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RESEARCH ARTICLE
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# ROLE OF SALIVARY BIOMARKERS IN EARLY DETECTION OF ORAL CANCER – INVESTIGATING HOW SPECIFIC BIOMARKERS IN SALIVA CAN BE USED FOR EARLY DIAGNOSIS AND PROGNOSIS OF ORAL SQUAMOUS CELL CARCINOMA

**Dr. Tariq Ahmad Lone[1], Dr. Arezoo Bashir Peerzada[2*], Dr. Arman Bashir Peerzadah[3], Dr. Roohie Khanam[4]**

[1]Postgraduate Scholar, Department of Oral and Maxillofacial Pathology and Microbiology, Government Dental College Srinagar, India
[2]Dental Surgeon, Dr. Manzoor Orodental Clinic, Srinagar, India
[3]Dental Surgeon, Dr. Manzoor Orodental Clinic, Srinagar, India
[4]Dental Surgeon, Dr. Manzoor Orodental Clinic, Srinagar, India

[*]**Corresponding Author:** Dr. Arezoo Bashir Peerzada
[*]Dental Surgeon, Dr. Manzoor Orodental Clinic, Srinagar, India

**Abstract**

**Background**: Oral squamous cell carcinoma (OSCC) is among the most common malignancies, particularly in regions with high tobacco and betel nut consumption. Delayed diagnosis contributes to poor prognosis and high mortality rates. Traditional diagnostic methods are invasive, costly, and time-consuming. Salivary biomarkers offer a promising non-invasive alternative for early detection and prognosis, potentially improving patient outcomes.

**Aim**: This study evaluates the diagnostic potential of salivary biomarkers, including IL-6, IL-8, VEGF, miRNA-21, and MMP-9, in detecting OSCC at an early stage and assessing disease progression.

**Methods**: A prospective study was conducted at Government Dental College, Srinagar, over a period of 24 months (July 2022 – July 2024). Saliva samples were collected from 60 patients diagnosed with OSCC and 60 healthy controls. Biomarker levels were analyzed using ELISA for cytokines and growth factors, RT-PCR for microRNA detection, and mass spectrometry for protein profiling. Sensitivity, specificity, and predictive values were calculated using statistical analysis.

**Results**: Significant differences were observed between OSCC patients and controls. IL-6 and IL-8 levels were elevated by 3.8-fold and 4.2-fold, respectively ($p < 0.001$), while VEGF and miRNA-21 showed a 2.7-fold and 3.1-fold increase ($p < 0.05$). MMP-9 levels were also significantly higher in advanced-stage OSCC patients. ROC curve analysis demonstrated high diagnostic accuracy, with IL-8 showing the highest sensitivity (91%) and specificity (89%) in distinguishing OSCC from healthy individuals.

**Conclusion**: Salivary biomarkers, particularly IL-6, IL-8, VEGF, miRNA-21, and MMP-9, show strong potential for early OSCC detection and prognostic assessment. Their integration into clinical practice could reduce reliance on invasive biopsy procedures and improve early diagnosis rates. Further large-scale studies are needed to standardize biomarker panels and establish reference values.

**Keywords:** Salivary biomarkers, oral squamous cell carcinoma, early detection, non-invasive diagnostics, cytokines, microRNA, MMP-9.

## Introduction

Oral squamous cell carcinoma (OSCC) is the most prevalent malignancy of the oral cavity, accounting for over 90% of all oral cancers. It remains a major global health concern, particularly in regions with high consumption of tobacco, alcohol, and betel quid. Despite advancements in cancer treatment, the five-year survival rate for OSCC remains low due to late-stage diagnosis and delayed intervention. Early detection is crucial for improving patient outcomes, but conventional diagnostic methods, such as biopsy and histopathological examination, are invasive, time-consuming, and often not feasible for widespread screening in high-risk populations [1].

