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Comparative Evaluation of Salivary Interleukin-1β and Interleukin-6 as Non-invasive Biomarkers in Oral Squamous Cell Carcinoma: A Cross-Sectional Analytical Study

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Abstract

Background

Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer and remains a major global health challenge due to delayed diagnosis and poor prognosis. Salivary inflammatory cytokines have emerged as promising non-invasive biomarkers for the early detection of OSCC. This study aimed to evaluate and compare salivary Interleukin-1 beta (IL-1β) and Interleukin-6 (IL-6) levels among healthy individuals and patients with histopathologically confirmed OSCC.

Methodology

This comparative case-control study included 126 participants, comprising 63 healthy controls and 63 patients with OSCC. Demographic characteristics and habit history, including tobacco and alcohol use, were recorded. Unstimulated whole saliva samples were collected using the passive drool technique, and salivary IL-1β and IL-6 concentrations were quantified using enzyme-linked immunosorbent assay (ELISA). Statistical analysis was performed using statistical software, and $p < 0.05$ was considered statistically significant.

Results

OSCC was more prevalent among men and individuals with tobacco-related habits, particularly smokeless tobacco use. The buccal mucosa was the most commonly affected anatomical site. Salivary IL-1β and IL-6 levels were significantly higher in patients with OSCC than in healthy controls (IL-1β: 69.26 ± 88.40 vs. 25.11 ± 59.50 pg/mL, $p = 0.001$; IL-6: 54.71 ± 65.90 vs. 3.36 ± 1.35 pg/mL, $p < 0.001$).

Conclusion

Salivary IL-1β and IL-6 concentrations were significantly elevated in patients with OSCC compared with healthy controls, indicating an association between these inflammatory cytokines and OSCC. Future multicenter prospective studies incorporating diagnostic accuracy assessments are warranted to establish their potential clinical utility.

Categories: Oncology, Oral Medicine, Dentistry
Keywords: biomarkers, early detection of oral cancer, enzyme-linked immunosorbent assay (elisa), inflammatory cytokines, interleukin-1beta, interleukin-6, mouth neoplasms, oral squamous cell carcinoma, saliva, salivary biomarkers

Introduction

Oral cancer is a major global health concern and one of the most common head and neck malignancies. Oral squamous cell carcinoma (OSCC) accounts for over 90% of oral cancers and commonly affects the buccal mucosa, tongue, floor of the mouth, gingiva, palate, and lips [1]. Despite advances in diagnosis and treatment, the five-year survival rate remains unsatisfactory, largely due to delayed diagnosis and advanced-stage presentation [2].

OSCC is a multifactorial disease resulting from interactions among genetic susceptibility, environmental factors, and chronic inflammation. Major risk factors of OSCC include tobacco use (smoked and smokeless), alcohol consumption, betel quid/areca nut chewing, and infection with high-risk human papillomavirus

(HPV) [3]. Early detection is crucial for improving prognosis and reducing treatment-related morbidity.

Conventional diagnostic approaches for OSCC include clinical examination, imaging, and histopathological evaluation. Clinical examination and most imaging modalities are generally non-invasive; definitive diagnosis requires histopathological examination of biopsy tissue, which involves an invasive procedure. Although clinical examination remains an important component of oral cancer screening, imaging and biopsy-based investigations are not routinely feasible for large-scale population-based screening [4]. Consequently, there is increasing interest in identifying reliable, non-invasive biomarkers for the early detection of OSCC. Saliva is an attractive diagnostic biofluid because its collection is non-invasive and inexpensive and reflects local and systemic pathological processes [5]. Among salivary biomarkers, inflammatory cytokines have gained considerable attention because of their role in oral carcinogenesis [6].

