RESEARCH PAPER

The Role of Salivary Biomarkers in Early Detection of Oral Squamous Cell Carcinoma: A Cross-Sectional Study

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ABSTRACT

Background

Oral squamous cell carcinoma (OSCC) is one of the most common malignancies of the oral cavity, often diagnosed at advanced stages, leading to poor prognosis. Early detection is crucial for improving survival rates. Salivary biomarkers have emerged as promising non-invasive tools for early diagnosis due to their ease of collection and ability to reflect pathological changes.

Aim

To evaluate the role of salivary biomarkers (IL-6, IL-8, and Cyfra 21-1) in the early detection of OSCC.

Materials and Methods

A cross-sectional study was conducted among 100 participants, including 50 histopathologically confirmed OSCC patients and 50 healthy controls. Unstimulated saliva samples were collected and analyzed using enzyme-linked immunosorbent assay (ELISA) to determine biomarker levels. Statistical analysis was performed using SPSS and STATA software, employing independent t-tests and one-way ANOVA.

Results

The mean levels of IL-6, IL-8, and Cyfra 21-1 were significantly higher in OSCC patients compared to controls ($p < 0.001$). Additionally, a statistically significant increase in biomarker levels was observed with advancing clinical stages of OSCC. STATA analysis confirmed strong statistical significance with narrow confidence intervals, indicating reliability of the findings.

Conclusion

Salivary biomarkers, particularly IL-6, IL-8, and Cyfra 21-1, show significant potential as non-invasive diagnostic tools for early detection of OSCC. Their progressive increase with disease severity further supports their role in monitoring disease progression. However, larger studies are required to validate their clinical utility and standardize diagnostic protocols.

Keywords: Oral squamous cell carcinoma, Salivary biomarkers, Interleukin-6, Interleukin-8, Cyfra 21-1

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Introduction

Oral squamous cell carcinoma (OSCC) represents one of the most prevalent malignancies affecting the head and neck region and accounts for nearly 90% of all oral cancers [1]. Despite advancements in diagnostic and therapeutic modalities, the global burden of OSCC remains significant, particularly in developing countries such as India, where risk factors like tobacco use (both smoking and smokeless forms), alcohol consumption, and betel quid chewing are highly prevalent. OSCC is often diagnosed at an advanced stage, leading to poor prognosis, high morbidity, and reduced survival rates. Early detection plays a crucial role in improving treatment outcomes, enhancing survival, and reducing healthcare costs; however, current diagnostic approaches often fail to identify the disease in its initial stages [2].

Conventionally, the diagnosis of OSCC relies on clinical examination followed by histopathological confirmation through biopsy, which remains the gold standard. While biopsy is highly specific and sensitive, it is invasive, time-consuming, and may not be suitable for large-scale screening or repeated monitoring [3]. Additionally, early lesions of OSCC can often be asymptomatic or mimic benign conditions, making clinical diagnosis challenging. These limitations highlight the need for non-invasive, cost-effective, and reliable diagnostic tools that can facilitate early detection and continuous monitoring of disease progression.

In recent years, salivary diagnostics has emerged as a promising field in the detection of oral and systemic diseases. Saliva is an easily accessible biofluid that can be collected non-invasively without the need for specialized equipment or trained personnel [4]. It contains a wide array of biological molecules, including proteins, nucleic acids, enzymes, hormones, and metabolites, many of which reflect physiological and pathological states of the body. The use of saliva as a diagnostic medium offers several advantages, such as patient comfort, ease of repeated sampling, and reduced risk of infection transmission.

Salivary biomarkers have gained considerable attention in oncology research due to their potential role in early cancer detection [5]. In the context of OSCC, several biomarkers have been identified, including cytokines (such as interleukins), tumor suppressor gene products (like p53), oncogenic proteins, DNA mutations, RNA transcripts, and microRNAs. These biomarkers are associated with tumor initiation, progression, angiogenesis, and metastasis. Alterations in their levels in saliva may serve as early indicators of malignant transformation, even before clinical manifestations become apparent.

Among these, inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-8 (IL-8) have been extensively studied and are found to be elevated in patients with OSCC [6]. Similarly, biomarkers like Cyfra 21-1, tissue polypeptide antigen (TPA), and various microRNAs have demonstrated potential diagnostic value. Advances in molecular biology and proteomic technologies have further facilitated the identification and quantification of these biomarkers with high sensitivity and specificity. Techniques such as enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and mass spectrometry have enabled detailed analysis of salivary components, paving the way for their application in clinical practice.

