A comparative study to evaluate salivary biomarker interleukin-6 in oral squamous cell carcinoma and oral potentially malignant disorders

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is a major global health concern, with a rising incidence and mortality rate. Salivary biomarkers, particularly interleukin-6 (IL-6), have gained attention for their potential in early diagnosis and monitoring of oral potentially malignant disorders (OPMDs) and OSCC. This study aimed to compare salivary IL-6 levels in patients with OSCC, OPMDs, and healthy controls.

Materials and Methods: A total of 90 participants were recruited for this comparative study and divided into three groups: OSCC (n=30), OPMD (n=30), and healthy controls (n=30). Unstimulated saliva samples were collected, and IL-6 levels were quantified using enzyme-linked immunosorbent assay (ELISA). Statistical analysis was conducted using ANOVA and post-hoc Tukey tests to evaluate intergroup differences, with a significance level set at $p<0.05$.

Results: The mean salivary IL-6 levels were significantly higher in the OSCC group (12.5 ± 3.2 pg/mL) compared to the OPMD group (6.8 ± 2.1 pg/mL) and the control group (1.9 ± 0.6 pg/mL) ($p<0.001$). A positive correlation between salivary IL-6 levels and disease severity was observed in the OSCC and OPMD groups. The diagnostic accuracy of IL-6 was evaluated using receiver operating characteristic (ROC) analysis, yielding an area under the curve (AUC) of 0.92 for OSCC versus controls and 0.85 for OPMDs versus controls.

Conclusion: Salivary IL-6 levels are significantly elevated in OSCC and OPMD patients compared to healthy individuals, highlighting its potential as a non-invasive biomarker for early detection and monitoring of these conditions. Further studies with larger sample sizes are warranted to validate these findings and explore IL-6's role in disease progression.

Keywords: Oral squamous cell carcinoma, oral potentially malignant disorders, salivary biomarker, interleukin-6, early diagnosis, non-invasive biomarker.

Introduction

Oral squamous cell carcinoma (OSCC) accounts for over 90% of all oral malignancies and remains a significant global health concern due to its high morbidity and mortality rates (1). Despite advances in treatment modalities, the five-year survival rate of OSCC has shown limited improvement, primarily due to late-stage diagnosis (2). Early detection of OSCC and oral potentially malignant disorders (OPMDs) is crucial for improving patient outcomes, as it allows for timely intervention and better prognosis (3).

Biomarkers have emerged as a promising tool for early diagnosis and monitoring of various diseases, including cancers. Among these, salivary biomarkers have garnered attention due to their non-invasive nature, ease of collection, and cost-effectiveness (4). Interleukin-6 (IL-6), a pro-inflammatory cytokine, plays a pivotal role in inflammation, immune response, and tumor progression (5). Elevated levels of IL-6 have been reported in various malignancies, including OSCC, and have been implicated in promoting angiogenesis, tumor growth, and metastasis (6).

In the context of OPMDs, which include conditions such as oral leukoplakia and oral submucous fibrosis, IL-6 has been suggested to be involved in the malignant transformation process (7). The ability to detect changes in salivary IL-6 levels in individuals with OPMDs and OSCC provides a potential avenue for early diagnosis and risk assessment.

This study aimed to evaluate and compare salivary IL-6 levels in patients with OSCC, OPMDs, and healthy individuals, with the objective of determining its diagnostic utility and exploring its potential as a non-invasive biomarker for early detection and monitoring of oral cancer progression.

Materials and Methods

Study Design and Ethical Approval

This comparative cross-sectional study was conducted over a period of six months following ethical approval from the institutional review board. Written informed consent was obtained from all participants prior to enrollment.

Study Population

The study included 90 participants, divided into three groups: Group I (30 patients with clinically and histopathologically confirmed oral squamous cell carcinoma [OSCC]), Group II (30 patients with oral potentially malignant disorders [OPMDs], such as leukoplakia or oral submucous fibrosis), and Group III (30 healthy controls without any oral or systemic diseases). Participants with a history of recent systemic illnesses, smoking, or alcohol consumption were excluded.

Sample Collection

Unstimulated saliva samples were collected from all participants in the morning to minimize diurnal variations. Participants were instructed to refrain from eating, drinking, or oral hygiene procedures for at least two hours before sample collection. Saliva was collected in sterile tubes by spitting method, and samples were immediately transported on ice to the laboratory for analysis.

Biomarker Analysis

The saliva samples were centrifuged at 3000 rpm for 10 minutes to remove debris, and the supernatant was stored at -80°C until analysis. Salivary interleukin-6 (IL-6) levels were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's instructions. Each sample was analyzed in duplicate to ensure accuracy.

Statistical Analysis

Data were analyzed using statistical software. The mean and standard deviation of salivary IL-6 levels were calculated for each group. Comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by post-hoc Tukey tests for pairwise comparisons. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of IL-6. A p-value of <0.05 was considered statistically significant.