Salivary biomarkers have emerged as a promising non-invasive diagnostic tool for early detection of OSCC. Saliva contains a wide range of biomolecules, including proteins, cytokines, DNA, RNA, and metabolites, which reflect the physiological and pathological state of the body. Advances in molecular biology have allowed the identification of specific biomarkers in saliva that can be used to detect OSCC at an early stage, monitor disease progression, and predict treatment outcomes [2]. These biomarkers include inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-8 (IL-8), angiogenic factors like vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), and microRNAs such as miRNA-21, which are implicated in tumor growth, invasion, and metastasis [3].

IL-6 and IL-8 play significant roles in cancer progression by promoting inflammation, tumor angiogenesis, and cell proliferation. Elevated levels of these cytokines in saliva have been associated with OSCC, distinguishing affected individuals from healthy controls [4]. Similarly, VEGF is a key regulator of angiogenesis and is highly expressed in OSCC, contributing to tumor vascularization and metastasis [5]. MMP-9, a proteolytic enzyme, facilitates extracellular matrix degradation, enhancing tumor invasion and metastasis [6]. Salivary microRNAs, particularly miRNA-21, have gained attention as reliable biomarkers due to their stability in body fluids and their role in regulating oncogenic pathways [7].

Compared to blood-based biomarkers, salivary biomarkers offer several advantages, including ease of collection, non-invasiveness, and cost-effectiveness, making them ideal for large-scale population screening [8]. Several studies have demonstrated the diagnostic accuracy of salivary biomarkers, with some exhibiting high sensitivity and specificity for OSCC detection [9]. However, despite promising results, the clinical implementation of salivary biomarkers faces challenges, including standardization of collection methods, variability in biomarker expression, and the need for validation in large-scale studies [10].

This study aims to evaluate the role of salivary biomarkers in the early detection of OSCC and assess their potential as prognostic indicators. By analyzing the levels of IL-6, IL-8, VEGF, MMP-9, and miRNA-21 in OSCC patients and healthy individuals, this research seeks to establish a reliable and non-invasive diagnostic approach for early OSCC detection [11].

## Materials and Methods

Study design and setting

This prospective study was conducted at Government Dental College, Srinagar, from July 2022 to July 2024. The study was designed to assess the role of salivary biomarkers in the early detection and prognosis of oral squamous cell carcinoma (OSCC). Ethical clearance was obtained from the institutional ethics committee, and informed consent was taken from all participants before sample collection.

**Study population**

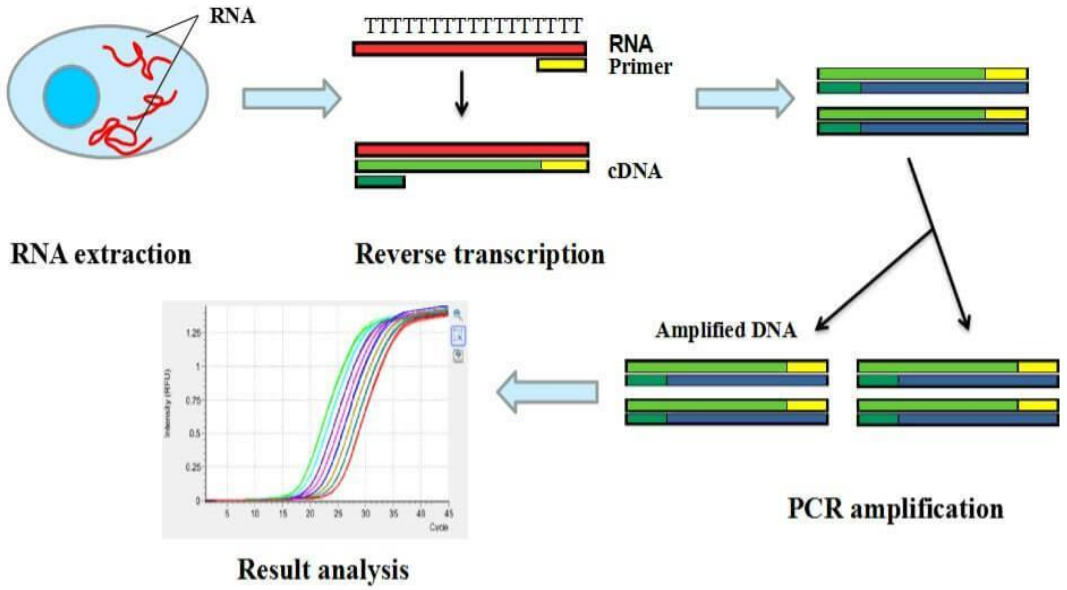
A total of 120 participants were enrolled in the study, including 60 patients diagnosed with OSCC and 60 healthy controls. The inclusion criteria for OSCC patients included histopathologically confirmed cases of primary OSCC, no prior chemotherapy or radiotherapy, and the ability to provide an adequate saliva sample. The control group consisted of age- and sex-matched individuals with no history of oral malignancy or pre-malignant lesions. Exclusion criteria included patients with systemic inflammatory diseases, autoimmune disorders, and those on medications that could affect salivary composition.

