Original Article

Evaluation of salivary biomarker interleukin-6 in oral squamous cell carcinoma and oral potentially malignant disorders – A comparative cross-sectional South Indian study

Venkata Naga Sirisha Chundru[1], Nirmal Madhavan R[2], Lalitha Chintala[3], Ramanarayana Boyapati[4], Meka Srikar[5]

[1]Department of Oral and Maxillofacial Pathology, Malla Reddy Dental College for Women, Suraram, Hyderabad, Telangana, [2]Department of Oral and Maxillofacial Pathology, Government Dental College, Cuddalore, Chidambaram, Tamil Nadu, [3]Department of Oral Medicine and Radiology, Malla Reddy Institute of Dental Sciences, Suraram, Hyderabad, Telangana, [4]Department of Periodontology, Sibar Institute of Dental Sciences, Takkellapadu, Guntur, Andhra Pradesh, [5]Department of Biological Sciences, BS-MS Programme, Indian Institute of Science Education and Research, Kolkata, West Bengal, India

Abstract

Background: Oral squamous cell carcinoma (OSCC) accounts for nearly 90% of oral malignancies and represents a major global health care problem. It is often preceded by oral potentially malignant disorders (OPMD). Although regular clinical examination forms the backbone for oral cancer screening, subtle lesions go unnoticed and there is a need for more sensitive and specific molecular biomarkers in mass screening of population. Salivary proteomics offer an attractive alternative to serum and tissue testing.

Aims: To find the diagnostic utility of salivary interleukin-6 (IL-6) in the differential diagnosis of OSCC, OPMD from healthy controls.

Study Design: *In vivo* study.

Methods: After approval from the Institutional Review Board, unstimulated whole saliva was collected from 90 subjects, 30 in each group of OSCC, OPMD and controls after ethical clearance. Salivary IL-6 was measured by ELISA, and the results were statistically analysed.

Results: Significant difference in salivary IL-6 was seen between OSCC, OPMD and controls. Receiver operating characteristic curve analysis showed the highest area under a curve of 0.982 in distinguishing OSCC from controls. It showed a sensitivity of 71% and specificity of 100% at a cut-off value of 33.4 pg/mL ($P = 0.000$). Moderately differentiated OSCC (MDSCC) showed a significant increase in salivary IL-6 concentration compared to well-differentiated OSCC (WDSCC).

Conclusion: Results of the present study showed strong predictive power of salivary IL-6 in distinguishing OSCC from controls. Its levels also increased with tumor aggressiveness from WDSCC to MDSCC. Thus, salivary IL-6 could have a diagnostic and/or prognostic significance in identifying high-risk groups in mass screening of the population.

Keywords: Biomarkers, cytokines, interleukin-6, oral squamous cell carcinoma, saliva

Address for correspondence: Dr. Venkata Naga Sirisha Chundru, Department of Oral and Maxillofacial Pathology, Malla Reddy Dental College for Women, Suraram, Hyderabad, Telangana, India.
E-mail: drsirishameka@gmail.com

Submitted: 30-Apr-2024, **Revised:** 20-Jun-2024, **Accepted:** 01-Aug-2024, **Published:** 15-Oct-2024

Access this article online

Quick Response Code:

Website: https://journals.lww.com/JPAT/

DOI: 10.4103/jomfp.jomfp_122_24

How to cite this article: Chundru VN, Madhavan RN, Chintala L, Boyapati R, Srikar M. Evaluation of salivary biomarker interleukin-6 in oral squamous cell carcinoma and oral potentially malignant disorders – A comparative cross-sectional South Indian study. J Oral Maxillofac Pathol 2024;28:387-92.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) accounts for nearly 90% of oral malignancies and represents a major global health care problem. Recently, there has been an alarming rise in cases among young, middle aged men and women. Despite improvement in treatment modalities, the overall 5-year survival rate is not more than 50%. Although regular comprehensive examination forms the backbone for oral cancer screening, subtle lesions may go unnoticed and there is a need for more sensitive and specific molecular biomarkers. Salivary diagnostics, being non-invasive, readily available and cost effective seems a feasible approach for mass screening compared to serum. Cytokines are molecular messengers that play a role in signalling, regulation, maintaining and inducing various cellular interactions. Their physiological activities are dysregulated during inflammation and carcinogenesis.[1]