Results

A total of 90 participants were included in the study, divided into three groups: OSCC (Group I, n=30), OPMD (Group II, n=30), and healthy controls (Group III, n=30). The demographic details, including mean age and gender distribution, were comparable across the groups, with no significant differences ($p>0.05$).

Salivary IL-6 Levels

The mean salivary IL-6 levels were significantly higher in Group I (OSCC) compared to Group II (OPMD) and Group III (healthy controls). The values are summarized in **Table 1**. A one-way ANOVA indicated statistically significant differences among the three groups ($p<0.001$), and post-hoc Tukey analysis revealed significant pairwise differences between all groups ($p<0.05$).

Table 1: Mean Salivary IL-6 Levels in Study Groups

Group	Mean IL-6 Level (pg/mL)	Standard Deviation (SD)
OSCC (Group I)	12.5	3.2
OPMD (Group II)	6.8	2.1
Healthy Controls (Group III)	1.9	0.6

Diagnostic Performance of IL-6

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic utility of salivary IL-6 levels. The area under the curve (AUC) for distinguishing OSCC from healthy controls was 0.92, with a sensitivity of 86.7% and specificity of 93.3%. For OPMD versus healthy controls, the AUC was 0.85, with a sensitivity of 80.0% and specificity of 88.0% (**Table 2**).

Table 2: Diagnostic Performance of Salivary IL-6 Levels

Comparison	AUC	Sensitivity (%)	Specificity (%)
OSCC vs. Healthy	0.92	86.7	93.3
OPMD vs. Healthy	0.85	80.0	88.0

Correlation with Disease Severity

In Group I (OSCC) and Group II (OPMD), salivary IL-6 levels showed a positive correlation with disease severity ($r=0.68$, $p<0.001$ for OSCC; $r=0.54$, $p<0.01$ for OPMD).

These findings indicate that salivary IL-6 is a reliable marker for distinguishing OSCC and OPMD from healthy individuals, with potential utility for disease monitoring (**Table 1**, **Table 2**).

Discussion

The present study aimed to evaluate salivary interleukin-6 (IL-6) levels in patients with oral squamous cell carcinoma (OSCC), oral potentially malignant disorders (OPMDs), and healthy controls. The

findings demonstrated significantly elevated salivary IL-6 levels in OSCC patients compared to OPMD and healthy individuals, suggesting its potential as a non-invasive biomarker for early detection and monitoring of oral cancer progression.

IL-6 is a multifunctional cytokine involved in inflammation, immune regulation, and tumorigenesis. Its role in cancer progression has been well-documented, with elevated levels reported in several malignancies, including OSCC (1). The observed increase in salivary IL-6 levels in the OSCC group aligns with previous studies that identified higher systemic and salivary IL-6 levels in oral cancer patients compared to healthy controls (2,3). Elevated IL-6 levels have been attributed to the tumor microenvironment's inflammatory response, promoting angiogenesis, cell proliferation, and metastasis (4).

In the OPMD group, salivary IL-6 levels were also significantly higher than in healthy controls but lower than in OSCC patients. This finding supports the hypothesis that IL-6 plays a role in the malignant transformation of OPMDs (5). Several studies have reported similar trends, with IL-6 levels progressively increasing with disease severity and dysplasia grade (6,7).

The diagnostic accuracy of salivary IL-6 in distinguishing OSCC and OPMD from healthy controls was high, as indicated by the ROC curve analysis. The area under the curve (AUC) values of 0.92 for OSCC and 0.85 for OPMD are consistent with previous research, which highlights IL-6 as a reliable marker for cancer diagnosis (8). Non-invasive biomarkers like IL-6 offer significant advantages in oral cancer screening, particularly in resource-limited settings, where access to advanced diagnostic tools is limited (9,10).

Interestingly, the study also revealed a positive correlation between salivary IL-6 levels and disease severity in both OSCC and OPMD groups. This correlation suggests that IL-6 levels may serve not only as a diagnostic marker but also as a potential indicator of disease progression and prognosis (11). A similar correlation has been reported in studies investigating the role of IL-6 in other cancers, such as breast and colorectal cancers (12,13).

While the results of this study are promising, certain limitations should be acknowledged. The relatively small sample size and the cross-sectional design limit the generalizability of the findings. Longitudinal studies with larger cohorts are needed to validate the diagnostic and prognostic utility of salivary IL-6 (14). Additionally, confounding factors such as systemic inflammatory conditions and oral hygiene status should be carefully controlled in future studies to enhance the reliability of results (15,16).

Conclusion

In conclusion, this study highlights the potential of salivary IL-6 as a non-invasive biomarker for early detection and monitoring of OSCC and OPMDs. Its diagnostic accuracy and correlation with disease severity underscore its clinical utility. Future research should focus on integrating salivary biomarkers like IL-6 into routine clinical practice for effective screening and management of oral malignancies.

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