Research Article

Periodontal Pathogens In Oral Squamous Cell Carcinoma Tissue: A PCR-Based Case-Control Study

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity, and increasing evidence suggests that periodontal dysbiosis may contribute to its development and progression. Periodontal pathogens may influence carcinogenesis through chronic inflammation, altered immune responses and microbial interactions within the tumour microenvironment.

Aim: To evaluate the prevalence and burden of selected periodontal pathogens in OSCC tissue and to compare them with non-malignant oral tissue using polymerase chain reaction (PCR).

Materials and Methods: This hospital-based case-control study included 40 participants at a tertiary care hospital, comprising 20 histopathologically confirmed OSCC patients and 20 non-malignant controls. Demographic characteristics, tobacco use, smoking, alcohol consumption and periodontal parameters including Plaque Index, Gingival Index, bleeding on probing, probing pocket depth and clinical attachment loss were recorded. Tissue samples were subjected to species-specific PCR for Porphyromonas gingivalis, Fusobacterium nucleatum, Tannerella forsythia, Treponema denticola and Aggregatibacter actinomycetemcomitans. Data were analysed using IBM SPSS Statistics version 27.0, with $p<0.05$ considered statistically significant.

Results: Tobacco chewing was significantly more frequent among OSCC cases than controls (80.00% vs 35.00%; $p=0.010$). All periodontal parameters were significantly higher in OSCC patients ($p<0.001$). Porphyromonas gingivalis was detected in 75.00% of cases versus 35.00% of controls ($p=0.025$), Fusobacterium nucleatum in 80.00% versus 40.00% ($p=0.022$), and Treponema denticola in 55.00% versus 20.00% ($p=0.048$). At least one periodontal pathogen was identified in 95.00% of OSCC tissues compared with 60.00% of controls ($p=0.020$). Two or more pathogens were detected in 85.00% versus 40.00% ($p=0.008$). High pathogen burden was significantly associated with advanced TNM stage ($p=0.019$) and lymph-node positivity ($p=0.020$).

Conclusion: OSCC tissues demonstrated significantly greater periodontal pathogen prevalence and polymicrobial burden than non-malignant tissues. The association of high pathogen burden with advanced stage and nodal involvement suggests that periodontal dysbiosis may be linked with OSCC severity.

Keywords: Oral squamous cell carcinoma; Periodontal pathogens; Fusobacterium nucleatum; Porphyromonas gingivalis; Polymerase chain reaction.

Introduction

Oral squamous cell carcinoma (OSCC) is the predominant histological form of malignancy arising in the oral cavity and remains an important challenge in head-and-neck oncology. It originates from the stratified squamous epithelium of sites such as the tongue, buccal mucosa, gingiva and floor of the mouth. Its development is a multistep process involving genetic and epigenetic alterations together with prolonged exposure to carcinogenic influences. Tobacco in smoked or smokeless forms, areca-nut consumption and alcohol use are major established risk factors, while poor oral hygiene, chronic mucosal irritation and host susceptibility may modify individual risk. Despite advances in treatment, early recognition remains essential because clinical outcome is strongly influenced by disease stage at diagnosis.[1] The oral cavity supports a highly diverse microbial ecosystem distributed across saliva,

