and Meta-analyses "PRISMA" guidelines were followed in this systematic review[11].

Research Question

The research question was designed based on the PICO format: "Does OSCC patient have alteration in salivary microbial composition?"

Population

Patients with oral squamous cell carcinoma (OSCC).

Intervention

Microbiome alteration.

Comparison

Healthy individuals or patients without OSCC.

Outcome

Changes in the oral microbiome composition in OSCC patient.

Data Sources And Search Strategy

Records were identified through a literature search in PubMed, SCOPUS, and Web of Science databases. For the search strategy, combining MeSH terms and free text words using Boolean operators such as: Microbiota AND ((oral cancer) OR (Squamous Cell Carcinoma of Head and Neck) OR (oral carcinoma)), "((Carcinoma) OR (Squamous Cell), "OR "(Head and Neck Neoplasms)"AND "(Metagenomics)","(Microbiota)," AND "(Mouth Neoplasms)," OR "(Squamous Cell Carcinoma of the Head and Neck)" were used in articles published from January 2022 to December 2023.

Eligibility Criteria

The inclusion criteria for selection of the article were:

1. Human studies
2. Articles published in English
3. Articles with (minimum of 10 patients or?)10 or more than 10 patients in the study group
4. Clinically and histopathologically diagnosed cases of oral squamous cell carcinoma with well–defined staging and grading

The Exclusion Criteria

1. Narrator review or systematic reviews, meta-analyses, case reports, and series; in vitro studies; in animal studies
2. Studies with less than 10 patients
3. Analysis of oral microbiome in patients affected by OSCC, during or after cancer therapy
4. Studies which are not clinically and histopathologically diagnosed

Literature Screening

A two-step procedure was performed in this literature screening. First, all the recognized citations' titles and abstracts were extracted and preliminarily screened for inclusion in the full-text review. Second, the inclusion and exclusion criteria indicated above were used to determine if entire texts were eligible. The PRISMA flowchart depicts an overview of the literature search and screening processes given in Figure 1.

1. Data Extraction
2. Data extracted from the literature search were: author, country, year, mean age, gender, type of study, study population, cancer stage, risk factor, samples collected, detection method, associated microbiome, α and β diversity, and results.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) software was used for analyzing the data.

Results

Literature Search And Study Selection

In the preliminary search, 360 articles (PubMed – 180, Web of Science = 60 and Scopus = 120) were selected. 250 articles were screened after the removal of duplication. Of these, 133 articles were removed by reviewing titles or abstracts and 117 articles were eligible for full-text view. Papers not in English (n =11), not relevant to the topic (n = 17), Narrative or systematic review, meta-analysis, case report, and series (n = 23), Studies with less than 10 patients (n=7), not clinically and histopathologically diagnosed cases of OSCC (n = 18), no well-defined classification (n =10), insufficient data (n = 10) were excluded, thus a total of 20 articles were finally included in the review. (Figure 1)

Aspects of Included Studies

In total, 20 articles were included in this review published from January 2022 to December 2023. Of this one article was a retrospective study and 19 were prospective studies (including case-control (n =10), cross-sectional (n= 7), and observational study (n = 2) *(Supplementary Table 1).*

Socioeconomic Details

Of the 20 articles, nine were from China, four were from the USA, three were from India, two were from Japan, and one each from Australia and Finland. The overall sample size ranged from 12 to 112 which included 961 cases. The mean age was 60.12 ± 7.63, and in these 656 (68%) were male and 461 (48%) were females. Eighteen studies showed male predilection, whereas in two studies females predominated. Collectively, there was significantly higher male predilection than females leading to a 2: 1 of M: F ratio.