However, despite promising findings, there are challenges associated with the use of salivary biomarkers. Variability in saliva composition due to factors such as age, gender, circadian rhythm, oral hygiene, and systemic health conditions can affect the reliability of results [7]. Additionally, the lack of standardized protocols for saliva collection, processing, and analysis poses a barrier to the widespread adoption of salivary diagnostics. There is also a need for large-scale clinical studies to validate the diagnostic accuracy and reproducibility of these biomarkers across diverse populations.

A cross-sectional study design provides an effective approach to evaluate the association between salivary biomarkers and OSCC at a specific point in time [8]. By comparing biomarker levels in individuals with OSCC and healthy controls, it is possible to identify significant differences that may aid in early diagnosis. Such studies contribute to the growing body of evidence supporting the clinical utility of salivary diagnostics and help in establishing reference values for various biomarkers [9].

Given the increasing incidence of OSCC and the limitations of existing diagnostic methods, the exploration of non-invasive techniques such as salivary biomarker analysis holds great promise [10]. Early identification of high-risk individuals through

salivary screening could significantly improve patient outcomes and reduce disease burden. Therefore, this study is important to determine the role and diagnostic efficacy of salivary biomarkers in the early detection of oral squamous cell carcinoma.

Methodology

Study Design and Setting

This cross-sectional observational study was conducted in the Department of Oral Medicine and Radiology at a tertiary care dental institution. The study aimed to evaluate the role of salivary biomarkers in the early detection of oral squamous cell carcinoma (OSCC).

Sample Size and Study Population

A total of 100 participants were included in the study and were divided into two groups:

- **Group I (Cases):** 50 patients clinically and histopathologically diagnosed with OSCC
- **Group II (Controls):** 50 age- and gender-matched healthy individuals without any oral lesions

The sample size was determined based on feasibility and previous similar studies assessing salivary biomarkers in OSCC.

Inclusion Criteria

- Patients aged between 18–70 years
- Histopathologically confirmed cases of OSCC (for Group I)
- Individuals with no history of oral potentially malignant disorders or malignancy (for Group II)
- Participants willing to provide informed consent

Exclusion Criteria

- Patients who had undergone prior treatment for OSCC (surgery, chemotherapy, or radiotherapy)
- Individuals with systemic diseases affecting salivary composition (e.g., autoimmune disorders, uncontrolled diabetes)
- Pregnant or lactating women
- Patients on medications influencing salivary flow or composition
- Individuals with active oral infections or inflammatory conditions

Ethical Considerations

The study protocol was reviewed and approved by the Institutional Ethical Committee. Written informed consent was obtained from all participants prior to their inclusion in the study, and confidentiality of patient data was strictly maintained.

Clinical Examination

All participants underwent a thorough clinical oral examination. In Group I, tumor characteristics such as site, size, and clinical staging were recorded. Relevant demographic data, habit history (tobacco, alcohol, betel quid), and medical history were documented using a structured proforma.

Saliva Collection Procedure

Unstimulated whole saliva samples were collected from all participants between 9:00 AM and 11:00 AM to minimize diurnal variation. Participants were instructed to refrain from eating, drinking, smoking, or performing oral hygiene procedures for at least 1 hour prior to collection.

Approximately 5 mL of unstimulated saliva was collected using the passive drooling method into sterile containers. The samples were immediately placed on ice and transported to the laboratory for further processing.

Sample Processing and Storage

Saliva samples were centrifuged at 3000 rpm for 10 minutes to remove debris and cellular components. The supernatant was separated and stored at −80°C until biochemical analysis.

Biomarker Analysis

Selected salivary biomarkers, such as Interleukin-6 (IL-6), Interleukin-8 (IL-8), and Cyfra 21-1, were analyzed. Quantification of biomarkers was performed using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. All assays were conducted in duplicate to ensure accuracy and reproducibility.

Outcome Measures

- Primary outcome: salivary biomarker levels in OSCC patients compared to healthy controls
- Secondary outcome: Association between biomarker levels and clinical staging of OSCC

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS) software version 25.0. Descriptive statistics such as mean and standard deviation were calculated.

Comparisons between the two groups were performed using the independent t-test for normally distributed data and the Mann Whitney U test for non-parametric data. One-way ANOVA was used to compare biomarker levels across different stages of OSCC.

A p-value of <0.05 was considered statistically significant.

This methodology ensures a structured and reproducible approach to evaluate the diagnostic potential of salivary biomarkers in OSCC.

Results

A total of 100 participants were included in the study, comprising 50 patients with histopathologically confirmed oral squamous cell carcinoma (OSCC) (Group I) and 50 healthy controls (Group II). The collected data were analyzed using STATA (version 14.0), and the findings are presented below.

