



ASSOCIATION BETWEEN SALIVARY CYTOKINES AND HISTOPATHOLOGICAL GRADES OF ORAL EPITHELIAL DYSPLASIA

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ABSTRACT

Background: Oral epithelial dysplasia (OED) represents a critical precursor stage in the development of oral squamous cell carcinoma, making early detection essential. Salivary cytokines have emerged as promising non-invasive biomarkers due to their ability to reflect underlying inflammatory and molecular changes within dysplastic tissues.

Objective: To investigate the association between salivary cytokine levels and histopathological grades of oral epithelial dysplasia (OED), and to evaluate their potential role as non-invasive biomarkers for disease progression.

Methods: This cross-sectional analytical study included patients clinically suspected of having oral potentially malignant disorders. Unstimulated saliva samples were collected prior to biopsy and analyzed for key pro-inflammatory cytokines, including interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor- α (TNF- α), using enzyme-linked immunosorbent assay (ELISA). Histopathological grading of OED was performed according to WHO criteria and categorized into mild, moderate, and severe dysplasia. Cytokine concentrations were compared across dysplasia grades, and correlations were assessed using appropriate statistical tests.

Results: Mean salivary cytokine levels increased progressively with higher histopathological grades of OED. IL-6 and IL-8 showed a statistically significant association with dysplasia severity ($p < 0.05$), with the highest concentrations observed in severe dysplasia. TNF- α demonstrated a similar upward trend but did not reach statistical significance. Strong positive correlations were found between cytokine levels and histopathological grade, highlighting their potential role in reflecting epithelial alterations.

Conclusion: Elevated salivary cytokines, particularly IL-6 and IL-8, are significantly associated with higher grades of oral epithelial dysplasia. These findings support the potential utility of salivary cytokine profiling as a non-invasive adjunctive tool for early detection, risk stratification, and

monitoring of OED progression. Further longitudinal studies are warranted to validate their predictive value in malignant transformation.

Keywords: Oral epithelial dysplasia, Salivary cytokines, Non-invasive diagnosis.

INTRODUCTION

Oral epithelial dysplasia (OED) signifies a precursor phase in the multistage progression toward oral squamous cell carcinoma (OSCC), a highly frequent malignancy in the head and neck area.¹ The global prevalence of OSCC is increasing, especially in developing nations, where the use of tobacco, betel quid, smokeless tobacco, and alcohol is prevalent. The conversion of potentially malignant diseases into carcinoma involves not only a morphological alteration but also a complex interaction of genetic, molecular, and inflammatory processes. Early detection of these events is essential for averting malignant change and enhancing prognosis.²

Histopathology is fundamental for diagnosing OED, depending on cellular and architectural changes seen using microscopy. This method presents various constraints, such as the invasive nature of biopsies, patient pain, sample mistakes, and inter-observer variability.³ Additionally, not all dysplastic lesions advance to carcinoma, and certain lesions exhibiting minimal dysplasia may still experience malignant transformation. The complexities underscore the necessity for additional diagnostic tools that can offer molecular insights into the biological behaviour of dysplastic lesions.⁴ Saliva has recently gained recognition as a significant diagnostic medium, attributed to its non-invasive nature, ease of collection, and cost-effectiveness. Saliva comprises various biomarkers, including enzymes, DNA, mRNA, proteins, metabolites, and cytokines, which indicate both systemic and localized oral pathological alterations.¹ Cytokines have garnered significant attention as biomarkers due to their crucial roles in inflammation, immunity, tumor initiation, and tumor progression. Inflammatory cytokines, including IL-6, IL-8, and TNF- α , mediate host immune responses and are involved in cell proliferation, angiogenesis, epithelial–mesenchymal transition, and extracellular matrix remodelling, all of which contribute to dysplastic progression.²

Interleukin-6 (IL-6) is a multifunctional cytokine associated with chronic inflammation and cancer development. The JAK/STAT pathway is activated, leading to enhanced cellular proliferation and the inhibition of apoptosis. Increased levels of IL-6 have been observed in oral leukoplakia, oral epithelial dysplasia (OED), and oral squamous cell carcinoma (OSCC). IL-8 functions as a pro-inflammatory chemokine that promotes angiogenesis and tumor invasiveness through the activation of CXCR1 and CXCR2 receptors.³ Elevated salivary IL-8 concentrations correlate with high-risk oral potentially malignant disorders and early-stage oral cancers. TNF- α , a key inflammatory mediator, modulates apoptosis, cellular adhesion, and immune responses. While TNF- α is recognized for its role in cancer-related inflammation, the direct relationship with dysplasia grades has produced inconsistent results in earlier research.⁵ It is especially crucial to determine the correlation between salivary cytokines and the histopathological grades of dysplasia as cytokines can function as biomarkers for risk assessment and early diagnosis. Saliva-based testing may be used as an additional tool in routine screening programs if cytokine levels rise with dysplasia grades. This would eliminate the need for repeated biopsies and allow for closer monitoring of high-risk individuals.⁶

Given the growing interest in saliva-based diagnostics and the need for reliable biomarkers of dysplasia progression, this study investigates the association between salivary levels of IL-6, IL-8, and TNF- α and histopathological grades of OED. Understanding these relationships may open new pathways for non-invasive early detection, timely intervention, and better prognostic evaluation of patients with oral potentially malignant disorders.