Interleukin-1 beta (IL-1β) and Interleukin-6 (IL-6) are key pro-inflammatory cytokines implicated in tumor initiation, progression, angiogenesis, invasion, and immune modulation. IL-1β promotes inflammatory signaling through activation of the nuclear factor-kappa B (NF-κB) pathway, while IL-6 facilitates tumor growth and survival via the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway [7,8]. Elevated salivary concentrations of both IL-1β and IL-6 cytokines have been reported in patients with OSCC, suggesting their potential as diagnostic biomarkers. However, regional variations in genetic background, environmental exposures, and tobacco-related habits necessitate population-specific validation. Maharashtra, India, has a substantial prevalence of smokeless tobacco use, according to the Global Adult Tobacco Survey (GATS-2), 2016-2017 [9], highlighting the importance of investigating salivary biomarkers in this population. Therefore, this study aimed to evaluate and compare salivary IL-1β and IL-6 concentrations between healthy individuals and patients with histopathologically confirmed OSCC in the Maharashtrian population. Additionally, the study evaluated the potential of these biomarkers as non-invasive diagnostic tools for the early detection of OSCC.

Materials And Methods

Study design

This cross-sectional analytical study was conducted in accordance with the ethical principles of the Declaration of Helsinki (2013 revision). Ethical approval was obtained from the Institutional Ethics Committee of Krishna Vishwa Vidyapeeth, Karad (Protocol No.: KVVDU/IEC/06/2023, dated 05/06/2023), and written informed consent was obtained from all participants before their inclusion in the study. This study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (Figure *1*).