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Among 961 cases, 352 (36.6%) were stage I & II, 258 (26.8%) were stage III & IV, and for 351 (36.6%) the

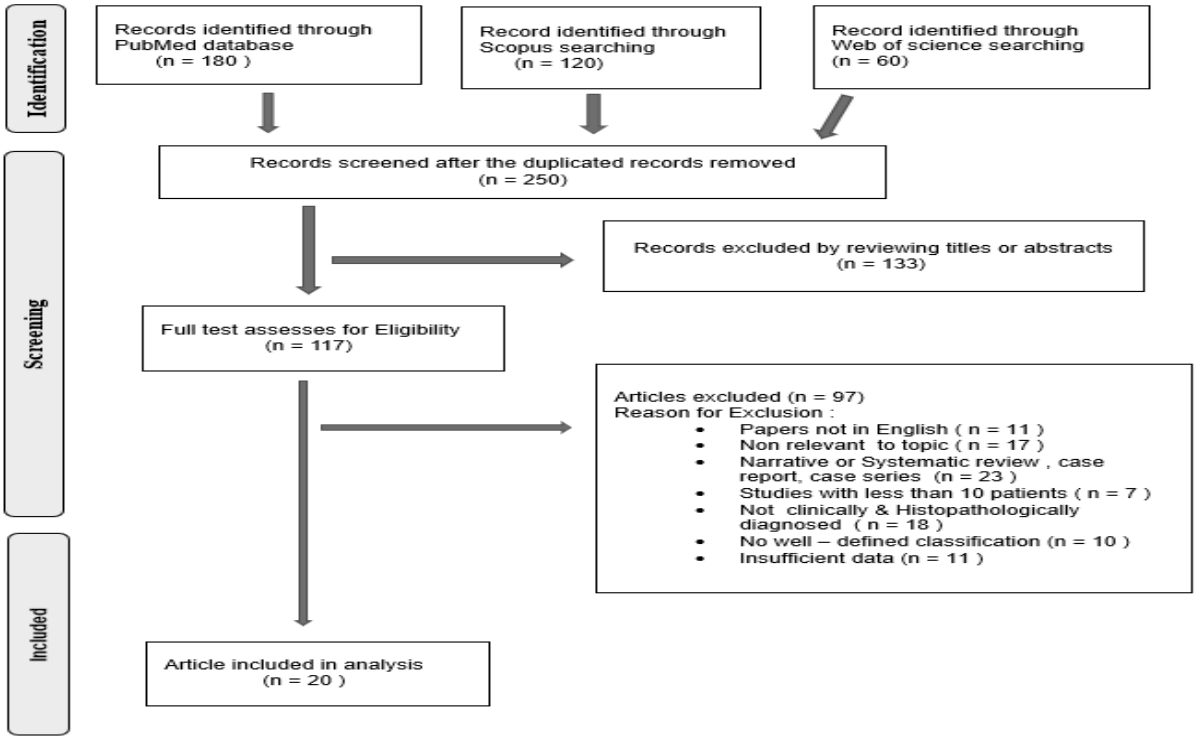

Figure 1: PRISMA figure depicting an overview of the literature search and screening processes

stages were not mentioned. The most commonly involved site was the tongue (n= ,50%), followed by the buccal mucosa (n=,30%), the gingiva (n=,15%), the floor of the mouth (n=, 10%), followed by (5%) each in other sites like the alveolar ridge, hard palate, faucial pillars, and retromolar areas and cancer site was not mentioned in 4 articles (table- 1).

Risk Factors

Major risk factors for oral cancer include alcohol, smoking, tobacco, and betel nut habits. In this review of 961 cases, 438 (45%) had a history of alcohol, 356 (37%) smoking, and 24 (2.49%) used tobacco. For 5 articles, 167 (17.3%) cases the habit history was not mentioned.

Samples and Detection Method

To observe changes in the oral microbiome in oral cancer patients, different types of samples were collected which included, 11 (55%) saliva samples, 8 (40%) tumor tissue samples, 6 (30%) oral swab samples, 2 (10%) tongue, and dental plaque samples. Numerous methods and commercial kits were available for the detection of microbiomes from the samples. In this review, we observed that out of 20 articles, 13 (65%) articles used the 16 S rRNA - V4 sequencing detection method, which is a principal method for microbiome investigation, followed by 9 (25%) articles that used DNA extraction method, other methods like 16 S rDNA sequencing, library construction, amplification, FISH Immunostaining,