Salivary cytokines have been an area of interest as markers of both cell proliferation and oral cancer. Interleukin-6 (IL-6) is a multifunctional cytokine that plays a role in inflammation and immune responses. It is associated with cancer cell growth, higher rate of metastasis and altered immune status.[2] For OSCC diagnosis, IL-6 has been proposed as one of the best molecular biomarkers.[3] Poor sensitivity, high false positive rates and paucity of large-scale global validation often limit the diagnostic utility of most markers. Analysis of salivary proteomic markers may unravel morbidity molecular signatures specific to OSCC. Previous studies on populations of different ethnicities showed proangiogenic, proinflammatory cytokines like Interleukin-6 (IL-6) has a role as surrogate molecular indicator of carcinogenic transformation from OPMD. Owing to the fact that an ideal biomarker should have widespread efficacy regardless of ethnicity, the present study aimed at pre-validating the same on the Indian population as well owing to their diverse tobacco-related habits, and products, and see if it could be used as a potential biomarker for distinguishing OSCC, oral potentially malignant disorders (OPMD) from healthy controls.

MATERIALS AND METHODS

Ethical approvals and informed consent: The current study was conducted as per the Declaration of Helsinki and its subsequent revisions. This multicentric study was approved by institutional ethical committees (ECR/227/INST/AP/2013/RR-16), (MRMCWIEC/AP/28/2019). All subjects were briefed about the purpose, and procedure and written informed consent was taken. All subjects participated in the study after signing the informed consent.

Patient selection

The study comprised 90 subjects categorised into three groups of 30 subjects in each group. **Group 1/OSCC** included subjects with clinically and histologically diagnosed OSCC cases and who had not undergone any form of therapy for OSCC. **Group 2/OPMD** included patients with clinically and histo-pathologically confirmed cases of leukoplakia, oral Lichen planus (OLP) and clinically confirmed cases of oral submucous fibrosis (OSMF) and none underwent any form of treatment for the same. Diagnosis of OSMF was made based on clinical symptoms like difficulty in mouth opening, and palpable fibrotic bands and graded with a clinical grading system. **Group 3/Controls** included age and gender-matched healthy individuals, free from oral inflammatory lesions or systemic illness.

Exclusion criteria included history of prior malignancy, diabetes, auto-immune disorders, hepatitis or HIV infection, systemic disorders, blood dyscrasias, recurrent or metastatic lesions to jaws, patients on drugs that induce hyposalivation or hypersalivation. Case history was taken, and data collected was entered into a detailed questionnaire. Habit history was taken to assess the type, duration and frequency. Periodontal health status was ascertained by community periodontal index (CPI). Tumor, node, metastasis (TNM) staging was obtained from medical records with the help of medical oncologist. The unstimulated whole saliva (UWS) was collected from subjects between 9 and 11 am by using simple drool method as per study by NAVAZESH.[4] Subjects were asked to refrain from eating, drinking, smoking, or oral hygiene procedure at least 1 hour prior to collection. Subjects were requested to swallow first, tilt their head forward and expectorate all saliva under non stimulatory conditions into sterile centrifuge tubes for 10 to 15 minutes without swallowing. A cooling centrifuge at 2500 rpm for 15 min at 4° C was used for centrifuging saliva to remove squamous cells and cell debris. Supernatant was separated into one ml aliquots and stored at − 70° C. Not more than one freeze thaw cycle allowed for each sample. For IL-6 estimation, solid phase sandwich enzyme linked immunosorbent assay (ELISA) was used. Salivary IL-6 was measured using specific ELISA kit Diaclone, France, using manufacturers recommendations. The colorimetric reaction developed is directly proportional to the concentration of IL-6 present in samples and standards and read at 450 nm wavelength in a microplate reader. The intensity of colour complex developed was read, and optical density (OD) values for each standard were plotted against expected concentration forming a standard curve. The concentration of IL-6 in the sample tested was measured using this standard curve.