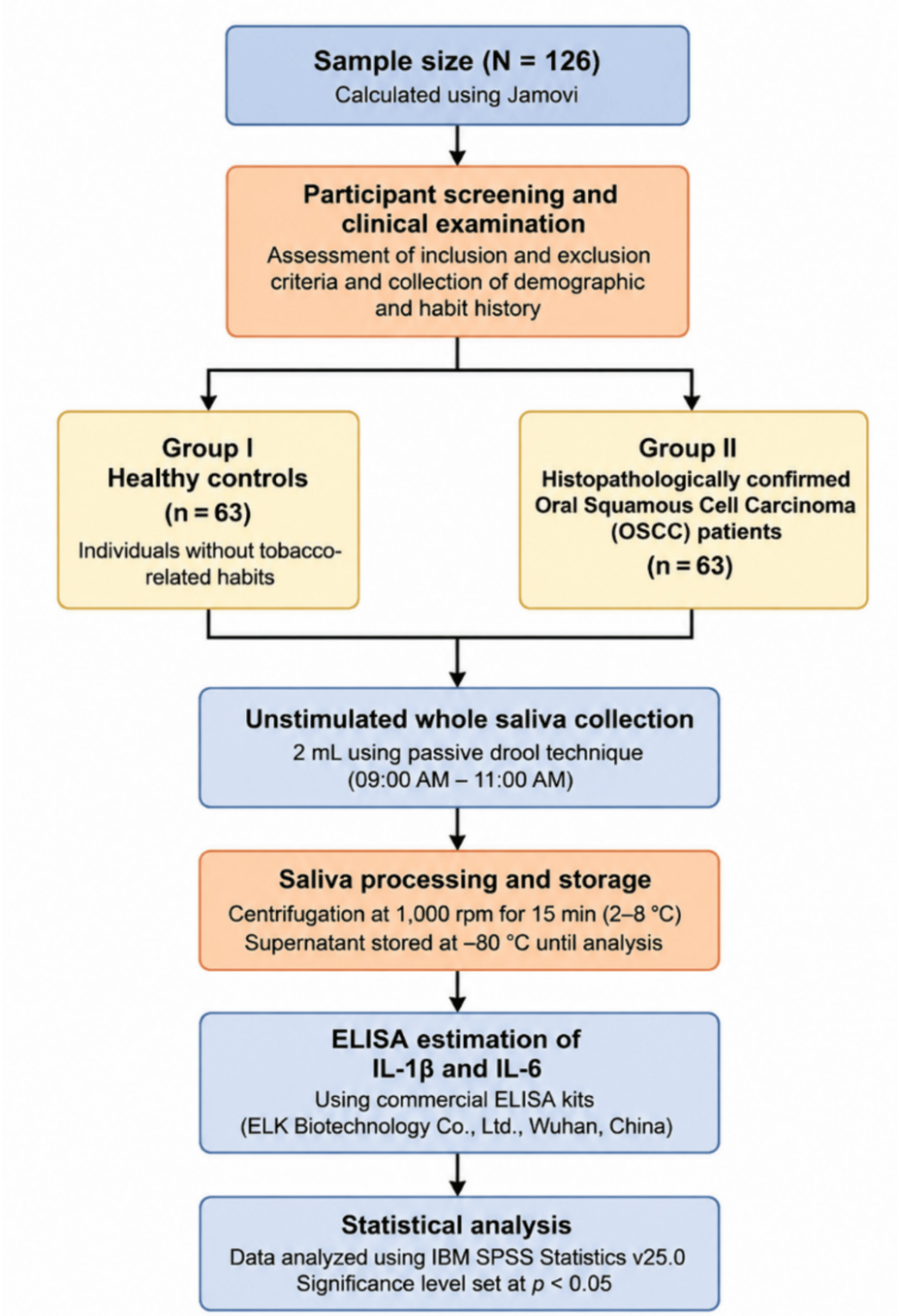


FIGURE 1: Schematic flow diagram of the study protocol.

IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; ELISA: enzyme-linked immunosorbent assay

Study population

Adult participants aged ⩾18 years of either sex presenting to the School of Dental Sciences, Krishna Vishwa Vidyapeeth, Karad, between June 2023 and June 2025 were screened for eligibility. Eligible participants were recruited by convenience sampling after satisfying the predefined inclusion and exclusion criteria. Data regarding demographic characteristics, socioeconomic status, medical history, and lifestyle-related habits, including tobacco use, were obtained using a validated structured proforma. The information obtained was systematically documented and utilized for subsequent statistical analyses.

Inclusion and exclusion criteria

The study included systemically healthy individuals with no history of tobacco use in any form, who

constituted the healthy control group. Patients with histopathologically confirmed OSCC constituted the oral cancer group. Individuals with any systemic illness or medical condition that could potentially influence the study outcomes, pregnant or lactating women, and those unwilling or unable to provide written informed consent were excluded from the study.

Clinical examination

All participants underwent a comprehensive oral examination conducted by a single Oral Medicine specialist trained in the clinical assessment of oral conditions. The use of a single examiner ensured consistency in clinical assessments and eliminated inter-examiner variability. The clinical examination included assessment of the anatomical site and clinical characteristics of OSCC lesions, along with documentation of tobacco- and alcohol-related habits.

Sample size estimation

The sample size was estimated a priori using Jamovi statistical software for the comparison of two independent means, assuming a two-sided significance level (α) of 0.05, statistical power ($1 - \beta$) of 80%, and a standardized effect size (Cohen's d) of 0.50. Based on these assumptions, the minimum required sample size was approximately 63 participants per group, yielding a total of 126 participants. The sample size was calculated using the following formula:

$$n=\frac{2(Z_{\alpha/2}+Z_{\beta})^2\sigma^2}{d^2}$$

where n represents the required sample size per group, $Z_{(\alpha/2)}$ is the standard normal deviate corresponding to a two-sided significance level of 0.05 (1.96), $Z_{(\beta)}$ is the standard normal deviate corresponding to a statistical power of 80% (0.84), σ represents the pooled standard deviation (SD), and d denotes the expected mean difference between the two groups. The minimum required sample size was estimated to be 63 participants per group, resulting in a total sample size of 126 participants.

Saliva sample collection and storage

A 2 mL unstimulated whole saliva sample was collected from all participants using the passive drool technique under standardized conditions. Saliva samples were collected between 09:00 AM and 11:00 AM to minimize circadian variation. Participants were asked to avoid eating, drinking, smoking, or performing oral hygiene procedures for a minimum of two hours prior to sampling. Participants were instructed to rinse their mouths thoroughly with water to remove food debris 10 minutes before saliva collection. Participants were seated comfortably in an upright position, with the head slightly tilted down, allowing saliva to pool in the floor of the mouth. The pooled saliva was then gently drooled into a sterile collection tube without swallowing or making exaggerated mouth movements. Saliva samples were transported on ice and immediately centrifuged at 2,500 × g for 15 minutes at 2°C-8°C. The resulting supernatant was carefully separated and transferred into Eppendorf tubes and stored immediately at −80°C until analysis by enzyme-linked immunosorbent assay (ELISA).