Table 1 General characteristics of included OSCC cases

Characteristics	n (%)
Age (mean ± S.D)	**60.12 ±7.63**
Sex	
Male	656 (68.26%)
Female	461 (47.97 %)
Type of Study	
Prospective study -	
Case-control study	10 (50%)
Cross-sectional study	7 (35%)
Observational study	2 (10%)
Retrospective study	1 (5 %)
Cancer stage	
Stage – I	129 (13.42 %)
Stage - II	134 (13.94 %)
Stage - III	52 (5.41 %)
Stage – IV	93 (9.67 %)
Stage – I/II	89 (9.26 %)
Stage – III/IV	79 (8.2 %)
Not mentioned	351 (36.5 %)
Risk factor	
Alcohol	438 (45.57 %)
Tobacco	24 (2.49 %)
Smoking	356 (37.04 %)
Not analysed	5 (0.52 %)
Site	
Buccal Mucosa	10 (50 %)
Tongue	8 (40 %)
Gingiva	15 (75 %)

610(63.5%

Floor of Mouth	7 (35 %)
Alveolar ridge	3 (15 %)
Hard palate	3 (15 %)
Retromolar Trigone ,	1(5%)
Faucial pillars	4 (20 %)
Not mentioned	

PCR each were used in 2 (10%) articles and also shotgun sequencing, gel electrophoresis, whole exome sequencing (WES), whole genome sequencing (WGS), metagenomic sequencing each was used in 1 (5%) article given in table -2.

Table 2 Different samples, detection method, and diversity of included cases

Characteristics	n (%)
Samples examined	
Saliva samples	11 (55 %)
Tissue samples	8 (40 %)
Oral swab	6 (30 %)
Tongue Plaque	2 (10 %)
Dental plaque	2 (10 %)
Detection Method	
16S rRNA Sequencing	13 (65 %)
16 S rDNA Sequencing	3 (15 %)
DNA Extraction	9 (45 %)
RNA Extraction	1 (5 %)
FISH	2 (10 %)
PCR	2 (10 %)
Whole – exome sequencing (WES)	2 (10 %)
Whole genome sequencing (WGS)	1 (5 %)
Shotgun Sequencing	1 (5 %)
Gel Electrophoresis	1 (5 %)
Metagenomic Sequencing	1 (5 %)
Library Construction	2 (10 %)
Diversity	
α – diversity	7 (35 %)
β - diversity	6 (30 %)
Both diversity	3 (15 %)
Not mentioned	2 (10 %)

Microbial Diversity

Diversity was calculated in two ways, namely alpha and beta diversity. Alpha diversity is the diversity occurring within a particular area or ecosystem. In contrast, beta diversity is the comparison of diversity between ecosystems, usually measured as the number of species changes between the ecosystems. In our review, 16 (80%) articles reported a change in diversity between diseased and healthy controls, in 2 (10%) articles there were no significant differences in diversity between the groups[12,13] and in the other 2 (10%) articles diversity between groups was not analyzed.[14,15] Out of 16 articles, 7 reported alpha diversity, 6 articles reported beta diversity and the remaining 4 articles showed changes in both alpha and beta diversity in cancerous samples.[16.17,18,19] Overall, In alpha diversity, 5 articles showed increased richness[20,21,22,23,24], and 4 articles showed decreased richness.[16,19.25,26] Eight articles showed significant changes in beta diversity in cancerous samples.[16,18,19,27,28,29,30,31]

Microbial Abundance

We observed that there was a significant difference in microbial composition between cancerous and non-cancerous patients. Out of 20 articles, 2 articles reported only with *fusobacterium species*, 1 with *prevotella*,1 with *fusobacterium, Actinobacteria*, 1 with *streptococcus and Gamella*, 3 with *streptococcus, Neisseria, Rothia* and *Capnocytophagia*, 12 with other microbiome species such as *Fusobacterium, prevotella, Porphyromonas, streptococcus, Bacteroides, Treponema, Filifactor, Rothia Aggregobacterium, Campylobacter, Leptotrichia, Pasteurellaceae, Velionella.* After combining the result,14 (70%) reported with a higher abundance of *Fusobacterium* at the species level, 8 (40%) of *Prevotella*, 6 (30%) of *Porphyromonas gingivalis*, 4 (20%) of *Peptostreptococcus*, 3 (15%) of *Bacteroides, Parvimonas, Capnocytophagia*, and 5% reported with others bacteria like *Actinobacteria, Treponema, Carnobacterium, Tanerella, Filifactor, Abiotrophia defective, Selemonas, Peptoanaerobacter, Gamella species, Ralstonia, Pedobacter, Aggregobacterium, Campylobacter, Leptotrichia, Pasteurellaceae, Velionella* which showed increased abundance in cancerous patient (figure 2).