tongue, teeth, gingival crevices and mucosal surfaces. Under healthy conditions, these microorganisms maintain a dynamic relationship with local immunity and epithelial barriers. Disturbance of this balance can result in dysbiosis, characterized by altered microbial composition and expansion of potentially pathogenic organisms. Interest in the oral microbiome has increased because microorganisms and their products remain in direct contact with the epithelium from which OSCC develops. Oral bacteria may influence carcinogenesis through chronic inflammation, microbial metabolites, oxidative stress, altered cell signalling and interference with host immune responses.[2] Periodontitis represents an important model of chronic oral dysbiosis. The periodontal pocket provides an ecological niche for anaerobic and Gram-negative microorganisms capable of producing virulence factors that repeatedly interact with surrounding tissues. Persistent periodontal inflammation promotes release of cytokines, reactive oxygen species, proteolytic enzymes and other mediators that may alter epithelial homeostasis. In the tumour microenvironment, sustained inflammatory signalling may facilitate cellular proliferation, angiogenesis, immune evasion and tissue invasion. Consequently, current research increasingly considers the relationship among periodontal disease, microbial imbalance and inflammatory pathways when exploring biological links between oral infection and OSCC.[3] Among the periodontal organisms receiving particular attention are Porphyromonas gingivalis and Fusobacterium nucleatum. Both are important components of periodontal dysbiosis and possess mechanisms capable of modifying epithelial behaviour and host immunity. Other organisms, including Tannerella forsythia, Treponema denticola and Aggregatibacter actinomycetemcomitans, may contribute to a polymicrobial environment that maintains chronic inflammation. Periodontal pathogens can influence cell-cycle regulation, inflammasome activity, DNA-damage responses and signalling pathways relevant to malignant transformation. Nevertheless, detection of these organisms in cancer tissue should not automatically be interpreted as proof of causation, because the altered tumour environment itself may favour colonization by anaerobic bacteria.[4] The biological significance of microbial detection also depends on the type of specimen examined. Saliva is easily obtained and useful for studying the overall oral microbial community, but it contains organisms shed from several anatomical surfaces. Tissue analysis provides a more direct means of determining whether microorganisms are present within or closely associated with the tumour microenvironment. Comparative examination of malignant and non-malignant oral tissues can therefore help distinguish generalized oral colonization from tumour-associated microbial patterns. Analysis of tissue pairs and saliva has emphasized that anatomical sampling site is an important consideration when interpreting microbiome findings in OSCC.[5] Molecular methods have substantially improved the identification of microorganisms that may be difficult to recover by conventional culture. Polymerase chain reaction (PCR) is particularly suitable for targeted microbial investigation because species-specific primers can detect bacterial DNA directly in clinical specimens. When combined with appropriate controls, standardized DNA extraction and strict contamination prevention, PCR offers a practical approach for evaluating selected periodontal pathogens in tissue. Broader sequencing studies have additionally shown that microbial communities vary across tumour tissue, adjacent mucosa, saliva and other oral niches, supporting clearly defined sampling and analytical methods in OSCC microbiome research.[6]

Materials and Methods

This hospital-based case-control study was conducted at a tertiary care hospital to evaluate the presence of selected periodontal pathogens in oral squamous cell carcinoma (OSCC) tissue using polymerase chain reaction (PCR). A total of 40 participants were included and divided into two groups: Group I (Cases) comprised 20 patients with histopathologically confirmed OSCC, and Group II (Controls) comprised 20 individuals without oral malignancy who underwent oral surgical procedures for benign conditions. Cases and controls were selected according to predefined eligibility criteria.

Inclusion Criteria: Adult patients aged ≥18 years with newly diagnosed and histopathologically confirmed primary OSCC who had not received chemotherapy or radiotherapy were eligible for inclusion in the case group. Controls included adults without any clinical or histopathological evidence of oral malignancy who required oral surgical procedures where clinically acceptable normal or non-malignant oral tissue could be obtained. Participants willing to undergo oral and periodontal examination and provide tissue samples were

included.

Exclusion Criteria: Patients who had received chemotherapy, radiotherapy or immunotherapy before sample collection were excluded. Participants with recurrent OSCC, other active malignancies, severe systemic infections, immunodeficiency disorders or prolonged immunosuppressive therapy were also excluded. Individuals who had received systemic antibiotics or professional periodontal therapy within the preceding three months were excluded because these interventions could alter the oral microbial profile.

Clinical and Demographic Assessment: Demographic and clinical information including age, sex, residence, tobacco chewing, smoking, alcohol consumption, oral hygiene practices, relevant systemic diseases and family history of malignancy was recorded using a structured proforma. In OSCC patients, the anatomical site of the lesion, clinical size, histopathological differentiation and tumour-node-metastasis (TNM) stage, wherever available, were documented.

Periodontal Examination: Periodontal status was evaluated using standard clinical parameters. These included Plaque Index (PI), Gingival Index (GI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment loss (CAL). Periodontal probing was performed using a calibrated periodontal probe. PPD and CAL were measured in millimetres at standard sites around the teeth. Periodontal status was categorized according to clinical findings, and the periodontal parameters were subsequently compared with the presence of periodontal pathogens.

Tissue Sample Collection: In the OSCC group, approximately 3–5 mm of fresh tumour tissue was obtained from a viable representative area during diagnostic biopsy or definitive surgical excision while avoiding necrotic tissue. In the control group, non-malignant oral mucosal tissue was collected during clinically indicated oral surgical procedures. Samples were collected under strict aseptic conditions using sterile instruments and transferred immediately into sterile, labelled microcentrifuge tubes containing an appropriate DNA-preservation medium or sterile phosphate-buffered saline. Samples were transported under cold-chain conditions and processed for molecular analysis.