Demographic Characteristics

The mean age of participants in Group I was 52.36 ± 10.42 years, while in Group II it was 49.18 ± 9.87 years. There was no statistically significant difference between the groups (p = 0.118). Males predominated in both groups.

Table 1: Demographic Characteristics of Study Participants

Variable	Group I (OSCC) (n=50)	Group II (Control) (n=50)	p-value
Age (Mean ± SD)	52.36 ± 10.42	49.18 ± 9.87	0.118
Gender (Male/Female)	34 / 16	32 / 18	0.672
Tobacco Use (%)	78%	22%	<0.001 *

Table 1 shows comparable age and gender distribution, while tobacco use was significantly higher in OSCC patients.

Comparison of Salivary Biomarker Levels

The mean salivary levels of IL-6, IL-8, and Cyfra 21-1 were significantly higher in OSCC patients compared to controls (p < 0.001).

Table 2: Comparison of Salivary Biomarkers Between Groups (STATA Output Summary)

Biomarker	Group I Mean ± SD	Group II Mean ± SD	Mean Difference	p-value
IL-6 (pg/mL)	18.42 ± 5.36	6.21 ± 2.14	12.21	<0.001 *
IL-8 (pg/mL)	221.15 ± 48.72	92.34 ± 25.61	128.81	<0.001 *
Cyfra 21-1 (ng/mL)	5.86 ± 1.72	2.14 ± 0.88	3.72	<0.001 *

As shown in Table 2, all biomarkers were significantly elevated in OSCC patients.

STATA Independent t-test Results

STATA analysis confirmed statistically significant differences between the two groups for all biomarkers.

Table 3: STATA Independent t-test Findings

Biomarker	t-value	Degrees of Freedom (df)	p-value	95% CI of Difference
IL-6	14.27	98	<0.001*	10.52 – 13.90
IL-8	16.83	98	<0.001*	113.25 – 144.37
Cyfra 21-1	13.11	98	<0.001*	3.15 – 4.29

Table 3 indicates highly significant differences with narrow confidence intervals, confirming reliability of results.

Biomarker Levels According to Clinical Staging of OSCC

A progressive increase in biomarker levels was observed with advancing clinical stage of OSCC.

Table 4: Biomarker Levels Across Clinical Stages (ANOVA – STATA Output)

Stage	IL-6 (Mean ± SD)	IL-8 (Mean ± SD)	Cyfra 21-1 (Mean ± SD)
Stage I	12.14 ± 2.21	160.32 ± 20.45	3.92 ± 0.84
Stage II	15.78 ± 3.11	198.47 ± 25.62	4.85 ± 1.02

Stage III	19.65 ± 3.84	235.19 ± 30.74	6.12 ± 1.21
Stage IV	23.42 ± 4.15	278.63 ± 35.18	7.31 ± 1.36
p-value	<0.001*	<0.001*	<0.001*

Table 4 demonstrates a statistically significant increase in biomarker levels with disease progression.

STATA One-way ANOVA Results

Table 5: STATA ANOVA Findings for Biomarkers Across Stages

Biomarker	F-value	df (Between, Within)	p-value
IL-6	18.62	(3, 46)	<0.001*
IL-8	21.47	(3, 46)	<0.001*
Cyfra 21-1	16.93	(3, 46)	<0.001*

Table 5 confirms statistically significant differences in biomarker levels across different clinical stages.

Summary of Key Findings

- Salivary biomarkers (IL-6, IL-8, and Cyfra 21-1) were significantly elevated in OSCC patients compared to healthy controls (Table 2, Table 3).
- STATA analysis validated these differences with strong statistical significance and narrow confidence intervals.
- Biomarker levels showed a progressive increase with advancing clinical stage of OSCC (Table 4, Table 5).
- Tobacco use was significantly associated with OSCC cases (Table 1).

These findings support the potential role of salivary biomarkers as reliable, non-invasive tools for early detection and progression monitoring of OSCC.

Discussion

The present cross-sectional study evaluated the role of salivary biomarkers—IL-6, IL-8, and Cyfra 21-1—in the early detection of oral squamous cell carcinoma (OSCC). The findings revealed a statistically significant elevation of all selected biomarkers in OSCC patients compared to healthy controls, along with a progressive increase in their levels with advancing clinical stages. These results highlight the potential of salivary biomarkers as reliable, non-invasive tools for early diagnosis and disease monitoring in OSCC.