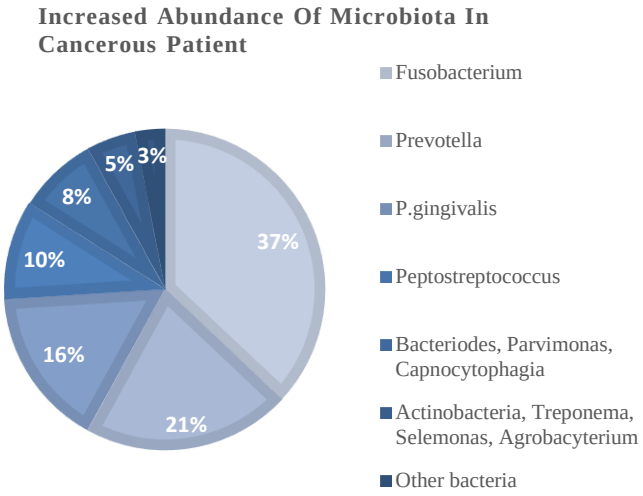

Figure 2: Increased Abundance of Microbiota in the sample of Cancerous patient

Out of 20 articles, 8 (40%) of *Streptococcus* species, 5 (25%) of *Neisseria*, 4 (20%) of *Firmicutes*, and 2 (10%) of *Rothia* showed increased abundance in healthy controls than in oral cancer patients.

Based on the evidence from this review, we observed a quantitatively increased abundance of salivary microbial composition in cancerous patients than in non-cancerous patients. Higher microbial abundance was seen with varying degrees of epithelial dysplasia, early & late stages of cancer, and also in patients with smoking, alcohol, and tobacco habits. *Fusobacterium, P.gingivalis*, and *Prevotella* were the most common

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species that showed higher abundance in all cancerous patients. *Capnocytophagia* gingivalis played an important role in OSCC by promoting OSCC invasion and metastasis. This study shows that OSCC significantly alters the dynamic balance between the host and the resident oral microflora of the oral cavity.

Discussion

Oral squamous cell carcinoma (OSCC) is one of the most common malignancies in the head and neck region. Recent researchers suggest that the oral microbiome plays an important role in the development of oral cancer, particularly OSCC. The oral cavity harbors about 500 - 700 diverse species of microorganisms.[32] The oral microbiome plays a role in maintaining a symbiotic relationship with the host; alteration in the microbial diversity and host - microbial interactions has been reported to be associated with the oral squamous cell carcinoma[5]. However, in this systematic review, our objective was to evaluate the specific bacterial species and their impact on oral cancer, particularly in oral squamous cell carcinoma (OSCC). In the overall comparison of results, the diversity and richness between healthy and tumor tissue showed variations.[33] In our review, changes in microbial diversity were more obvious when comparing cancerous patients with healthy patients.

In the 20 articles reviewed, different samples were collected such as saliva, tissue, oral swabs, and plaques. The type of sample collected may affect the result in evaluating the relationship between oral microbiota and cancer. Salivary samples and oral swabs may have colonizing microbiota from the superficial surface, whereas tissue samples may reveal more significant potential microbiota from a deeper surface.[34] Various factors such as salivary pH, redox potential, and oral hygiene status may influence surface microbial communities. Saliva is the optimal sampling site for acquiring oral microbiota DNA for analysis as it represents the microbiota found in all oral sites and their related diseases, and it is also used for exploring different biomarkers. There is no significant difference in stimulated, unstimulated, and mouth rinses given by Ryutaro et al.[35] Mouth rinse is the most reliable sample for detection in specific patients with low saliva flow and in elderly patients.