The minimum detectable dose of IL-6 using this Diaclone IL-6 ELISA kit was found to be 2 pg/mL.

Statistical analysis

Data obtained was entered into MS-Excel and analyzed in IBM SPSS ver. 21 (IBM Corp., Armonk, NY. USA) Descriptive statistics mean, standard deviation, median with interquartile range and standard error were calculated. Shapiro wilk test was applied to find normality. Chi-square test, Fisher Exact test, Kruskal-Wallis test, and Mann-Whitney U test was applied to find significance. Correlations were done using the Spearman rank test. Simple, multiple Logistic regression (LR) analysis with backward LR was done. Receiver operating characteristic (ROC) analysis was done and area under curve (AUC) was calculated. Sensitivity and specificity were calculated. A $P < 0.05$ was considered statistically significant.

RESULTS

The sample of 90 subjects was divided into three groups of 30 each. The ages of the patients ranged from 21 to 77 years. The age, gender and demographic data of subjects are shown in Table S1. Among the habit types, tobacco chewing with or without occasional alcohol was predominant followed by smoking in study groups. None of the subjects except one had any habit in the control group. Regarding habit duration, 50% (n = 15) of group 1 had habit duration of more than 20 years, whereas 66.7% (n = 20) of Group 2 had habit duration of less than 10 years. 23.3% (n = 07) of Groups 1 and 2 had habit duration between 10 to 20 years. Buccal mucosa formed the predominant site of involvement in Groups 1 and 2 followed by the tongue in Group 1. Comparison of CPI for periodontal health among various groups for different age groups showed statistically no significant difference except for the age range 31 to 40 years [Table S2]. Group 2 had 70% (n = 21) cases of OSMF, 23.3%(n = 07) leukoplakia and 6.7% (n = 02) OLP cases.

With regards to TNM staging in Group 1, 63.3% (n = 19) were in stage 4, 26.7% (n = 8) in stage 3 and 10% (n = 3) in stage 2. On histological grading, 73.3% (n = 22) were well-differentiated OSCC (WDSCC), 23.3% (n = 7) moderately differentiated OSCC (MDSCC) and 3.3% (n = 1) poorly differentiated OSCC (PDSCC) in Group 1. Comparison of salivary IL-6 among different groups showed a significant increase in IL-6 when all three groups were compared ($P < 0.001$). The levels of salivary IL-6 showed a very significant increase in Group 1 (median 170.40, IQR 232.85) compared to Group 3 (median 5.83, IQR 3.29) ($P < 0.001$). Similarly, there was a significant increase in salivary IL-6 in Group 1 (median 170.40, IQR 232.85) compared to Group 2 (median 7.50, IQR 8.70) ($P < 0.001$). Group 2 showed increased salivary IL-6 compared to Group 3 though statistically not significant [Table 1 and Figure 1].

To explore if salivary IL-6 has any diagnostic utility in differentiating between various groups, LR analysis using ROC and AUC was used. Comparison of Group 1 to Group 3 using ROC curve analysis showed an AUC of 0.982 (95% confidence interval [CI]:0.955-1.000) ($P = 0.000$). It showed 71% sensitivity and 100% specificity at a cut-off value of 33.4000 pg/mL [Table 2 and Figure 2].