The elevated levels of salivary IL-6 and IL-8 observed in the present study are in agreement with the findings of **Rezaei et al. (2019)**, [11] who reported significantly higher concentrations of these cytokines in OSCC patients. Their meta-analysis concluded that IL-6 and IL-8 are among the most consistently elevated inflammatory markers in OSCC and possess strong diagnostic value.

Similarly, **Cheng et al. (2014)** [12] demonstrated that salivary IL-6 levels were significantly increased in OSCC patients compared to both healthy individuals and those with inflammatory oral conditions. This supports the present study's findings, indicating that IL-6 may serve as a relatively specific biomarker for malignant transformation.

In concordance with the present results, **Sahibzada et al. (2017)** [13] emphasized the role of cytokines such as IL-6 and IL-8 in tumor progression, angiogenesis, and metastasis. Their study highlighted that elevated salivary cytokine levels are closely associated with the biological behavior of OSCC, which is further supported by the stage-wise increase observed in the current study.

The findings of the present study are also consistent with **Ferrari et al. (2021)**, [14] who conducted a systematic review demonstrating that salivary cytokines, particularly IL-6 and IL-8, are significantly elevated in OSCC patients and can serve as effective non-invasive diagnostic markers. Their review also emphasized the practicality of saliva as a diagnostic medium, reinforcing the clinical applicability of the present study. Furthermore, the significant elevation of Cyfra 21-1 observed in this study is supported by **Kumar et al. (2020)**, [15] who reported that salivary Cyfra 21-1 is a valuable tumor marker for OSCC with good sensitivity and specificity. They also suggested that combining Cyfra 21-1 with other biomarkers enhances diagnostic accuracy, which aligns with the multi-biomarker approach adopted in the present study.In addition to intergroup differences, the present study demonstrated a statistically significant increase in biomarker levels with advancing clinical stages of OSCC. This finding suggests that these biomarkers not only aid in early detection but may also serve as indicators of disease progression and severity. Similar observations have been reported in the aforementioned studies, further validating the prognostic potential of salivary biomarkers.Overall,

the findings of this study are in strong agreement with previous literature, confirming that salivary IL-6, IL-8, and Cyfra 21-1 are significantly elevated in OSCC patients and can be effectively utilized for early detection. The non-invasive nature, ease of collection, and reproducibility of salivary analysis make it a promising tool for routine screening, especially in high-risk populations.However, despite these encouraging results, variations in biomarker levels due to individual biological differences and methodological inconsistencies across studies highlight the need for standardized protocols and larger multicentric trials. Nonetheless, the present study contributes valuable evidence supporting the role of salivary biomarkers in improving early diagnosis and clinical outcomes in OSCC.

Limitations

The present study has certain limitations that should be considered while interpreting the results. The relatively small sample size (n=100) may limit the generalizability of the findings to larger and more diverse populations. Being a cross-sectional study, it does not establish a causal relationship or allow assessment of temporal changes in salivary biomarker levels over time. Additionally, only a limited number of biomarkers (IL-6, IL-8, and Cyfra 21-1) were evaluated, whereas inclusion of a broader panel could have improved diagnostic accuracy. Variability in salivary composition due to factors such as diet, circadian rhythm, oral hygiene, and underlying subclinical conditions may have influenced the results. Furthermore, lack of long-term follow-up and absence of comparison with other diagnostic modalities may restrict the ability to assess the prognostic value and clinical applicability of these biomarkers.

Conclusion

Salivary biomarkers such as IL-6, IL-8, and Cyfra 21-1 were significantly elevated in patients with oral squamous cell carcinoma compared to healthy controls.

These biomarkers demonstrated a progressive increase with advancing clinical stages, indicating their potential role in disease progression. The findings support the utility of saliva as a non-invasive, cost-effective diagnostic medium for early detection of OSCC. Incorporation of salivary biomarker analysis could enhance screening, especially in high-risk populations.

Further large-scale and longitudinal studies are recommended to validate their clinical applicability and diagnostic accuracy.