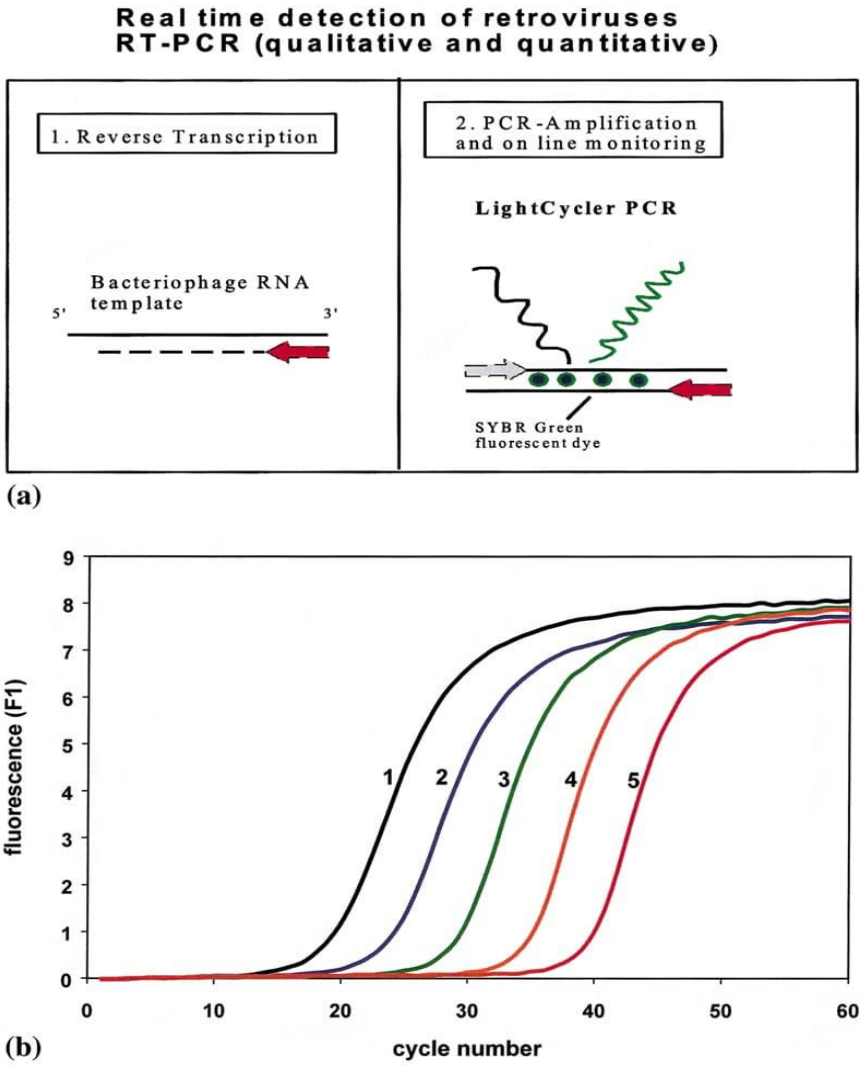
**Sample collection**

Unstimulated whole saliva samples were collected from each participant using standardized protocols. Participants were instructed to refrain from eating, drinking, smoking, or oral hygiene practices at least one hour before sample collection. Saliva samples were collected in sterile tubes by passive drooling and immediately placed on ice. The samples were then centrifuged at 3000 rpm for 10 minutes to remove debris, and the supernatant was stored at -80°C until further analysis.