Comparison of Group 2 to Group 3 showed an AUC of 0.611 (95% CI: 0.453-0.769) and a *P*-value of 0.157. It showed 70% sensitivity and 54% specificity at a cut-off value of 5.93 pg/mL. Comparison of Group 1 to Group 2 showed an AUC of 0.611 (95% CI: 0.453-0.769) and a *P*-value of 0.157. It showed 63% sensitivity and 57% specificity at a cut-off value of 6.65 pg/mL. When salivary IL-6 was compared for clinical TNM staging in Group 1, it was statistically not significant. Comparison of IL-6 for histological grading of OSCC in Group 1 showed a statistically significant difference between WDSCC and MDSCC ($P = 0.03$) although PDSCC was only one case [Table S3]. Comparison of salivary IL-6 for clinical grading of OSMF showed statistically no significant difference.

DISCUSSION

Oral cancer is the sixth most common cancer worldwide and India has one of the highest incidence rates accounting for nearly 1/3 of world burden of oral cancer.[5] Despite advances in treatment modalities, long-term survival of patients has not improved significantly owing to advanced disease state at the time of presentation to clinician. This could be partially due to initial asymptomatic nature of the disease and subtle lesions, which may sometimes go unnoticed during clinical examination. Early detection is key to successful management and measurement of molecular markers in saliva seems more amenable in screening large population or high-risk groups. The clinical significance of salivary biomarkers in various malignancies was studied by several investigators.[6] Newer amplification techniques and highly sensitive assays make saliva an attractive alternative to serum. Cytokines are intercellular signalling proteins that play a role in normal growth, proliferation, tissue repair and angiogenesis.[7] The development of oral cancer has been shown to be closely associated with altered cytokine

Table 1: Comparison of salivary IL-6 among different groups

Variable	Group	Min.	Max.	Median	IQR	*P*			
						1 vs 2 vs 3	1 vs 3	2 vs 3	1 vs 2
IL-6	OSCC	9.60	263.10	170.40	232.85	<0.001	<0.001	0.16	<0.001
	OPMD	1.60	51.80	7.50	8.70				
	CONTROL	1.40	31.90	5.83	3.29				

IL-6, Interleukin-6; OSCC, oral squamous cell carcinoma; OPMD, oral potentially malignant disorders

Table 2: Area Under Curve, Sensitivity and Specificity for salivary IL-6 between Group 1 (OSCC) and Group 3 (controls)

Area Under the Curve-IL-6					Sensitivity and Specificity of IL-6 between Group 1 vs Group 3			
Area	SE	*P*	Asymptotic 95% Confidence Interval		Variable	Cut off	Sensitivity	Specificity
			Lower Bound	Upper Bound				
0.982	0.014	0.000	0.955	1.000	IL-6	33.4000	71%	100%