The reliability of microbial investigations is primarily dependent on molecular biology techniques. In our review, 16S rRNA gene sequencing and amplification is the principal detection method used. It is cost-effective and it provides gene-level taxonomic classification.[36] In the 16S rRNA technique, V3-V4 regions were the most commonly sequenced region. Along with 16S rRNA gene sequencing other detection techniques such as DNA extraction, 16S rDNA sequencing, shotgun sequencing, FISH Immunostaining, PCR, RNA extraction, gel electrophoresis, whole exome sequencing (WES), whole genome sequencing (WGS), library construction, amplification, metagenomic sequencing were also used.

Oral microbiota such as *Fusobacteria, Firmicutes*, and *Bacteroidetes* were predominant in cancer patients in several studies.[37] From this review, *Fusobacterium, Porphyromonas gingivalis, prevotella,* and *Peptostreptococcus* showed greater abundance in oral cancer patients compared to other bacterial species. Dysbiosis or disturbance in homeostasis, has a significant effect on the host immune system, and eventually results in local and systemic cancer[38]. Various research studies on colorectal and breast cancer focused mainly on *Fusobacterium species.* Recently, the presence of *fusobacterium* has been identified in oesophageal cancer (ESCC).[39] Studies show that it promotes tumor growth, and metastasis, and alters host immune responses. In *fusobacterium* infection, there is chronic inflammation and it also alters the antiapoptotic pathways by inducing NF-kB signaling. It activates β-catenin signaling via IL 6, STAT3, binding to E-cadherin and also through LPS. The wnt transcriptional activity is increased with activation of pro-inflammatory cytokines. FadA is the virulent factor of *fusobacterium* that causes methylation of cyclin-dependent kinase inhibitor 2A (CDKN2A) promoter and alters macrophage infiltration in cancer cells. In addition, it activates p38, resulting in the secretion of Cyclin D1, MMP-9, MMP-13, and the expression of c-myc oncogenes which are involved in tumor invasion and metastasis.[40]

Porphyromonas gingivalis has a malignant potential in oesophageal, gastric, and pancreatic cancer.[41] It is a common oral commensal, proved to be found in OSCC sites. Studies showed that it undergoes chronic inflammation, apoptosis, epithelial-mesenchymal transition (EMT), cell proliferation, and tumor invasion. *Porphyromonas gingivalis* secrete an anti-apoptotic enzyme NDK (Nucleoside diphosphate kinase), modulates ATP / P2X7 – signaling, and produces ROS (Reactive oxygen species). ROS is a key mediator, associated with chronic inflammation and tumor development. *Porphyromonas gingivalis* is NF-B-dependent and produces cysteine proteinases called gingipains, it cleaves the MMP-9 pro-enzyme and activates MMP-9 which promotes tumor cell migration and invasion. In the anti-apoptotic pathway, it inactivates Bad (pro-apoptotic) through Akt / Jak 1 / Stat3 signaling. It also alters the cyclin / CDK (cyclin-dependent kinase) activity by inactivating the p53 tumor suppressor gene.[42]

In this review, other anaerobic bacteria such as *Peptostreptococcus, prevotella, Aggregatibacter*, and *Bacteroides* were also highly abundant in OSCC samples. On the other hand, *Streptococcus, Neisseria, firmicutes*, and *Rothia* showed decreased abundance in OSCC samples when compared to other species. Apart from carcinogenic bacteria, there is insufficient data on the involvement of viruses, parasites, and fungi in oral cancer [6]. There is evidence that the presence of periodontal disease is one of the high-risk factors for the development of OSCC[43]. Inflammation is the link

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between periodontitis and cancer and it is considered to be the seventh hallmark for cancer. In periodontitis, there is an increased release of inflammatory mediators such as cytokines which may promote damage in DNA, thereby causing tumorigenesis.[44] From this review, we additionally found that microbiome can vary according to the degree of dysplasia and stages of cancer. Oral microbiota is comparatively low in mild and moderate dysplasia when compared to severe dysplasia. In the early stage, there is a decreased abundance of microbiota when compared to the late stage of cancer. Increased abundance of microbiota was seen in severe dysplasia and advanced-stage cancer.

Analyzing the results, the oral microbiome in cancerous patients differs from that of healthy patients, and the microbiome may also play an important role in the progression, differentiation, invasion, and metastasis of cancer[45].