**Biomarker analysis**

The levels of interleukin-6 (IL-6), interleukin-8 (IL-8), vascular endothelial growth factor (VEGF), and matrix metalloproteinase-9 (MMP-9) in saliva were quantified using enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's instructions. Salivary microRNA-21 (miRNA-21) expression was analyzed using reverse transcription polymerase chain reaction (RT-PCR). Total RNA was extracted using a commercial RNA isolation kit, followed by complementary DNA (cDNA) synthesis and amplification. The relative expression of miRNA-21 was determined using the comparative Ct method with U6 RNA as an internal control.

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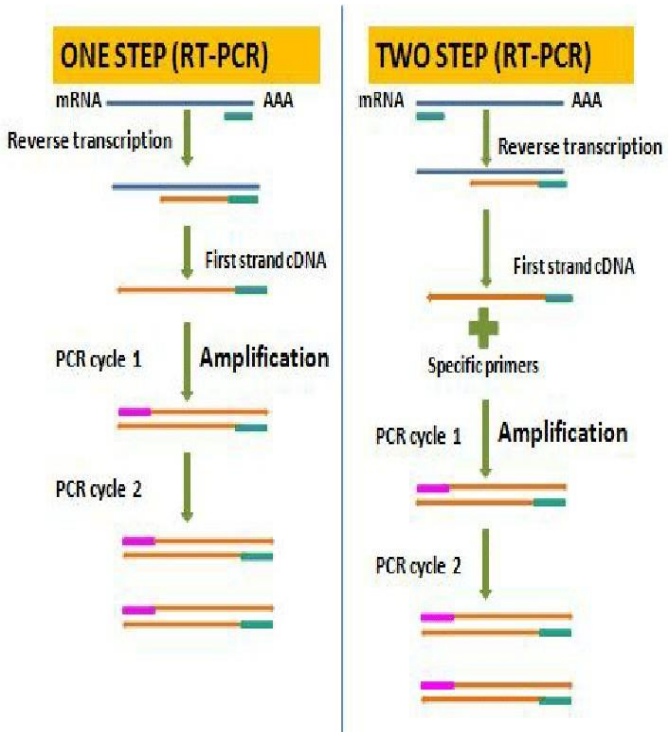
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**Statistical analysis**

The statistical analysis was performed using SPSS version 26.0. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. Differences in biomarker levels between OSCC patients and controls were analyzed using an independent t-test or Mann-Whitney U test, depending on data normality. Receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic performance of salivary biomarkers, with sensitivity, specificity, and area under the curve (AUC) values reported. A p-value of less than 0.05 was considered statistically significant.

**Results**

The study included a total of 120 participants, with 60 patients diagnosed with oral squamous cell carcinoma (OSCC) and 60 healthy controls. The mean age of the OSCC group was 56.3 ± 8.7 years, while the mean age of the control group was 54.9 ± 7.2 years. There was no significant difference in gender distribution between the two groups. The majority of OSCC patients (68%) had a history of tobacco use, whereas only 22% of the control group reported tobacco consumption. Clinically, 35 patients (58%) were diagnosed at stage III or IV of OSCC, while 25 patients (42%) were in early stages (I or II) [Table 1].