Biomarker estimation

The concentrations of IL-1β and IL-6 salivary biomarkers were quantified using commercially available ELISA kits (ELK Biotechnology Co., Ltd., Wuhan, China). A sandwich ELISA principle was employed according to the manufacturer's instructions and protocols, and biomarker concentrations were expressed as picograms per milliliter (pg/mL). Prior to the assay, all reagents and samples were equilibrated to room temperature to ensure optimal reaction conditions. Each biomarker was analyzed independently in duplicate to ensure accuracy, and the mean value of duplicate measurements was used for statistical analysis. The optical density values were quantified using an ELISA microplate reader (LisaQuant TS, Tulip Diagnostics Pvt. Ltd., Goa, India). The corresponding biomarker concentrations were calculated from standard calibration curves generated for each assay. Laboratory personnel performing the ELISA assays were blinded to the clinical diagnosis of the participants. Quality control procedures were performed according to the manufacturer's recommendations throughout the assay.

Statistical analysis

All data were compiled, coded, and analyzed using IBM Statistical Package for the Social Sciences (SPSS) software, version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize demographic characteristics, habit profile, anatomical site of OSCC, and salivary biomarker levels. Categorical variables were expressed as frequencies and percentages, whereas continuous variables were presented as mean ± SD. Data normality was assessed using the Shapiro-Wilk test before applying parametric tests. Associations between categorical variables were evaluated using Pearson's chi-squared (χ^2) test. Fisher's exact test was applied when the assumptions of the chi-squared test were not satisfied due to small expected cell frequencies. For contingency tables larger than 2 × 2 with sparse data, the Fisher-Freeman-Halton exact test was used, where appropriate. Mean salivary IL-1β and IL-6 concentrations were compared between the two groups using the independent-samples t-test. All statistical tests were two-

tailed, and a p-value of less than 0.05 was considered statistically significant. No missing data were observed; therefore, complete-case analysis was performed.

Results

Age distribution

The highest proportion of participants belonged to the 41-50 years age group (34.9%). The age distribution differed significantly between healthy participants and OSCC patients ($\chi^2 = 24.15$, df = 4, $p < 0.001$). Healthy participants were predominantly in the 31-40 years age group (42.9%), whereas OSCC patients were mainly in the 41-50 years age group (46.0%), indicating a statistically significant difference in age distribution between healthy participants and OSCC patients (Table *1*).

Age group (years)	Healthy participants (n = 63), n (%)	OSCC patients (n = 63), n (%)	Total (N = 126), n (%)
20-30	10 (15.9%)	0 (0.0%)	10 (7.9%)
31-40	27 (42.9%)	12 (19.0%)	39 (31.0%)
41-50	15 (23.8%)	29 (46.0%)	44 (34.9%)
51-60	7 (11.1%)	16 (25.4%)	23 (18.3%)
≥60	4 (6.3%)	6 (9.5%)	10 (7.9%)
Chi-squared test	$\chi^2 = 24.15$	df = 4	$p < 0.001$*

TABLE 1: Age-wise distribution of study participants.

Values are presented as number (percentage). Percentages were calculated using the respective group totals (n = 63 for each group), whereas percentages in the total column were calculated using the overall sample size (N = 126). Pearson's chi-squared test was used to compare the age-group distributions between healthy participants and OSCC patients.

*Statistically significant at $p < 0.05$.

OSCC: oral squamous cell carcinoma; χ^2: Pearson's chi-squared statistic; df: degrees of freedom

Gender distribution

The gender distribution of study participants is presented in Table *2*. Men constituted 62.7% (79/126) of the study population, while women accounted for 37.3% (47/126). Male predominance was observed in both healthy participants (57.1%) and OSCC patients (68.3%). However, the difference in gender distribution between the two groups was not statistically significant ($\chi^2 = 1.663$, df = 1, $p = 0.197$) (Table *2*).

Groups	Males, n (%)	Females, n (%)	Total, n (%)
Healthy participants	36 (57.1%)	27 (42.9%)	63 (100%)
OSCC	43 (68.3%)	20 (31.7%)	63 (100%)
Total	79 (62.7%)	47 (37.3%)	126 (100%)

TABLE 2: Gender-wise distribution of participants.

Values are presented as number (percentage). Percentages for males and females were calculated using the respective study-group totals (n = 63), whereas percentages in the total row were calculated using the overall sample size (N = 126). Pearson's chi-squared test was used to compare gender distribution between healthy participants and OSCC patients.