Conclusion

Based on the current evidence, we conclude that there is a significant dysbiosis in the oral microbiome which leads to changes in oral microbial diversity in cancer patients and healthy controls. This shows that the oral microbiome plays a significant role in the development and progression of OSCC. This review also highlights that the *Fusobacterium, Porphyromonas gingivalis, Prevotella*, and *Peptosptreptococcus* species showed increased abundance in OSCC than other bacterial species, this microbiome represents a valuable prognostic factor for OSCC.

References

1. Zhou Y, Tang Y, Luo J, Yang Yet al. High expression of HSP60 and survivin predicts poor prognosis for oral squamous cell carcinoma patients. *BMC Oral health*. 2023; 23:629. https://doi.org/10.1186/s12903-023-03311-5
2. Singhania N, Mishra A. Alcohol consumption, tobacco use, and viral infections: a multifactorial approach to understanding head and neck cancer risk. *Int J Appl Health* Care Anal. 2024; 9:44-57.
3. Tan Y, Wang Z, Xu M, Li Bet al. Oral squamous cell carcinomas: state of the field and emerging directions. *Int J Oral Sci*. 2023 Sep 22;15(1):44. https://doi.org/10.1038/s41368-023-00249-w
4. Anju VT, Busi S, Mohan MS, Dyavaiah M. Human Microbiome and the Susceptibility to Infections. In:Probiotics, Prebiotics, Synbiotics, and Postbiotics: human microbiome and human health. 2023 p 117-138. Singapore: Springer Nature https://doi.org/10.1007/978-981-99-1463-0_7
5. Cai L, Zhu H, Mou Q, Wong PY, et al. Integrative analysis reveals associations between oral microbiota dysbiosis and host genetic and epigenetic aberrations in oral cavity squamous cell carcinoma. *NPJ Biofilms Microbiomes*. 2024;10:39. https://doi.org/10.1038/s41522-024-00511-x
6. Sun J, Tang Q, Yu S, Xie M et al. Role of the oral microbiota in cancer evolution and progression. Cancer Med. 2020; 9:6306-63021. https://doi.org/10.1002/cam4.3206
7. Li R, Xiao L, Gong T, Liu J, et al. Role of oral microbiome in oral oncogenesis, tumor progression, and metastasis. *Mol Oral Microbiol*. 2023; 38:9-22.https://doi.org/10.1111/omi.12403
8. Reyes VE. Helicobacter pylori and its role in gastric cancer. *Microorgan*. 2023; 11:1312.https://doi.org/10.3390/microorganisms11051312
9. Romanescu M, Oprean C, Lombrea A, Badescu B, et al. Current state of knowledge regarding WHO high priority pathogens—resistance mechanisms and proposed solutions through candidates such as essential oils: a systematic review. *Int J Mol Sci.* 2023;24:9727. https://doi.org/10.3390/ijms24119727
10. Wang B, Deng J, Donati V, Merali N, et al. The roles and interactions of porphyromonas gingivalis and fusobacterium nucleatum in oral and gastrointestinal carcinogenesis: a narrative review. *Pathog*. 2024; 13:93. https://doi.org/10.3390/pathogens13010093
11. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med*. 2009; 151:264-269. https://doi.org/10.1016/j.ijsu.2010.02.007
12. Zhu H, Yip HC, Cheung MK, Chan HC, et al. Convergent dysbiosis of upper aerodigestive microbiota between patients with esophageal and oral cavity squamous cell carcinoma. *Int J Cancer*. 2023 May 1; 152:1903-1915. https://doi.org/10.1002/ijc.34460
13. Yan K, Auger S, Diaz A, Naman J, et al. Microbial changes associated with oral cavity cancer progression. *Otolaryngol Head Neck Surg.* 2023; 168:1443-1452. https://doi.org/10.1002/ohn.211
14. Li Z, Fu R, Wen X, Wang Q, et al. The significant clinical correlation of the intratumor oral microbiome in oral squamous cell carcinoma based on tissue-derived sequencing. *Front Physiol.* 2023; 13:1089539. https://doi.org/10.3389/fphys.2022.1089539
15. Kaliamoorthy S, Sayeeram SP, SundarRaj S, Balakrishnan J, et al. Investigating the association between fusobacterium nucleatum and oral squamous cell carcinoma: a pilot case-control study on tissue samples. *Cureus*. 2023;15. doi: 10.7759/cureus.47238
16. Haider K, Masooma S, Mehtab M, Ali SM, et al. The role of the oral microbiome in oral cancer pathogenesis. *J Popul Ther Clin Pharmacol*. 2024;