DNA Extraction: Genomic DNA was extracted from tissue specimens using a commercially available DNA extraction kit according to the manufacturer's instructions. Tissue samples were homogenized and subjected to enzymatic lysis, DNA purification and elution. The concentration and purity of extracted DNA were assessed using spectrophotometric measurement, and DNA samples were stored under appropriate refrigerated or frozen conditions until PCR analysis.

PCR Detection of Periodontal Pathogens: Species-specific PCR assays were performed for important periodontal pathogens, including Porphyromonas gingivalis, Fusobacterium nucleatum, Tannerella forsythia, Treponema denticola and Aggregatibacter actinomycetemcomitans. PCR amplification was carried out using specific primers, extracted DNA, PCR master mix and nuclease-free water in a thermal cycler. Each run included an appropriate positive control and a negative control to monitor amplification accuracy and contamination. Amplified PCR products were subjected to agarose gel electrophoresis, stained using an appropriate nucleic-acid stain and visualized under a gel documentation system. Detection of an amplification product of the expected molecular size was considered positive for the respective pathogen. Where feasible, selected samples were tested in duplicate to ensure reproducibility.

Primary and Secondary Outcome Measures: The primary outcome was the prevalence of individual periodontal pathogens in OSCC tissue compared with non-malignant control tissue. Secondary parameters included the presence of multiple periodontal pathogens, total pathogen positivity, association of individual pathogens with periodontal status, tobacco exposure, tumour site, histopathological differentiation and TNM stage. The coexistence of two or more periodontal pathogens within the same tissue specimen was also evaluated.

Statistical Analysis

Data were entered into Microsoft Excel and analysed using IBM SPSS Statistics version 27.0. Continuous variables were expressed as mean ± standard deviation or median with interquartile range according to data distribution, while categorical variables were presented as frequencies and percentages. Normality was assessed using the Shapiro–Wilk test. Independent-samples t-test or Mann–Whitney U test was used for comparison of continuous variables between groups. Chi-square test or Fisher's exact test was applied for categorical variables, including comparison of pathogen prevalence between OSCC cases and controls. Odds ratios with 95% confidence intervals were calculated to estimate the strength of association between

periodontal pathogens and OSCC. A two-tailed p-value <0.05 was considered statistically significant.

Results

A total of 40 participants were included in the study, consisting of 20 patients with histopathologically confirmed oral squamous cell carcinoma (OSCC) in Group I and 20 non-malignant controls in Group II.

Table 1. Comparison of Demographic and Risk-Factor Characteristics Between OSCC Cases and Controls

The mean age of participants in the OSCC group was 54.65 ± 10.72 years, compared with 50.10 ± 11.15 years in the control group. Although the mean age was numerically higher among OSCC cases, the difference between the two groups was not statistically significant (p=0.196). In relation to sex distribution, males constituted 75.00% (15/20) of the OSCC group and 65.00% (13/20) of the control group, whereas females accounted for 25.00% (5/20) and 35.00% (7/20), respectively. The difference in sex distribution was not statistically significant (p=0.731). Regarding place of residence, 60.00% (12/20) of OSCC patients were from rural areas and 40.00% (8/20) were from urban areas. Among controls, 55.00% (11/20) were rural residents and 45.00% (9/20) were urban residents. No statistically significant difference was observed between the groups with respect to residence (p=1.000). Tobacco chewing was reported by 80.00% (16/20) of OSCC patients compared with 35.00% (7/20) of controls. This difference was statistically significant (p=0.010). Smoking was reported in 55.00% (11/20) of OSCC patients and 25.00% (5/20) of controls; however, the difference did not reach statistical significance (p=0.105). Similarly, alcohol consumption was present in 45.00% (9/20) of OSCC cases compared with 20.00% (4/20) of controls, with no statistically significant difference between the groups (p=0.176).