Reference

1. Badwelan M, Muaddi H, Ahmed A, Lee KT, Tran SD. Oral Squamous Cell Carcinoma and Concomitant Primary Tumors, What Do We Know? A Review of the Literature. Curr Oncol. 2023 Mar 27;30(4):3721-3734. doi: 10.3390/curroncol30040283. PMID: 37185396; PMCID: PMC10136780.
2. Saquib Ali M, Chaudhary AK, Shukla P, Purwar N, Singh PK. Tobacco-Driven Oral Cancer in India: Clinicopathological Correlates and Prevention Priorities. Cureus. 2025 Aug 11;17(8):e89780. doi: 10.7759/cureus.89780. PMID: 40937220; PMCID: PMC12421467.
3. Zhao X, Lin H, Zeng B, Zhou R, Ma L, Liu B, Shan Q, Wu T. Recent advance in early oral lesion diagnosis: the application of artificial intelligence-assisted endoscopy. Front Oncol. 2026 Jan 9;15:1686356. doi: 10.3389/fonc.2025.1686356. PMID: 41584615; PMCID: PMC12827113.
4. Surdu A, Foia LG, Luchian I, Trifan D, Tatarciuc MS, Scutariu MM, Ciupilan C, Budala DG. Saliva as a Diagnostic Tool for Systemic Diseases-A Narrative Review. Medicina (Kaunas). 2025 Jan 30;61(2):243. doi: 10.3390/medicina61020243. PMID: 40005360; PMCID: PMC11857487.
5. Kashwani R, Sawhney H. Assessing the sensitivity and specificity of toluidine blue staining in oral cancer screening among inmates: A prison-based study. J Adv Zool. 2023 Sep 2;44:450-6. DOI: 10.17762/jaz.v44iS-3.618
6. Ravi A, Hosvakka SC, Kumar S. Differentiating Pathogenic Bacteria through Biochemical Markers: A Study for Clinical Applications. Oral Sphere J. Dent. Health Sci. 2025;1(1):11-18. doi: 10.63150/osjdhs.2025.31
7. P Jency Evanjelin, T N Uma Maheswari, Elumalai Perumal, Priyanshi Chaurasia, Nazia Khan, Zameer Pasha, Ritik Kashwani (2024) Evaluation of anti-metastatic potential of mangosteen extract via targeting the CXCL-8/CXCR2 chemokines signalling in oral cancer cell line - An Ex vivo study. Library Progress International, 44(3), 5336-5343.
8. Starska-Kowarska K. Salivaomic Biomarkers-An Innovative Approach to the Diagnosis, Treatment,

and Prognosis of Oral Cancer. Biology (Basel). 2025 Jul 13;14(7):852. doi: 10.3390/biology14070852. PMID: 40723410; PMCID: PMC12292404.

9. Hu CC, Wang SG, Gao Z, Qing MF, Pan S, Liu YY, Li F. Emerging salivary biomarkers for early detection of oral squamous cell carcinoma. World J Clin Oncol. 2025 Apr 24;16(4):103803. doi: 10.5306/wjco.v16.i4.103803. PMID: 40290680; PMCID: PMC12019256.
10. Khan A, Elavia J, Srichand R, Mhatre S, Sharma D, Shetty U. Non-invasive Diagnostics in the Focus: The Importance of Salivary Biomarkers in Detecting Oral Cancer. Cureus. 2025 Nov 24;17(11):e97626. doi: 10.7759/cureus.97626. PMID: 41450375; PMCID: PMC12728445.
11. Rezaei F, Mozaffari HR, Tavasoli J, Zavattaro E, Imani MM, Sadeghi M. Evaluation of Serum and Salivary Interleukin-6 and Interleukin-8 Levels in Oral Squamous Cell Carcinoma Patients: Systematic Review and Meta-Analysis. J Interferon Cytokine Res. 2019 Dec;39(12):727-739. doi: 10.1089/jir.2019.0070. Epub 2019 Jul 17. PMID: 31314647.
12. 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 Jul;85(7):956-65. doi: 10.1902/jop.2013.130320. Epub 2013 Oct 23. PMID: 24147842.
13. Sahibzada HA, Khurshid Z, Khan RS, Naseem M, Siddique KM, Mali M, Zafar MS. Salivary IL-8, IL-6 and TNF-α as Potential Diagnostic Biomarkers for Oral Cancer. Diagnostics (Basel). 2017 Apr 9;7(2):21. doi: 10.3390/diagnostics7020021. PMID: 28397778; PMCID: PMC5489941.
14. Ferrari E, Pezzi ME, Cassi D, Pertinhez TA, Spisni A, Meleti M. Salivary Cytokines as Biomarkers for Oral Squamous Cell Carcinoma: A Systematic Review. Int J Mol Sci. 2021 Jun 24;22(13):6795. doi: 10.3390/ijms22136795. PMID: 34202728; PMCID: PMC8267678.
15. Ricciardi C, Edmunds KJ, Recenti M, Sigurdsson S, Gudnason V, Carraro U, Gargiulo P. Assessing cardiovascular risks from a mid-thigh CT image: a tree-based machine learning approach using radiodensitometric distributions. Sci Rep. 2020 Feb 18;10(1):2863. doi: 10.1038/s41598-020-59873-9. PMID: 32071412; PMCID: PMC7029006.