  doi:10.60014/pmjg.v13i2.374

31:285-293. https://doi.org/10.53555/jptcp.v31i1.3988
17. Zhang Z, Feng Q, Li M, Li Z, et al. Age-related cancer-associated microbiota potentially promotes oral squamous cell cancer tumorigenesis by distinct mechanisms. *Front Microbiol*. 2022; 13:852566. https://doi.org/10.3389/fmicb.2022.852566
18. Ganly I, Hao Y, Rosenthal M, Wang H, et al. Oral microbiome in nonsmoker patients with oral cavity squamous cell carcinoma, defined by metagenomic shotgun sequencing. *Cancers*. 2022; 14:6096.https://doi.org/10.3390/cancers14246096
19. Saxena R, Prasoodanan PKV, Gupta SV, Gupta S, et al. Assessing the effect of smokeless tobacco consumption on oral microbiome in healthy and oral cancer patients. *Front Cell Infect Microbiol*. 2022; 12:841465. https://doi.org/10.3389/fcimb.2022.841465
20. Yamamoto Y, Kamiya T, Yano M, Huyen VT, et al. Oral microbial profile analysis in patients with oral and pharyngeal cancer reveals that tumoral fusobacterium nucleatum promotes oral cancer progression by activating yap. *Microorgan*. 2023; 11:2957.https://doi.org/10.3390/microorganisms11122957
21. Zhou J, Wang L, Yuan R, Yu X, et al. Signatures of mucosal microbiome in oral squamous cell carcinoma identified using a random forest model. *Cancer Manag Res*. 2020:5353-5363. doi:10.1126/science.116080919460998
22. Yang J, He P, Zhou M, Li S, et al. Variations in the oral microbiome and its predictive functions between tumorous and healthy individuals. *J Med Microbiol.* 2022; 71:001568. doi 10.1099/jmm.0.001568
23. Nie F, Wang L, Huang Y, et al. Characteristics of microbial distribution in different oral niches of oral squamous cell carcinoma. *Front Cell Infect Microbiol*. 2022; 12:905653. https://doi.org/10.3389/fcimb.2022.905653
24. Hashimoto K, Shimizu D, Ueda S, Miyabe S, et al. Feasibility of oral microbiome profiles associated with oral squamous cell carcinoma. *J Oral Microbiol.* 2022; 14:2105574. https://doi.org/10.1080/20002297.2022.2105574
25. Mäkinen AI, Pappalardo VY, Buijs MJ, Brandt BW, et al. Salivary microbiome profiles of oral cancer patients analyzed before and after treatment. *Microbiome*. 2023; 11:171. https://doi.org/10.1186/s40168-023-01613-y
26. Michikawa C, Gopalakrishnan V, Harrandah AM, Karpinets TV, et al. Fusobacterium is enriched in oral cancer and promotes induction of programmed death-ligand 1 (PD-L1). *Neoplasia*. 2022; 31:100813. https://doi.org/10.1016/j.neo.2022.100813
27. Benjamin WJ, Wang K, Zarins K, Bellile E, et al. Oral microbiome community composition in head and neck squamous cell carcinoma. *Cancers*. 2023; 15:2549. https://doi.org/10.3390/cancers15092549
28. Lan Q, Zhang C, Hua H, Hu X. Compositional and functional changes in the salivary microbiota related to oral leukoplakia and oral squamous cell carcinoma: a case control study. *BMC Oral Health*. 2023; 23:1021. https://doi.org/10.1186/s12903-023-03760-y
29. Liu Y, Li Z, Qi Y, Wen X, et al. Metagenomic analysis reveals a changing microbiome associated with the depth of invasion of oral squamous cell carcinoma. *Front Microbiol.* 2022; 13:795777. https://doi.org/10.3389/fmicb.2022.795777
30. Zhu W, Shen W, Wang J, Xu Y, et al. Capnocytophaga gingivalis is a potential tumor promotor in oral cancer. Oral Diseases. 2024; 30:353-362. https://doi.org/10.1111/odi.14376
31. Pandey D, Szczesniak M, Maclean J, Yim HC, et al. Dysbiosis in head and neck cancer: determining optimal sampling site for oral microbiome collection. *Pathog*. 2022; 11:1550. https://doi.org/10.3390/pathogens11121550
32. Deo PN, Deshmukh R. Oral microbiome: unveiling the fundamentals. J Oral Maxillofac Pathol. 2019; 23:122-128. doi: 10.4103/jomfp.JOMFP_304_18
33. Zhao H, Chu M, Huang Z, Yang X, et al. Variations in oral microbiota associated with oral cancer. *Sci Repo*. 2017; 7:11773. https://doi.org/10.1038/s41598-017-11779-9
34. Gopinath D, Menon RK, Wie CC, Banerjee M, et al. Differences in the bacteriome of swabs, saliva, and tissue biopsies in oral cancer. *Sci Rep*. 2021; 11:1181. https://doi.org/10.1038/s41598-020-80859-0
35. Jo R, Nishimoto Y, Umezawa K, Yama K, et al. Comparison of oral microbiome profiles in stimulated and unstimulated saliva, tongue, and mouth-rinsed water. *Sci Rep*. 2019; 9:16124. https://doi.org/10.1038/s41598-019-52445-6
36. Tedersoo L, Albertsen M, Anslan S, Callahan B. Perspectives and benefits of high-throughput long-read sequencing in microbial ecology. Appl and Environ Microbiol. 2021;87: e00626-21. https://doi.org/10.1128/AEM.00626-21
37. Sufiawati I, Piliang A, Ramamoorthy VR. Oral microbiota in oral cancer patients and healthy individuals: a scoping review. *Dent J Maj Kedokt Gigi* 2022; 55:186-193. doi: 10.20473/j.djmkg.v55.i4.p186–193
38. Ge Y, Wang X, Guo Y, Yan J, et al. Gut microbiota influence tumor development and alter interactions with the human immune system. *J Exp Clin Cancer Res*. 2021; 40:1-9. https://doi.org/10.1186/s13046-021-01845-6
39. Yano Y, Etemadi A, Abnet CC. Microbiome and cancers of the esophagus: a review. *Microorgan*. 2021; 9:1764. https://doi.org/10.3390/microorganisms9081764