Table 2. Comparison of Periodontal Parameters Between OSCC Cases and Controls

The periodontal examination demonstrated higher mean values for all evaluated periodontal parameters among OSCC cases compared with controls. The mean Plaque Index (PI) was 2.13 ± 0.47 in the OSCC group and 1.48 ± 0.42 in the control group. This difference was statistically significant (p<0.001). Similarly, the mean Gingival Index (GI) was significantly higher among OSCC cases, with a value of 1.92 ± 0.51, compared with 1.31 ± 0.45 among controls (p<0.001). The mean percentage of bleeding on probing (BOP) was 56.40 ± 18.75% in OSCC patients compared with 33.15 ± 14.60% in controls, and the difference was statistically significant (p<0.001). The mean probing pocket depth (PPD) was also greater in the OSCC group, measuring 4.38 ± 0.86 mm, compared with 3.21 ± 0.72 mm in the control group (p<0.001). The mean clinical attachment loss (CAL) was 4.96 ± 1.12 mm among OSCC cases and 3.45 ± 0.91 mm among controls. This difference was also statistically significant (p<0.001).

Table 3. PCR Detection of Periodontal Pathogens in OSCC and Control Tissues

PCR analysis demonstrated differences in the detection frequency of individual periodontal pathogens between OSCC and control tissue samples. Porphyromonas gingivalis was detected in 75.00% (15/20) of OSCC tissues compared with 35.00% (7/20) of control tissues. The difference was statistically significant (p=0.025). Fusobacterium nucleatum was the most frequently detected pathogen among OSCC cases and was identified in 80.00% (16/20) of OSCC tissue samples compared with 40.00% (8/20) of control tissue samples. This difference was statistically significant (p=0.022). Tannerella forsythia was detected in 60.00% (12/20) of OSCC samples and 25.00% (5/20) of control samples. Although the frequency was numerically higher in OSCC tissues, the difference did not reach the conventional level of statistical significance (p=0.054). Treponema denticola was identified in 55.00% (11/20) of OSCC tissues compared with 20.00% (4/20) of control tissues. The difference between the groups was statistically significant (p=0.048). Aggregatibacter actinomycetemcomitans was detected in 30.00% (6/20) of OSCC samples and 15.00% (3/20) of controls, with no statistically significant difference (p=0.451). When overall pathogen positivity was considered, at least one of the selected periodontal pathogens was detected in 95.00% (19/20) of OSCC tissue samples compared with 60.00% (12/20) of control tissue samples. The difference in overall periodontal pathogen detection between the two groups was statistically significant (p=0.020).

Table 4. Comparison of Periodontal Pathogen Burden Between OSCC Cases and Controls

The distribution of participants according to the number of periodontal pathogens detected showed a higher pathogen burden among OSCC cases. No periodontal pathogen was identified in only 5.00% (1/20) of OSCC cases, whereas 40.00% (8/20) of control participants had no detectable pathogen. Detection of only one pathogen was observed in 10.00% (2/20) of OSCC cases and 20.00% (4/20) of controls. Two periodontal pathogens were detected in 25.00% (5/20) of OSCC tissue samples compared with 20.00% (4/20) of control samples. In contrast, three or more periodontal pathogens were detected in 60.00% (12/20) of OSCC cases, whereas this degree of pathogen positivity was observed in only 20.00% (4/20) of controls. The difference in the frequency of three or more pathogens between the two groups was statistically significant (p=0.022). When the microbial burden was categorized as the presence of two or more periodontal pathogens, 85.00% (17/20) of OSCC cases were positive compared with 40.00% (8/20) of controls. This difference was statistically significant (p=0.008).

Table 5. Association of High Periodontal Pathogen Burden With Clinicopathological Characteristics Among OSCC Cases

Among the 20 OSCC patients, high periodontal pathogen burden was defined as the detection of three or more periodontal pathogens. According to TNM stage, 8 patients had Stage I–II disease and 12 patients had Stage III–IV disease. High pathogen burden was detected in 25.00% (2/8) of patients with Stage I–II disease compared with 83.33% (10/12) of patients with Stage III–IV disease. This association was statistically significant (p=0.019). With respect to tumour size, 9 patients had T1–T2 tumours, of whom 33.33% (3/9) showed a high pathogen burden. Among the 11 patients with T3–T4 tumours, 81.82% (9/11) had a high pathogen burden. Although the proportion was higher among patients with larger tumours, the association did not reach statistical significance (p=0.065). Regarding lymph-node status, 10 patients were classified as N0 and 10 patients had lymph-node-positive disease. High pathogen burden was present in 30.00% (3/10) of N0 patients compared with 90.00% (9/10) of patients with positive lymph-node involvement. The association between lymph-node status and high pathogen burden was statistically significant (p=0.020). Histopathologically, 9 patients had well-differentiated OSCC, of whom 33.33% (3/9) demonstrated a high periodontal pathogen burden. Among 11 patients with moderately or poorly differentiated OSCC, 81.82% (9/11) had a high pathogen burden. The difference was not statistically significant at the 5% level (p=0.065). Tobacco exposure was present in 16 of the 20 OSCC patients. Among tobacco-exposed patients, 68.75% (11/16) showed a high periodontal pathogen burden, compared with 25.00% (1/4) among patients without tobacco exposure. However, the association between tobacco exposure and high periodontal pathogen burden was not statistically significant (p=0.255).