The difference was not statistically significant ($\chi^2 = 1.663$, df = 1, $p = 0.197$).

OSCC: oral squamous cell carcinoma; χ^2: Pearson's chi-squared statistic; df: degrees of freedom

Habit-wise distribution of study participants

Smokeless tobacco use was the most frequently reported tobacco habit among patients with OSCC,

accounting for 69.8% (44/63), followed by smoked tobacco use (22.2%; 14/63) and combined smoked and smokeless tobacco use (7.9%; 5/63). All healthy participants were non-tobacco users, consistent with the predefined study-group selection criteria. Alcohol consumption was reported by 34.9% of OSCC patients compared with 7.9% of healthy participants, representing a statistically significant difference ($p < 0.001$). Spicy food consumption was uncommon in both groups, with no statistically significant difference ($p = 1.000$). The habit-wise distribution of participants is presented in Table 3.

Habit	Healthy participants (n = 63), n (%)	OSCC patients (n = 63), n (%)	Total (N = 126), n (%)	Statistical test	p-value
No tobacco use	63 (100.0%)	1 (1.6%)	64 (50.8%)	$\chi^2 = 122.06$	<0.001*
Smokeless tobacco use	0 (0%)	44 (69.8%)	44 (34.9%)	Fisher's exact	<0.001*
Smoked tobacco use	0 (0%)	14 (22.2%)	14 (11.1%)	Fisher's exact	<0.001*
Both smoked and smokeless tobacco use	0 (0%)	5 (7.9%)	5 (4.0%)	Fisher's exact	0.058
Alcohol consumption	5 (7.9%)	22 (34.9%)	27 (21.4%)	$\chi^2 = 13.62$	<0.001*
Use of spicy food	1 (1.6%)	2 (3.2%)	3 (2.4%)	Fisher's exact	1.000

TABLE 3: Habit-wise distribution of study participants.

Values are presented as number (percentage). Percentages for healthy participants and OSCC patients were calculated using the respective group totals (n = 63), whereas percentages in the total column were calculated using the overall sample size (N = 126). Pearson's chi-squared test or Fisher's exact test was applied, as appropriate.

*Statistically significant at $p < 0.05$.

χ^2: Pearson's chi-squared statistic; OSCC: oral squamous cell carcinoma

Site- and gender-wise distribution of OSCC lesions

Among the 63 OSCC patients, the buccal mucosa was the most frequently affected site (47.6%), followed by the alveolus (25.4%). Lesions involving the labial mucosa accounted for 9.5% of cases, while the tongue and palate each represented 7.9%; the lip was the least commonly affected site (1.6%). Although men accounted for the majority of lesions at most anatomical sites, no statistically significant association was observed between gender and lesion site (Fisher-Freeman-Halton exact test, $p = 0.64$) (Table 4).

Site	Males, n (%)	Females, n (%)	Total, N (%)
Buccal mucosa	23 (36.5%)	7 (11.1%)	30 (47.6%)
Labial mucosa	4 (6.3%)	2 (3.2%)	6 (9.5%)
Alveolus	10 (15.9%)	6 (9.5%)	16 (25.4%)
Lip	1 (1.6%)	0 (0 %)	1 (1.6%)
Tongue	2 (3.2%)	3 (4.8%)	5 (7.9%)
Palate	3 (4.8%)	2 (3.2%)	5 (7.9%)
Total	43 (68.3%)	20 (31.7%)	63 (100%)

TABLE 4: Site- and gender-wise distribution of OSCC lesions.

Values are presented as number (percentage). Percentages were calculated using the total number of OSCC patients (N = 63), irrespective of gender. Each patient was classified according to the primary anatomical site of the lesion. The Fisher-Freeman-Halton exact test was used to assess the association between gender and anatomical site of OSCC lesions because of small expected cell frequencies. No statistically significant association was observed (p = 0.64).