  doi:10.60014/pmjg.v13i2.374

40. McIlvanna E, Linden GJ, Craig SG, Lundy FT, et al. Fusobacterium nucleatum and oral cancer: a critical review. *BMC Cancer*. 2021; 21:1-1. https://doi.org/10.1186/s12885-021-08903-4
41. Kong J, Liu Y, Qian M, Xing L, Gao S. The relationship between porphyromonas gingivalis and oesophageal squamous cell carcinoma: a literature review. *Epidemiol Infect.* 2023:1-29. https://doi.org/10.1017/S0950268823000298
42. Singh S, Singh AK. Porphyromonas gingivalis in oral squamous cell carcinoma: a review. *Microbes Infect.* 2022; 24:104925. https://doi.org/10.1016/j.micinf.2021.104925
43. Gopinath D, Menon RK, Veettil SK, Botelho MG, et al. Periodontal diseases as putative risk factors for head and neck cancer: systematic review and meta-analysis. *Cancers*. 2020; 12:1893. https://doi.org/10.3390/cancers12071893
44. Jain P, Hassan N, Khatoon K, Mirza MA, et al. Periodontitis and systemic disorder—an overview of relation and novel treatment modalities. *Pharm.* 2021; 13:1175. https://doi.org/10.3390/pharmaceutics13081175
45. Huang X, Pan T, Yan L, Jin T, et al. The inflammatory microenvironment and the urinary microbiome in the initiation and progression of bladder cancer. *Genes Dis.* 2021; 8:781-97. https://doi.org/10.1016/j.gendis.2020.10.002

  doi:10.60014/pmjg.v13i2.374