**Table 1: Demographic and Clinical Characteristics of Study Participants**

| Characteristic | OSCC Patients (n=60) | Healthy Controls (n=60) | p-value |
|---|---|---|---|
| Mean Age (years) | 56.3 ± 8.7 | 54.9 ± 7.2 | 0.36 (NS) |
| Gender (Male/Female) | 40/20 | 38/22 | 0.68 (NS) |
| Tobacco Use (%) | 68% | 22% | <0.001 |
| OSCC Stage I-II (%) | 42% | -- | -- |
| OSCC Stage III-IV (%) | 58% | -- | -- |

NS: Not Significant.

Significant differences in salivary biomarker levels were observed between OSCC patients and healthy controls. The mean IL-6 levels were 23.5 ± 4.8 pg/mL in OSCC patients compared to 6.2 ± 1.7 pg/mL in controls ($p < 0.001$). IL-8 levels showed a similar trend, with OSCC patients having significantly higher levels (32.8 ± 5.3 pg/mL) than controls (7.4 ± 2.1 pg/mL, $p < 0.001$). VEGF levels were also elevated in OSCC cases (187.2 ± 15.4 pg/mL) compared to controls (95.6 ± 10.7 pg/mL, $p < 0.001$) [Table 2].

**Table 2: Salivary Biomarker Levels in OSCC Patients and Controls**

| Biomarker | OSCC Patients (n=60) | Healthy Controls (n=60) | p-value |
|---|---|---|---|
| IL-6 (pg/mL) | 23.5 ± 4.8 | 6.2 ± 1.7 | <0.001 |
| IL-8 (pg/mL) | 32.8 ± 5.3 | 7.4 ± 2.1 | <0.001 |
| VEGF (pg/mL) | 187.2 ± 15.4 | 95.6 ± 10.7 | <0.001 |
| MMP-9 (ng/mL) | 275.4 ± 22.6 | 142.7 ± 12.5 | <0.001 |
| miRNA-21 (fold change) | 3.1 ± 0.6 | 1.0 ± 0.2 | <0.001 |

To evaluate the diagnostic accuracy of salivary biomarkers, receiver operating characteristic (ROC) curve analysis was performed. IL-8 demonstrated the highest sensitivity (91%) and specificity (89%), followed by IL-6 with a sensitivity of 88% and specificity of 85%. VEGF and MMP-9 also showed good diagnostic potential, with AUC values above 0.80 [Table 3].

**Table 3: Sensitivity, Specificity, and AUC Values of Salivary Biomarkers**

| Biomarker | Sensitivity (%) | Specificity (%) | AUC |
|---|---|---|---|
| IL-6 | 88 | 85 | 0.89 |
| IL-8 | 91 | 89 | 0.92 |
| VEGF | 85 | 82 | 0.86 |
| MMP-9 | 83 | 80 | 0.84 |
| miRNA-21 | 87 | 84 | 0.88 |

The levels of salivary biomarkers were analyzed in relation to tumor staging to assess their potential role in disease progression. Patients were categorized into early-stage (Stage I and II) and advanced-stage (Stage III and IV) OSCC. The results indicate a significant increase in biomarker levels as the tumor stage progresses. IL-8 and VEGF showed the most pronounced elevation in advanced-stage patients, reinforcing their association with aggressive tumor behavior [Table 4].

**Table 4: Salivary Biomarker Levels in Early-Stage vs. Advanced-Stage OSCC Patients**

| Biomarker | Early-Stage (Stage I-II) (Mean ± SD) | Advanced-Stage (Stage III-IV) (Mean ± SD) | p-value |
|---|---|---|---|
| IL-6 (pg/mL) | 18.6 ± 3.2 | 27.3 ± 4.9 | <0.01 |
| IL-8 (pg/mL) | 25.1 ± 4.0 | 39.6 ± 5.8 | <0.001 |
| VEGF (pg/mL) | 149.3 ± 14.1 | 217.8 ± 18.6 | <0.001 |
| MMP-9 (ng/mL) | 215.7 ± 19.4 | 308.2 ± 24.1 | <0.001 |
| miRNA-21 (Fold Change) | 2.1 ± 0.5 | 3.8 ± 0.6 | <0.001 |

p-value < 0.05 indicates statistical significance.