IL-6, Interleukin-6

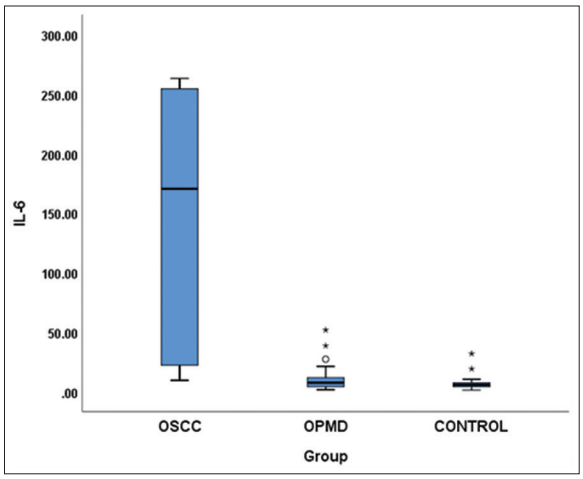

Figure 1: Comparison of salivary IL-6 among different groups

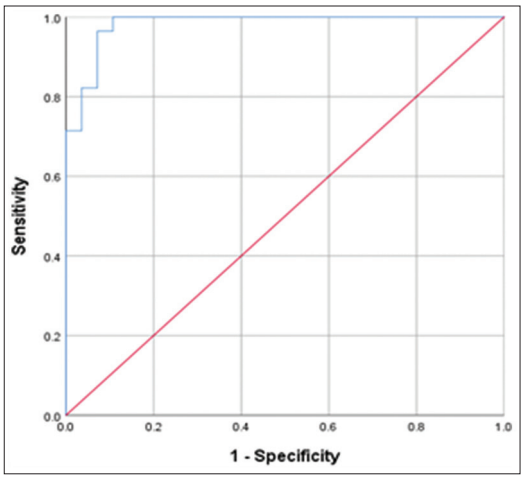

Figure 2: ROC curve and AUC for Groups 1 and 3

response. IL-6 is one such cytokine. In normal cells, cytokine stimulation results in growth inhibition but in oral cancer, cytokine stimulation leads to upregulation of positive cell cycle regulators NF-κB, signal transducers, activators of transcription and mitogen activated protein kinase pathway.[8] IL-6 has various biological functions and one among them is development of cancer. Transcription factor AP-2 secretion is upregulated by IL-6, which in turn causes activation of Ras and cerB2 oncogenes. IL-6 inactivates P^{53} tumor suppressor gene causing suppression of apoptosis and leading to uncontrolled cell proliferation.[9] Elevation of IL-6 promotes immune unresponsivenesss, induction of wasting, cachexia and hypercalcemia all of which are seen in OSCC patients with poor prognosis.[10] In the present study, the predominance of males over females in Groups 1 and 2 suggested increased incidence of OSCC and OPMD in men compared to women. This is in consistency with various studies.[9,11,12] Increased habit duration was seen in OSCC compared to OPMD. Except for the age range 31 to 40 years, no significant difference was observed between the groups for periodontal health as assessed by CPI index in our study. However, few studies showed that salivary IL-6 was significantly higher in OSCC compared to chronic periodontitis.[3,13] This outweighed any potential inflammatory background to elevated IL-6 expression rather than OSCC. The present study showed very significant increase in salivary IL-6 in OSCC compared to OPMD and controls ($P < 0.001$). A very significant increase in salivary IL-6 in OSCC compared to controls seen in our study is in line with various studies.[1,5,9,13-23] However, John *et al*.[10] found no significant difference in salivary IL-6 between OSCC and controls. The increased levels seen in our study and many studies could be result of altered cytokine production occurring chiefly in oral cavity due to constant contact of saliva with oral cancerous lesion. Increased salivary IL-6 reflects local production of cytokines in OSCC rather than local inflammation, periodontitis, and smoking.[13,19] Tumor infiltrating lymphocytes and oral cancer cells are responsible for increased IL-6.[24] Our study showed very significant increase in salivary IL-6 in OSCC

compared to OPMD ($P < 0.001$). This was in accordance with various studies.[5,9,16,20,22,23] Higher concentration of salivary IL-6 might reflect development of OSCC from OPMD. On histological grading of OSCC, our study showed significant increase in salivary IL-6 in MDSCC compared to WDSCC ($P = 0.03$). This was consistent with the study of Dinesh *et al*.[5] who also showed significant correlation with histopathological grading, suggesting increased salivary IL-6 levels are associated with tumor aggressiveness and severity. However, study by Panneer *et al*.[9] and Rani *et al*.[13] showed no such difference between histological grades of OSCC. This could be due to unequal distribution of cases with histologically proven OSCC. For various TNM stages of OSCC, our study showed increased salivary IL-6 in stage IV compared to stage III and II though statistically not significant. This was similar to the study by Dineshkumar *et al*.,[5] whereas Panneer *et al*.[9] found no significant difference between all other stages except stage II and IV. The present study showed an increase in salivary IL-6 in OPMD compared to controls though statistically not significant. This was in accordance with a few studies.[1,19,22] However, some studies showed significant difference in salivary IL-6 between OPMD and controls.[5,9,15,20,23,25,26] Studies by Kaur and Jacobs[8] and Zhu *et al*.[27] showed significant increase in salivary IL-6 with increasing severity of dysplasia. A significant increase in salivary IL-6 was seen in erosive OLP compared to reticular OLP.[27] Our study showed no significant difference between salivary IL-6 and clinical grading of OSMF. This could be due to unequal distribution of cases in Group 2 and most of our OSMF cases are clinical grade 1 and 2. Dinesh *et al*.[5] and Panneer *et al*.[9] also showed no significant difference in salivary IL-6 levels based on histological, clinical grading of OPMD, clinical types of leukoplakia and between high risk and low risk sites of leukoplakia.