Table 1. Comparison of demographic and risk-factor characteristics between OSCC cases and controls

Parameter	Group I: OSCC Cases (n=20)	Group II: Controls (n=20)	p-value
Age (years), Mean ± SD	54.65 ± 10.72	50.10 ± 11.15	0.196
Sex			
Male	15 (75.00%)	13 (65.00%)	0.731
Female	5 (25.00%)	7 (35.00%)	
Residence			
Rural	12 (60.00%)	11 (55.00%)	1.000
Urban	8 (40.00%)	9 (45.00%)	
Tobacco chewing	16 (80.00%)	7 (35.00%)	**0.010**
Smoking	11 (55.00%)	5 (25.00%)	0.105
Alcohol consumption	9 (45.00%)	4 (20.00%)	0.176

Table 2. Comparison of periodontal parameters between OSCC cases and controls

Periodontal Parameter	Group I: OSCC Cases (n=20), Mean ± SD	Group II: Controls (n=20), Mean ± SD	p-value
Plaque Index (PI)	2.13 ± 0.47	1.48 ± 0.42	**<0.001**
Gingival Index (GI)	1.92 ± 0.51	1.31 ± 0.45	**<0.001**
Bleeding on Probing (BOP), %	56.40 ± 18.75	33.15 ± 14.60	**<0.001**
Probing Pocket Depth (PPD), mm	4.38 ± 0.86	3.21 ± 0.72	**<0.001**
Clinical Attachment Loss (CAL), mm	4.96 ± 1.12	3.45 ± 0.91	**<0.001**

Table 3. PCR detection of periodontal pathogens in OSCC and control tissues

Periodontal Pathogen	Group I: OSCC Cases (n=20)	Group II: Controls (n=20)	p-value
Porphyromonas gingivalis	15 (75.00%)	7 (35.00%)	**0.025**
Fusobacterium nucleatum	16 (80.00%)	8 (40.00%)	**0.022**
Tannerella forsythia	12 (60.00%)	5 (25.00%)	0.054
Treponema denticola	11 (55.00%)	4 (20.00%)	**0.048**
Aggregatibacter actinomycetemcomitans	6 (30.00%)	3 (15.00%)	0.451
At least one periodontal pathogen detected	19 (95.00%)	12 (60.00%)	**0.020**

Table 4. Comparison of periodontal pathogen burden between OSCC cases and controls

Number of Periodontal Pathogens Detected	Group I: OSCC Cases (n=20)	Group II: Controls (n=20)	p-value
No pathogen detected	1 (5.00%)	8 (40.00%)	—
One pathogen	2 (10.00%)	4 (20.00%)	—
Two pathogens	5 (25.00%)	4 (20.00%)	—
Three or more pathogens	12 (60.00%)	4 (20.00%)	**0.022**
Two or more pathogens	**17 (85.00%)**	**8 (40.00%)**	**0.008**

Table 5. Association of high periodontal pathogen burden with clinicopathological characteristics among OSCC cases

High pathogen burden was defined as detection of ≥3 periodontal pathogens.

Clinicopathological Parameter	Total n	High Pathogen Burden n (%)	p-value
TNM Stage			
Stage I–II	8	2 (25.00%)	**0.019**
Stage III–IV	12	10 (83.33%)	

Tumour Size			
T1–T2	9	3 (33.33%)	0.065
T3–T4	11	9 (81.82%)	
Lymph Node Status			
N0	10	3 (30.00%)	**0.020**
N-positive	10	9 (90.00%)	
Histopathological Differentiation			
Well differentiated	9	3 (33.33%)	0.065
Moderately/Poorly differentiated	11	9 (81.82%)	
Tobacco Exposure			
Present	16	11 (68.75%)	0.255
Absent	4	1 (25.00%)	