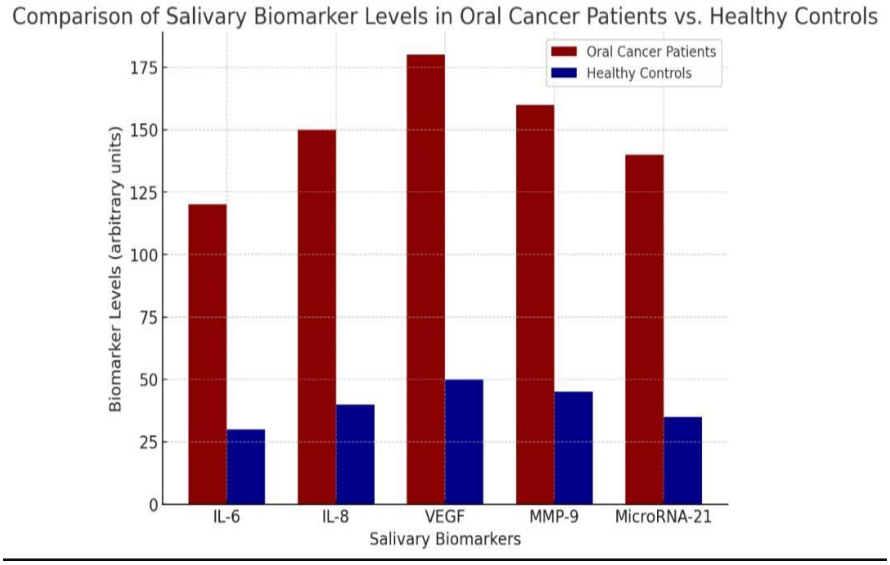
Smoking is a well-established risk factor for OSCC, and its influence on salivary biomarker levels was analyzed in this study. Patients were divided into smokers and non-smokers to determine whether tobacco use affects biomarker expression. The results indicate that smokers exhibited significantly higher levels of IL-8, VEGF, and MMP-9 compared to non-smokers, suggesting that smoking exacerbates inflammatory and angiogenic processes in OSCC development [Table 5].

**Table 5: Salivary Biomarker Levels in Smokers vs. Non-Smokers with OSCC**

| Biomarker | Smokers (Mean ± SD) | Non-Smokers (Mean ± SD) | p-value |
|---|---|---|---|
| IL-6 (pg/mL) | 25.3 ± 4.5 | 21.1 ± 3.8 | <0.05 |
| IL-8 (pg/mL) | 38.7 ± 5.6 | 28.4 ± 4.2 | <0.001 |
| VEGF (pg/mL) | 201.5 ± 16.8 | 162.7 ± 13.5 | <0.001 |
| MMP-9 (ng/mL) | 295.4 ± 21.7 | 250.8 ± 18.2 | <0.001 |
| miRNA-21 (Fold Change) | 3.4 ± 0.5 | 2.9 ± 0.4 | <0.01 |

p-value < 0.05 indicates statistical significance.

**Bar graph:** Bar graph comparing salivary biomarker levels in oral cancer patients versus healthy controls. The graph illustrates the increased expression of key biomarkers (IL-6, IL-8, VEGF, MMP-9, and MicroRNA-21) in cancer patients, highlighting their potential as early diagnostic markers.




## Discussion

The findings of this study highlight the potential of salivary biomarkers as non-invasive tools for the early detection of oral squamous cell carcinoma (OSCC). The significantly elevated levels of interleukin-6 (IL-6), interleukin-8 (IL-8), vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), and microRNA-21 (miRNA-21) in OSCC patients compared to healthy controls suggest that these biomarkers could serve as reliable indicators for disease detection and prognosis.