OSCC: oral squamous cell carcinoma

Salivary concentrations of IL-1β and IL-6

The salivary concentrations of IL-1β and IL-6 in healthy participants and OSCC patients are presented in Figure 2. OSCC patients demonstrated substantially higher mean salivary concentrations of both biomarkers compared with healthy controls. Independent samples t-test analysis demonstrated statistically significant differences between the two groups. IL-1β levels were markedly increased in OSCC patients ($t = -3.29$, $p = 0.001$), and IL-6 levels were significantly elevated ($t = -6.18$, $p < 0.001$) (Figure 2).

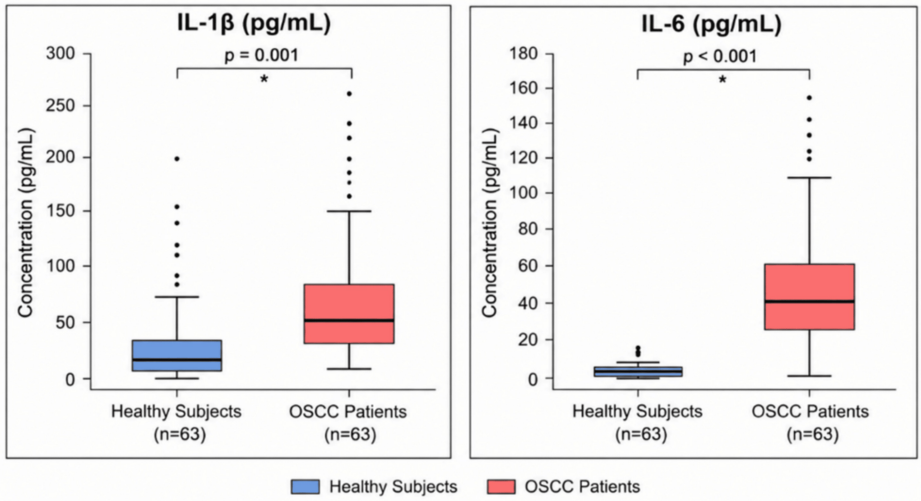


FIGURE 2: Comparison of salivary IL-1β and IL-6 concentrations between healthy participants and OSCC patients.

*Statistically significant at $p < 0.05$.

OSCC: oral squamous cell carcinoma; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6

Discussion

The global distribution of oral cancers is uneven, with an incidence of approximately 1%-4% in Western countries. In contrast, the disease is substantially more prevalent in developing Asian regions, where it is associated with considerable morbidity and mortality [10]. Oral cancer constitutes a major public health burden in low- and middle-income countries. Delayed diagnosis often results in advanced disease, poorer

prognosis, and increased healthcare costs. The development of sensitive and specific biomarkers is essential for the early detection of oral cancer. Such biomarkers may facilitate timely diagnosis, prompt treatment, and improved clinical outcomes [11]. Among the salivary cytokines investigated in patients with oral cancers, IL-1β, IL-6, and IL-8 have been recognized as reliable biomarkers owing to their significant association with disease development and progression [12].

Age distribution

Age is a well-recognized risk factor for OSCC because prolonged exposure to tobacco, areca nut, and alcohol, together with cumulative genetic and epigenetic alterations, promotes malignant transformation. In the present study, OSCC patients were predominantly in the 41-50 years age group (46.0%), whereas healthy participants were mainly distributed in the 31-40 years age group (42.9%). The age distribution differed markedly between the two groups ($\chi^2 = 24.15$, $p < 0.001$), suggesting that increasing age is associated with the occurrence of OSCC. These findings are consistent with those of Warnakulasuriya, who reported that the incidence of OSCC increases with advancing age due to the cumulative effects of exposure to established risk factors and progressive molecular alterations in the oral epithelium [13]. Johnson et al. observed that the majority of patients with OSCC are diagnosed during the fifth and sixth decades of life, reflecting the long latency period required for carcinogenesis [14]. Our observations are also comparable to those of Liao et al., who retrospectively analyzed 827 patients with OSCC and reported that most patients presented in the fifth decade or later, indicating that OSCC predominantly affects middle-aged and older adults [15]. Bhattacharjee et al. identified that the majority of OSCC patients belonged to the 41-60 years age group, which closely resembles the age distribution observed in the present study [16]. Our observations are also in accordance with the studies conducted by Gole et al. [17] and Sahu and Kumar in the Indian population [18]. The relatively younger age at presentation observed in this study may reflect the early initiation and widespread use of smokeless tobacco and betel quid in India compared with reports from Western populations. Genetic susceptibility, poor oral hygiene, nutritional deficiencies, and socioeconomic factors may also contribute to earlier development of OSCC in developing countries.