Discussion

The present study included 40 participants, with 20 histopathologically confirmed OSCC cases and 20 non-malignant controls. The mean age of OSCC patients was 54.65 ± 10.72 years, compared with 50.10 ± 11.15 years among controls (p=0.196), while males constituted 75.00% and 65.00% of the respective groups (p=0.731). Dhanuthai et al. (2018), in a large multicentre study of 6,151 oral cancer patients, reported a mean age of approximately 58.37 years and found that 68.90% were males, which is comparable with the age and male predominance observed in the present study.[7] Tobacco chewing showed the clearest risk-factor difference in the present study, being reported by 80.00% of cases versus 35.00% of controls (p=0.010). Smoking (55.00% vs 25.00%, p=0.105) and alcohol consumption (45.00% vs 20.00%, p=0.176) were also more frequent among cases, although statistical significance was not achieved. Periodontal examination revealed consistently poorer periodontal parameters among OSCC patients. The mean Plaque Index was 2.13 ± 0.47 versus 1.48 ± 0.42, Gingival Index 1.92 ± 0.51 versus 1.31 ± 0.45, BOP 56.40 ± 18.75% versus 33.15 ± 14.60%, PPD 4.38 ± 0.86 versus 3.21 ± 0.72 mm, and CAL 4.96 ± 1.12 versus 3.45 ± 0.91 mm, with all differences being statistically significant (p<0.001). These findings are comparable with those of Komlós et al. (2021), who studied 100 oral cancer patients and 100 controls and reported a mean plaque index of 2.6 ± 0.8 in cases versus 1.6 ± 0.9 in controls, mean PPD of 5.6 ± 1.3 versus 2.5 ± 1.1 mm, and mean CAL of 6.2 ± 1.3 versus 2.8 ± 1.1 mm.[8] Their mean BOP was also higher among cancer patients (44.9 ± 30.4%) than controls (31.4 ± 23.2%), although the BOP difference in their study was not significant (p=0.09). Furthermore, 72.1% of their oral cancer patients had stage IV periodontitis, whereas 51.6% of controls predominantly had stage II periodontitis. Thus, although the absolute periodontal measurements vary between studies, both demonstrate a distinctly poorer periodontal profile among oral cancer patients. The PCR findings of the present study demonstrated a particularly strong association with Porphyromonas gingivalis, which was detected in 75.00% (15/20) of OSCC tissues compared with 35.00% (7/20) of control tissues (p=0.025). Chang et al. (2019) similarly demonstrated enrichment of periodontal pathogens in OSCC tissues and reported P. gingivalis in 60.7% of OSCC tissues, compared with 32.8% of paracancerous tissues and only 13.3% of normal tissues.[9] Their study further showed that P. gingivalis positivity was associated with later clinical stage, lower tumour differentiation, lymph-node metastasis, deeper periodontal pockets and greater clinical attachment loss. The higher prevalence of P. gingivalis in both the OSCC and control groups of the present study may reflect differences in underlying periodontal status, population characteristics and PCR methodology.

Fusobacterium nucleatum was the most frequently detected pathogen in the present study, occurring in 80.00% (16/20) of OSCC tissues compared with 40.00% (8/20) of controls, with a statistically significant difference (p=0.022). Kaliamoorthy et al. (2023) examined 12 OSCC tissues and 12 non-cancerous oral mucosal tissues using PCR and demonstrated F. nucleatum positivity in 3 of 12 OSCC specimens (25.00%), whereas none of the 12 control specimens showed positive amplification.[10] Although the absolute prevalence in their study was considerably lower than the 80.00% observed in the present study, both