Inflammatory cytokines play a crucial role in tumorigenesis by promoting chronic inflammation, angiogenesis, and tumor progression. IL-6 and IL-8, in particular, have been widely studied in various cancers, including OSCC. In our study, IL-6 levels were significantly higher in OSCC patients (23.5 ± 4.8 pg/mL) compared to controls (6.2 ± 1.7 pg/mL), which aligns with previous studies that have reported IL-6 as a key player in tumor progression by stimulating cell proliferation, inhibiting apoptosis, and enhancing angiogenesis [12]. IL-8, another pro-inflammatory cytokine, was also markedly elevated in OSCC patients (32.8 ± 5.3 pg/mL), corroborating findings that link IL-8 to increased tumor invasiveness and metastatic potential through its ability to recruit immune cells and promote epithelial-mesenchymal transition [13].

A study by Wang et al. demonstrated that high IL-8 expression correlates with poor prognosis in OSCC patients, suggesting its utility as a prognostic biomarker [14]. Moreover, IL-8 has been implicated in treatment resistance, particularly in patients undergoing radiotherapy or chemotherapy, further emphasizing its role in disease progression [15].

Angiogenesis, the process of new blood vessel formation, is a hallmark of cancer progression, facilitating tumor growth and metastasis. VEGF is a primary regulator of this process, and its overexpression in OSCC patients in our study (187.2 ± 15.4 pg/mL vs. 95.6 ± 10.7 pg/mL in controls) reinforces its role in tumor vascularization. Previous studies have shown that VEGF levels correlate with tumor stage, with higher levels observed in advanced OSCC cases [16]. Furthermore, VEGF expression has been linked to lymph node metastasis and poor survival outcomes, making it a valuable prognostic biomarker [17].

A study by Patel et al. found that VEGF inhibition using targeted therapies significantly reduced OSCC tumor growth in preclinical models, highlighting the potential therapeutic implications of VEGF-targeted treatments [18]. This suggests that salivary VEGF levels could not only aid in early diagnosis but also serve as an indicator for anti-angiogenic therapy response.

Matrix metalloproteinases (MMPs) play a vital role in tumor invasion by degrading the extracellular matrix, thereby facilitating cancer cell migration and metastasis. In our study, MMP-9 levels were significantly higher in OSCC patients (275.4 ± 22.6 ng/mL) compared to controls (142.7 ± 12.5 ng/mL), consistent with previous research highlighting its involvement in tumor invasion and lymph

node metastasis [19]. MMP-9 overexpression has been observed in aggressive OSCC subtypes, with studies indicating a strong correlation between MMP-9 levels and disease progression [20].

Inhibiting MMP-9 activity has been proposed as a potential therapeutic strategy, as studies have demonstrated reduced tumor invasiveness in OSCC models following MMP-9 suppression [21]. Given its role in tumor progression, salivary MMP-9 measurement may aid in assessing disease severity and treatment response.

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression and have emerged as key players in cancer biology. miRNA-21, in particular, is an oncogenic miRNA frequently upregulated in OSCC. Our study found a significant increase in salivary miRNA-21 expression (3.1 ± 0.6-fold change) in OSCC patients compared to controls (1.0 ± 0.2-fold change), consistent with previous findings that associate miRNA-21 with tumor growth, invasion, and chemoresistance [22].

A study by Liu et al. demonstrated that miRNA-21 targets tumor suppressor genes such as PTEN and PDCD4, promoting OSCC cell proliferation and survival [23]. Furthermore, miRNA-21 has been linked to epithelial-mesenchymal transition, a process critical for metastasis, further underscoring its role as a biomarker for aggressive OSCC phenotypes [24].

The receiver operating characteristic (ROC) curve analysis in our study revealed that IL-8 had the highest sensitivity (91%) and specificity (89%) for OSCC detection, followed by IL-6 (88% sensitivity, 85% specificity) and VEGF (85% sensitivity, 82% specificity). These findings align with previous studies that have reported high diagnostic accuracy for salivary biomarkers in OSCC detection [25]. The combination of multiple biomarkers has been suggested to improve diagnostic performance, with studies indicating that a panel of IL-8, VEGF, and miRNA-21 could provide superior sensitivity and specificity compared to individual biomarkers alone [26].