Gender distribution

Male predominance was observed in both healthy participants (57.1%) and OSCC patients (68.3%) in the present study. Overall, men constituted 62.7% of the total study population, with a male-to-female ratio of 1.68:1. The male-to-female ratio finding is lower than those reported by Bhattacharjee et al. (2.5:1) and Tranby et al. (3:1), suggesting possible differences in the study population, geographic region, or exposure to risk factors [16,19]. The results of our study are in agreement with the study conducted by Gopala Krishnan et al. [20]. A retrospective review by Liao et al. confirmed a pronounced male predominance, with 90%-93% of cases occurring in men and only 7%-10% in women [15]. Warnakulasuriya reported that the incidence of oral cancer is consistently higher among men worldwide, although the gender gap has narrowed in recent years because of increasing tobacco and alcohol use among women in several populations [13]. Johnson et al. emphasized that the higher prevalence of tobacco- and alcohol-related risk behaviors among men largely accounts for the observed gender disparity in oral cancer incidence [14]. The higher prevalence of OSCC among men is likely due to greater exposure to tobacco, betel quid, and alcohol. These carcinogenic lifestyle habits are more common among men in many populations, contributing to the observed gender disparity.

Habit-wise distribution of study participants

The present study revealed a strong association between tobacco-related habits and OSCC. Smokeless tobacco use was the most prevalent habit among OSCC patients (69.8%), followed by smoked tobacco use (22.2%), whereas all healthy participants reported no tobacco use. Statistically significant differences were observed for no tobacco use, smokeless tobacco use, smoked tobacco use, and alcohol consumption ($p < 0.001$), highlighting the important role of these lifestyle factors in the development of OSCC. In contrast, combined smoked and smokeless tobacco use ($p = 0.057$) and spicy food consumption ($p = 1.000$) were not associated with OSCC in the present study. The predominance of smokeless tobacco use among OSCC patients is consistent with the epidemiological profile of oral cancer in India and other South Asian countries, where smokeless tobacco products such as gutkha, khaini, mawa, and betel quid with tobacco are widely consumed. Smokeless tobacco contains carcinogens that induce DNA damage and oxidative stress, leading to chronic inflammation, epithelial dysplasia, and malignant transformation. The findings of the present study are consistent with Acharya et al., who reported that smokeless tobacco is one of the strongest independent risk factors for oral cancer in the Indian subcontinent [21]. Warnakulasuriya emphasized that tobacco use, in both smoked and smokeless forms, is the principal modifiable risk factor for oral cancer worldwide [13].

Alcohol consumption was more common among OSCC patients than healthy participants ($p < 0.001$). Alcohol enhances mucosal permeability to tobacco carcinogens, promotes acetaldehyde formation and oxidative stress, and impairs DNA repair, thereby increasing the risk of oral cancer. The combined exposure to tobacco and alcohol has been shown to exert a synergistic effect, substantially increasing the risk of oral cancer compared with either exposure alone. Jun et al. confirmed that individuals with combined tobacco and alcohol use have a significantly higher risk of developing oral cancer than non-users [22].

The present study did not find a significant association in participants who used both smoked and smokeless

tobacco (p = 0.057) and spicy food consumption (p = 1.000). This finding is likely attributable to the relatively small number of participants with combined habits. Spicy foods may cause transient mucosal irritation in susceptible individuals; there is insufficient evidence to support a direct carcinogenic role.

Site-wise and gender-wise distribution of OSCC lesions

In the present study, the buccal mucosa was the most frequently affected site, accounting for 47.6% of OSCC cases, followed by the alveolus (25.4%), labial mucosa (9.5%), tongue, and palate (7.9%), while the lip (1.6%) was the least commonly affected site. Men constituted the majority of cases at most anatomical sites; no statistically significant association was observed between gender and the site of lesion (p = 0.64). This finding suggests that while OSCC is more prevalent among men, the anatomical distribution of lesions is independent of gender. The predominance of buccal mucosa involvement observed in the present study is consistent with numerous studies conducted in the Indian subcontinent. This distribution is attributed to the common practice of placing smokeless tobacco or betel quid in the buccal vestibule for prolonged periods.