investigations showed preferential detection of F. nucleatum in malignant tissue. Differences in sample size, geographic and dietary background, periodontal disease prevalence, tumour sampling sites, nucleic-acid extraction procedures and PCR sensitivity may account for variation in detection rates. The finding that F. nucleatum was also detected in 40.00% of controls in the present study suggests that its mere presence is not specific for malignancy; rather, the significantly greater frequency in OSCC tissue may be more relevant. Among the other periodontal microorganisms evaluated, Treponema denticola was detected in 55.00% of OSCC tissues versus 20.00% of controls (p=0.048), while Tannerella forsythia occurred in 60.00% versus 25.00% (p=0.054) and Aggregatibacter actinomycetemcomitans in 30.00% versus 15.00% (p=0.451). Kaliamoorthy et al. (2021) investigated T. denticola in 30 OSCC specimens and 30 non-cancerous mucosal specimens and detected the organism in 8 of 30 OSCC tissues (26.6%), whereas none of their control samples were positive.[11] Thus, the 55.00% positivity observed in the present OSCC group was higher than the 26.6% reported by Kaliamoorthy et al., while both investigations demonstrated greater detection in malignant tissue. In the present study, the higher frequencies of T. forsythia and A. actinomycetemcomitans among cases did not attain statistical significance, indicating that the strength of association was not uniform across all periodontal species. An important finding of the present investigation was that OSCC tissues showed not only greater individual pathogen positivity but also a substantially greater polymicrobial burden. At least one periodontal pathogen was detected in 95.00% of OSCC cases compared with 60.00% of controls (p=0.020). Three or more organisms were detected in 60.00% versus 20.00% (p=0.022), while the presence of two or more pathogens was recorded in 85.00% of cases compared with 40.00% of controls (p=0.008). Ganly et al. (2019) similarly demonstrated a strong relationship between a periodontal-pathogen-associated microbiome and oral cavity squamous cell carcinoma; their cancer-associated microbial profile showed an odds ratio of 15.33 (95% CI 2.80–83.89; p=0.0011).[12] The relationship between pathogen burden and tumour advancement was also noteworthy. High pathogen burden, defined as the presence of three or more periodontal pathogens, was detected in only 25.00% (2/8) of Stage I–II OSCC cases but in 83.33% (10/12) of Stage III–IV cases (p=0.019). A similar numerical pattern was noted for tumour size, with high pathogen burden in 33.33% of T1–T2 tumours versus 81.82% of T3–T4 tumours (p=0.065), and for histopathological differentiation, with rates of 33.33% in well-differentiated versus 81.82% in moderately/poorly differentiated tumours (p=0.065). Yang et al. (2018) also documented progressive alteration of the oral microbiome with advancing OSCC stage in 197 OSCC patients and 51 healthy controls.[13] They observed that the relative abundance of Fusobacteria increased progressively from 2.98% in healthy controls to 4.35% in Stage I, 6.24% in Stages II–III and 7.92% in Stage IV OSCC. Although their study assessed relative abundance rather than the number of PCR-positive periodontal species, the progressive microbiological change with tumour stage is consistent with the substantially greater high-pathogen burden identified among Stage III–IV patients in the present study. The non-significant trends observed for tumour size and differentiation should, however, be interpreted cautiously because only 20 OSCC cases were evaluated. Lymph-node involvement showed one of the strongest clinicopathological relationships with periodontal pathogen burden in the present study. High pathogen burden was found in 90.00% (9/10) of node-positive OSCC patients compared with 30.00% (3/10) of N0 patients (p=0.020). Eun et al. (2021) evaluated the oral microbiome in 54 patients with OSCC, of whom 20 (37.04%) had lymph-node metastasis and 34 (62.96%) had no lymph-node metastasis, and demonstrated a significant difference in microbial β-diversity between metastatic and non-metastatic groups.[14] Their metastatic group was enriched with several bacterial taxa, demonstrating that microbial community composition can differ according to nodal status. In the present study, high pathogen burden was additionally found in 68.75% (11/16) of tobacco-exposed patients compared with 25.00% (1/4) of patients without tobacco exposure, although this difference was not statistically significant (p=0.255).

Conclusion

The present study demonstrated a significantly higher prevalence and burden of periodontal pathogens in OSCC tissue compared with non-malignant control tissue. Fusobacterium nucleatum, Porphyromonas gingivalis and Treponema denticola showed significant associations with OSCC. Periodontal parameters were also significantly worse among OSCC patients. A high pathogen burden was significantly associated

with advanced TNM stage and lymph-node positivity, indicating a possible relationship between periodontal dysbiosis and tumour severity.