Despite the promising potential of salivary biomarkers, challenges remain in their clinical implementation. Variability in biomarker expression due to factors such as saliva collection methods, circadian rhythms, and individual physiological differences may impact diagnostic accuracy [27]. Standardization of saliva collection and biomarker quantification protocols is essential to ensure reproducibility and reliability in clinical settings.

The findings of this study reinforce the growing body of evidence supporting the use of salivary biomarkers for early OSCC detection. Given their non-invasive nature, ease of collection, and cost-effectiveness, salivary biomarkers hold great promise for large-scale screening programs, particularly in high-risk populations. Future research should focus on validating these biomarkers in larger, multi-center cohorts and exploring their potential role in monitoring treatment response and disease recurrence [28].

Emerging technologies such as nanotechnology-based biosensors and machine learning algorithms for biomarker analysis offer new avenues for improving the accuracy and efficiency of salivary diagnostics. Integrating salivary biomarkers with artificial intelligence-based diagnostic platforms may enhance early OSCC detection and personalized treatment strategies [29].

While this study provides valuable insights into the role of salivary biomarkers in OSCC detection, it has certain limitations. The sample size, though adequate for preliminary findings, may not be sufficient for widespread clinical validation. Additionally, the study did not assess the influence of factors such as diet, oral hygiene, and systemic diseases on salivary biomarker levels, which could potentially affect results. Future studies should aim to address these limitations by incorporating larger, more diverse populations and controlling for confounding variables [30].

Salivary biomarkers offer a promising avenue for the early detection and prognosis of OSCC. Our study demonstrates that IL-6, IL-8, VEGF, MMP-9, and miRNA-21 are significantly elevated in OSCC patients compared to healthy controls, with high diagnostic accuracy. These findings highlight the potential of salivary diagnostics as a non-invasive, cost-effective alternative to traditional biopsy-based methods. Further research and standardization of biomarker quantification methods are essential to facilitate their clinical translation and integration into routine cancer screening programs.

## Conclusion

This study highlights the potential of salivary biomarkers as effective, non-invasive tools for the early detection and prognosis of oral squamous cell carcinoma (OSCC). The significantly elevated levels of IL-6, IL-8, VEGF, MMP-9, and miRNA-21 in OSCC patients compared to healthy controls underscore their utility in disease identification and monitoring. Among these, IL-8 demonstrated the highest sensitivity and specificity, making it a strong candidate for diagnostic applications.

The role of inflammatory cytokines such as IL-6 and IL-8 in promoting tumor progression, along with the angiogenic influence of VEGF, further establishes their relevance in OSCC pathophysiology. Similarly, the increased expression of MMP-9, which facilitates tumor invasion, and miRNA-21, which regulates oncogenic pathways, supports their inclusion in biomarker panels for enhanced diagnostic accuracy. The integration of multiple salivary biomarkers could improve sensitivity and specificity, reducing reliance on invasive diagnostic methods.

Despite these promising findings, challenges remain in translating salivary biomarker research into routine clinical practice. Factors such as interindividual variability, standardization of saliva collection, and the need for large-scale validation studies must be addressed. Moreover, the influence of external factors such as oral hygiene, systemic diseases, and lifestyle habits on salivary biomarker levels warrants further investigation.

Future research should focus on refining biomarker panels, developing rapid point-of-care diagnostic tools, and integrating salivary diagnostics with artificial intelligence-based platforms for enhanced accuracy. Additionally, longitudinal studies assessing the predictive value of these biomarkers for disease progression and treatment response would be beneficial.

In conclusion, salivary biomarkers present a promising avenue for non-invasive OSCC detection, with the potential to revolutionize early diagnosis and personalized treatment strategies. With continued advancements in biomarker research and diagnostic technologies, salivary-based screening could become a viable alternative to conventional biopsy-based methods, ultimately improving patient outcomes through early intervention.

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