Our data was subjected to ROC curve analysis to evaluate the predictive power and AUC was calculated as measure of utility of IL-6 in detecting OSCC and OPMD from controls. The present study with largest AUC of 0.982 under ROC curve for salivary IL-6 showed the strongest predictive power in differentiating OSCC from controls. It yielded a sensitivity of 71% and a specificity of 100% in differentiating OSCC from controls at a cut-off value of 33.4000 pg/mL ($P = 0.000$).

Limitations

The present study did not include a comparison of salivary IL-6 with different histological grades of dysplasia in leukoplakia and oral Lichen planus.

CONCLUSION

The present study indicated that altered cytokine production occurs in OSCC and OPMD and salivary IL-6 could be held as a promising biomarker. Our study revealed the strong predictive power of salivary IL-6 in differentiating OSCC from controls and higher salivary IL-6 expression was associated with aggressiveness of tumor. The findings of the present study could aid researchers and clinicians in post-operative management of OSCC patients and monitor treatment outcomes or disease recurrence after therapy completion. More longitudinal studies with large sample sizes, inclusion of wide variables and possible elimination of factors that may influence salivary IL-6 are required for its diagnostic applicability in identifying high-risk groups in mass screening of the Indian population and development of future point-of-care salivary diagnostics.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Babiuch K, Kuśnierz-Cabala B, Kęsek B, Okoń K, Darczuk D, Chomyszyn-Gajewska M. Evaluation of proinflammatory, NF-kappaB dependent cytokines: IL-1α, IL-6, IL-8, and TNF-α in tissue specimens and saliva of patients with oral squamous cell carcinoma and oral potentially malignant disorders. J Clin Med 2020;9:867.
2. Prasad G, McCullough M. Chemokines and cytokines as salivary biomarkers for the early diagnosis of oral cancer. Int J Dent 2013;2013:813756.
3. Lisa Cheng YS, Jordan L, Gorugantula LM, Schneiderman E, Chen HS, Rees T. Salivary interleukin-6 and -8 in patients with oral cancer and patients with chronic oral inflammatory diseases. J Periodontol 2014;85:956-65.
4. Navazesh M. Methods for collecting saliva. Ann N Y Acad Sci 1993;694:72-7.
5. Dineshkumar T, Ashwini BK, Rameshkumar A, Rajashree P, Ramya R, Rajkumar K. Salivary and serum interleukin-6 levels in oral premalignant disorders and squamous cell carcinoma: Diagnostic value and clinicopathologic correlations. Asian Pac J Cancer Prev 2016;17:4899-906.
6. Bigler LR, Streckfus CF, Dubinsky WP. Salivary biomarkers for the detection of malignant tumors that are remote from the oral cavity. Clin Lab Med 2009;29:71–85.
7. Chundru VNS, Nirmal RM, Srikanth B, Bojji M, Midhun N, Lakshmi BJ. Salivaomics for oral cancer detection: An insight. J Pharm Bioallied Sci 2021;13(Suppl 1):S52-6.
8. Kaur J, Jacobs R. Proinflammatory cytokine levels in oral lichen planus, oral leukoplakia, and oral submucous fibrosis. J Korean Assoc Oral Maxillofac Surg 2015;41:171-5.
9. Panneer Selvam N, Sadaksharam J. Salivary interleukin-6 in the detection of oral cancer and precancer. Asia -Pac J Clin Oncol 2015;11:236-41.
10. St John MA, Li Y, Zhou X, Denny P, Ho CM, Montemagno C, *et al*. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity

and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2004;130:929–35.
11. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009;45:309–16.
12. Sankaranarayanan R. Oral cancer in India: An epidemiologic and clinical review. Oral Surg Oral Med Oral Pathol 1990;69:325–30.
13. Rani NAJ, Harshavardhan BG, Srinivasan S, Gopal SK. Evaluation of salivary interleukin-6 in patients with oral squamous cell carcinoma, oral potentially malignant disorders, chronic periodontitis and in healthy controls: A cross-sectional comparative study. Ann Maxillofac Surg 2023;13:70-5.
14. Vucicević Boras V, Cikes N, Lukać J, Virag M, Cekić-Arambasin A. Salivary and serum interleukin 6 and basic fibroblast growth factor levels in patients with oral squamous cell carcinoma. Minerva Stomatol 2005;54:569–73.
15. Rhodus NL, Ho V, Miller CS, Myers S, Ondrey F. NF-kappaB dependent cytokine levels in saliva of patients with oral preneoplastic lesions and oral squamous cell carcinoma. Cancer Detect Prev 2005;29:42–5.
16. Rhodus NL, Cheng B, Myers S, Miller L, Ho V, Ondrey F. The feasibility of monitoring NF-kappaB associated cytokines: TNF-alpha, IL-1alpha, IL-6 and IL-8 in whole saliva for the malignant transformation of oral lichen planus. Mol Carcinog 2005;44:77–82.
17. Katakura A, Kamiyama I, Takano N, Shibahara T, Muramatsu T, Ishihara K, *et al*. Comparison of salivary cytokine levels in oral cancer patients and healthy subjects. Bull Tokyo Dent Coll 2007;48:199–203.
18. SahebJamee M, Eslami M, AtarbashiMoghadam F, Sarafnejad A. Salivary concentration of TNFalpha, IL1alpha, IL6, and IL8 in oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal 2008;3:E292–5.
19. Brailo V, Vucicevic-Boras V, Lukac J, Lukenda DB, Alajbeg IZ, Milenovic A, *et al*. Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer. Med Oral Patol Oral Cir Bucal 2012;17:e10–5.
20. Abdel-Fattah M, El Gaaly K, Al-Refai M, Galwash D. Estimation of salivary levels of interleukine-6 (IL-6) and interleukine-8 (IL-8) in the oral pre-malignant and malignant lesions. Egypt Dent J 2016;62:193-201.
21. Al Talabani, Shanaz M, Abdul-Wahab R. Serum and salivary levels of proinflammatory cytokines as potential biomarkers in the diagnosis of oral squamous cell carcinoma. J Bagh College Dentistry 2009;21:60-5.
22. Patil S, Kaswan S, Rahman F, Dhoni B, Wadhawan R. Salivary and serum tumor necrosis factor alpha, interleukin 1 alpha, interleukin 1 beta, interleukin 6 and interleukin 8 in patients with oral carcinoma and leukoplakia. J Nepal Dent Asso 2013;13:33-8.
23. Rajkumar K, Kumar AR, Ramyamalini V, Nandhini G, Kumar TD, Ashwini BK, *et al*. Estimation of serological and salivary biomarkers in patients with oral squamous cell carcinoma, premalignant lesions and conditions. SRM Uni J Dent Sci 2010;1:14–9.
24. Pries R, Wollenberg B. Cytokines in head and neck cancer. Cytokine Growth Factor Rev 2006;17:141–6.
25. Brailo V, Vucićević-Boras V, Cekić-Arambasin A, Alajbeg IZ, Milenović A, Lukac J. The significance of salivary interleukin 6 and tumor necrosis factor alpha in patients with oral leukoplakia. Oral Oncol 2006;42:370–3.
26. Sharma M, Bairy I, Pai K, Satyamoorthy K, Prasad S, Berkovitz B, *et al*. Salivary IL-6 levels in oral leukoplakia with dysplasia and its clinical relevance to tobacco habits and periodontitis. Clin Oral Investig 2011;15:705–14.
27. Zhu ZD, Ren XM, Zhou MM, Chen QM, Hua H, Li CL. Salivary cytokine profile in patients with oral lichen planus. J Dent Sci 2022;17:100-5.