References

1. Chamoli A, Gosavi AS, Shirwadkar UP, Wangdale KV, Behera SK, Kurrey NK, et al. Overview of oral cavity squamous cell carcinoma: risk factors, mechanisms, and diagnostics. Oral Oncol. 2021;121:105451. doi:10.1016/j.oraloncology.2021.105451. Available from: https://doi.org/10.1016/j.oraloncology.2021.105451.
2. Li Q, Hu Y, Zhou X, Liu S, Han Q, Cheng L. Role of oral bacteria in the development of oral squamous cell carcinoma. Cancers (Basel). 2020;12(10):2797. doi:10.3390/cancers12102797. Available from: https://doi.org/10.3390/cancers12102797.
3. Rai AK, Panda M, Das AK, Rahman T, Das R, Das K, et al. Dysbiosis of salivary microbiome and cytokines influence oral squamous cell carcinoma through inflammation. Arch Microbiol. 2021;203(1):137–152. doi:10.1007/s00203-020-02011-w. Available from: https://doi.org/10.1007/s00203-020-02011-w.
4. Yao Y, Shen X, Zhou M, Tang B. Periodontal pathogens promote oral squamous cell carcinoma by regulating ATR and NLRP3 inflammasome. Front Oncol. 2021;11:722797. doi:10.3389/fonc.2021.722797. Available from: https://doi.org/10.3389/fonc.2021.722797.
5. Yang K, Wang Y, Zhang S, Zhang D, Hu L, Zhao T, et al. Oral microbiota analysis of tissue pairs and saliva samples from patients with oral squamous cell carcinoma: a pilot study. Front Microbiol. 2021;12:719601. doi:10.3389/fmicb.2021.719601. Available from: https://doi.org/10.3389/fmicb.2021.719601.
6. Nie F, Wang L, Huang Y, Yang P, Gong P, Feng Q, et al. Characteristics of microbial distribution in different oral niches of oral squamous cell carcinoma. Front Cell Infect Microbiol. 2022;12:905653. doi:10.3389/fcimb.2022.905653. Available from: https://doi.org/10.3389/fcimb.2022.905653.
7. Dhanuthai K, Rojanawatsirivej S, Thosaporn W, Kintarak S, Subarnbhesaj A, Darling M, et al. Oral cancer: a multicenter study. Med Oral Patol Oral Cir Bucal. 2018;23(1):e23–e29. doi:10.4317/medoral.21999. Available from: https://doi.org/10.4317/medoral.21999.
8. Komlós G, Csurgay K, Horváth F, Pelyhe L, Németh Z. Periodontitis as a risk for oral cancer: a case-control study. BMC Oral Health. 2021;21(1):640. doi:10.1186/s12903-021-01998-y. Available from: https://doi.org/10.1186/s12903-021-01998-y.
9. Chang C, Geng F, Shi X, Li Y, Zhang X, Zhao X, et al. The prevalence rate of periodontal pathogens and its association with oral squamous cell carcinoma. Appl Microbiol Biotechnol. 2019;103(3):1393–1404. doi:10.1007/s00253-018-9475-6. Available from: https://doi.org/10.1007/s00253-018-9475-6.
10. Kaliamoorthy S, Priya Sayeeram S, SundarRaj S, Balakrishnan J, Nagarajan M, Samidorai A. Investigating the association between Fusobacterium nucleatum and oral squamous cell carcinoma: a pilot case-control study on tissue samples. Cureus. 2023;15(10):e47238. doi:10.7759/cureus.47238. Available from: https://doi.org/10.7759/cureus.47238.
11. Kaliamoorthy S, Saranyan R, Govindasamy A, Balakrishnan J. Exploring the presence of Treponema denticola in oral squamous cell carcinoma. J Med Pharm Allied Sci. 2021;10(5):3686–3688. doi:10.22270/jmpas.V10I5.1679. Available from: https://doi.org/10.22270/jmpas.V10I5.1679.
12. Ganly I, Yang L, Giese RA, Hao Y, Nossa CW, Morris LGT, et al. Periodontal pathogens are a risk factor of oral cavity squamous cell carcinoma, independent of tobacco and alcohol and human papillomavirus. Int J Cancer. 2019;145(3):775–784. doi:10.1002/ijc.32152. Available from: https://doi.org/10.1002/ijc.32152.
13. Yang CY, Yeh YM, Yu HY, Chin CY, Hsu CW, Liu H, et al. Oral microbiota community dynamics associated with oral squamous cell carcinoma staging. Front Microbiol. 2018;9:862. doi:10.3389/fmicb.2018.00862. Available from: https://doi.org/10.3389/fmicb.2018.00862.
14. Eun YG, Lee JW, Kim SW, Hyun DW, Bae JW, Lee YC. Oral microbiome associated with lymph node metastasis in oral squamous cell carcinoma. Sci Rep. 2021;11(1):23176. doi:10.1038/s41598-021-02638-9. Available from: https://doi.org/10.1038/s41598-021-02638-9.