Similar findings have been reported by Bhattacharjee et al. [16], Gole et al. [17], and Sahu and Kumar [18], who have identified the buccal mucosa as the predominant site of OSCC among Indian patients. Warnakulasuriya highlighted geographic variations in oral cancer distribution, with buccal mucosa cancers predominating in South Asia and tongue and floor-of-mouth cancers being more common in Western populations due to cigarette smoking and alcohol consumption [13]. No statistically significant association was observed between gender and lesion location. This suggests that the anatomical site of involvement is influenced more by the location of carcinogen exposure and individual tobacco-use practices than by gender itself.

Salivary concentrations of IL-1β and IL-6

The present study identified that the salivary concentrations of IL-1β and IL-6 were markedly elevated in patients with OSCC compared with healthy controls. IL-1β promotes chronic inflammation, angiogenesis, extracellular matrix degradation, and tumor invasion through activation of cyclooxygenase-2 (COX-2), matrix metalloproteinases (MMPs), and vascular endothelial growth factor (VEGF). Tobacco-derived carcinogens stimulate IL-1β production via the NF-κB pathway, contributing to oral carcinogenesis. IL-6 promotes tumor proliferation, angiogenesis, epithelial-mesenchymal transition, and immune evasion through JAK/STAT3 signaling. Elevated salivary IL-6 levels in this study likely reflect increased inflammatory activity and tumor burden in OSCC. These findings indicate that both cytokines are associated with oral cancer and support their potential role as non-invasive salivary biomarkers for the early detection of OSCC. The elevated salivary IL-1β and IL-6 concentrations observed in this study are consistent with reports from Croatia, Sri Lanka, Taiwan, and India, indicating that these cytokines are consistently associated with OSCC across different populations [12,23-29].

Limitations and future perspectives

The present study was limited by its single-center, cross-sectional design and relatively small sample size, which may limit the generalizability of the findings. In addition, only IL-1β and IL-6 were evaluated, and patients with oral potentially malignant disorders (OPMDs) were not included. Furthermore, the association of salivary biomarker concentrations with tumor stage, histopathological grade, treatment response, and patient survival was not investigated. Future multicenter prospective studies with larger and more diverse populations are required to validate these findings. Inclusion of patients with OPMDs, evaluation of additional salivary biomarkers, and assessment of their correlation with clinicopathological parameters may enhance the diagnostic and prognostic utility of salivary biomarker profiling. Furthermore, the development of multiplex salivary biomarker panels integrated with point-of-care diagnostic technologies may facilitate the early detection, risk stratification, and longitudinal monitoring of OSCC.

Conclusions

The present study demonstrated significantly higher salivary IL-1β and IL-6 concentrations in patients with OSCC than in healthy controls, highlighting their association with oral carcinogenesis. Smokeless tobacco use emerged as the predominant risk factor, and the buccal mucosa was the most frequently affected anatomical site. Salivary IL-1β and IL-6 show promise as non-invasive biomarkers for distinguishing patients with OSCC from healthy individuals. These biomarkers may serve as simple, cost-effective adjuncts to conventional clinical examination, particularly for early detection and population-based screening of high-risk individuals. However, large multicenter prospective studies incorporating multiplex biomarker panels and longitudinal follow-up are required to validate their diagnostic performance before routine clinical implementation.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the

work.

Concept and design: Girish Suragimath, Ashwinirani SR, Disha G. Suragimath

Acquisition, analysis, or interpretation of data: Girish Suragimath, Ashwinirani SR, Disha G. Suragimath, Satish Patil, Satish V. Kakade

Drafting of the manuscript: Girish Suragimath, Ashwinirani SR, Disha G. Suragimath

Critical review of the manuscript for important intellectual content: Girish Suragimath, Ashwinirani SR, Disha G. Suragimath, Satish Patil, Satish V. Kakade

Supervision: Girish Suragimath

Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. Ethics Committee of Krishna Vishwa Vidyapeeth, Karad issued approval KVVDU/IEC/06/2023. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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