SUPPLEMENTARY MATERIAL

Table S1: Demographic data of study subjects

	Category	OSCC		OPMD		CONTROL	
		Count	%	Count	%	Count	%
Age	21–30	1	3.3	10	33.3	9	30.0
	31–40	8	26.7	12	40.0	9	30.0
	41–50	8	26.7	7	23.3	8	26.7
	51–60	4	13.3	1	3.3	2	6.7
	>60	9	30.0	0	0	2	6.7
	Total	30	100.0	30	100.0	30	100.0
Gender	Males	21	70.0	25	83.3	15	50.0
	Females	9	30.0	5	16.7	15	50.0
	Total	30	100.0	30	100.0	30	100.0
Habit duration in years	No habit	1	3.3	1	3.3	29	96.7
	<10 years	7	23.3	20	66.7	1	3.3
	10 to 20 years	7	23.3	7	23.3	0	0
	>20 years	15	50.0	2	6.7	0	0
	Total	30	100.0	30	100.0	30	100.0

OSCC, Oral squamous cell carcinoma; OPMD, oral potentially malignant disorders

Table S2: Distribution of periodontal status (highest CPI score) among study groups

Age	Group	Highest CPI Score										*P*
		0		1		2		3		4		
		Count	%	Count	%	Count	%	Count	%	Count	%	
21–30	OSCC	0	0.0	1	100.0	0	0.0	0	0	0	0	0.12
	OPMD	1	10.0	4	40.0	5	50.0	0	0	0	0	
	CONTROL	5	55.6	3	33.	1	11.1	0	0	0	0	
31–40	OSCC	0	0.0	1	12.5	7	87.5	0	0.0	0	0	0.003
	OPMD	1	8.3	0	0.0	10	83.3	1	8.3	0	0	
	CONTROL	0	0.0	7	77.8	1	11.1	1	11.1	0	0	
41–50	OSCC	0	0.0	1	12.5	6	75.0	1	12.5	0	0	0.35
	OPMD	0	0.0	2	33.3	2	33.3	2	33.3	0	0	
	CONTROL	2	25.0	1	12.5	4	50.0	1	12.5	0	0	
51–60	OSCC	0	0	0	0	3	75.0	0	0.0	1	25.0	0.31
	OPMD	0	0	0	0	0	0.0	1	100.0	0	0.0	
	CONTROL	0	0	0	0	1	50.0	1	50.0	0	0.0	
>60	OSCC	0	0	0	0	4	50.0	4	50.0	0	0	0.19
	CONTROL	0	0	0	0	0	0.0	2	100.0	0	0	

Healthy (0) Bleeding (1) Calculus (2) Periodontal pocket of 4—5 mm (3)

Periodontal pocket 6 mm or more (4)

CPI, community periodontal index; OSCC, oral squamous cell carcinoma; OPMD, oral potentially malignant disorders

Table S3: Comparison of Salivary IL-6 for histological grading of OSCC

Variable	Histological grading	Minimum	Maximum	Median	IQR	*P*
IL-6	WDSCC	9.60	258.10	110.75	232.33	0.03
	MDSCC	247.30	263.10	253.00	9.70	

Note: Poorly differentiated OSCC is only one sample. WDSCC, well-differentiated OSCC; MDSCC, moderately differentiated OSCC; IL-6, Interleukin-6