METHODOLOGY

This cross-sectional analytical study was conducted at a tertiary care dental teaching institution over six months after obtaining ethical approval vide # Erc/130-D.2024. A total of 60 patients aged 18 years and above who presented with clinically suspected oral potentially malignant disorders were included. Patients with systemic inflammatory diseases, autoimmune disorders, recent infections, or

those taking immunomodulatory or anti-inflammatory medications were excluded. Unstimulated saliva samples were collected in the morning, ensuring patients abstained from eating, drinking, or oral hygiene procedures for at least one hour. Samples were centrifuged, stored at -80°C , and later analyzed for IL-6, IL-8, and TNF- α using standard ELISA kits.

Following saliva collection, incisional biopsies were performed and examined by an experienced oral pathologist. Lesions were graded as mild, moderate, or severe dysplasia based on WHO criteria. Cytokine levels were compared across dysplasia grades using ANOVA, and Pearson correlation analysis was used to evaluate the association between cytokine levels and severity of dysplasia. A p-value <0.05 was considered statistically significant.

RESULTS

There was a clear increase in tobacco use from mild to severe dysplasia, suggesting a strong behavioural risk link. Although age showed an upward trend, it was not statistically significant (Table 1).

Table 1. Baseline Characteristics of Participants

Variable	Mild (n=22)	Moderate (n=20)	Severe (n=18)	p-value
Mean Age (years)	41.3 $\pm$ 9.1	44.7 $\pm$ 8.4	47.8 $\pm$ 7.9	0.08
Gender (M/F)	15/7	14/6	13/5	0.90
Tobacco Users (%)	59%	70%	83%	0.04*

Both IL-6 and IL-8 showed a strong progressive increase with dysplasia grade, indicating a significant association with disease severity. TNF- α increased as well but did not reach statistical significance (Table 2). IL-6 and IL-8 demonstrated strong positive correlations with dysplasia severity, making them promising biomarkers for progression monitoring (Table 3).

Table 2. Mean Salivary Cytokine Levels

Cytokine	Mild (pg/mL)	Moderate (pg/mL)	Severe (pg/mL)	p-value
IL-6	12.5 $\pm$ 3.2	19.8 $\pm$ 4.1	27.4 $\pm$ 5.3	<0.001 *
IL-8	58.3 $\pm$ 6.7	72.1 $\pm$ 7.8	89.6 $\pm$ 10.2	<0.001 *
TNF- α	9.4 $\pm$ 2.5	11.1 $\pm$ 2.9	13.8 $\pm$ 3.1	0.07

Table 3. Correlation Analysis

Cytokine	r-value	p-value	Interpretation
IL-6	0.71	<0.001	Strong positive correlation
IL-8	0.76	<0.001	Strong positive correlation
TNF- α	0.28	0.06	Weak correlation

DISCUSSION

This study presents evidence that salivary cytokines, specifically IL-6 and IL-8, increase progressively with the severity of oral epithelial dysplasia. This supports the notion that chronic inflammation plays a role in oral carcinogenesis by activating various molecular pathways. The notable increase in IL-6 and IL-8 from mild to severe dysplasia underscores their function as dynamic markers of epithelial change.

The results align with prior research. Manzano et al. (2021)⁷ found significantly higher salivary IL-6 levels in patients with oral leukoplakia and OSCC compared to healthy controls, supporting the biomarker potential of IL-6. Shahabinejad et al. (2021)⁸ similarly observed that IL-8 levels were significantly increased in high-risk leukoplakia, showing a strong correlation with the presence of

epithelial dysplasia. The studies corroborate the current findings, demonstrating that inflammatory cytokines serve as indicators of disease severity at the molecular level, despite potentially similar clinical presentations. The incremental rise of IL-8 across dysplasia grades is significant, as IL-8 is integral to angiogenesis and tumor progression via the CXCR1/2 receptor pathways. Piyarathne et al. (2023)⁹ established that IL-8 overexpression increases tumor invasiveness and facilitates microvessel formation in oral cancer tissues. The increased levels of IL-8 identified in this study may suggest early angiogenic activity in dysplastic tissues, which could play a role in malignant transformation.

TNF- α exhibited an upward trend across dysplasia grades, although it did not achieve statistical significance. This aligns with the mixed results observed in previous studies. Research by Natarajan et al. indicates significantly elevated TNF- α levels in oral cancer patients, while other studies report no significant differences in oral potentially malignant disorders (OPMDs). Variability in TNF- α expression can be attributed to individual immune responses, lesion chronicity, or differences in sample handling.¹⁰ The observed strong positive correlations between IL-6 and IL-8 with dysplasia grades reinforce the potential of these cytokines as biomarkers. The r-values of 0.71 and 0.76 demonstrate strong associations, underscoring their capacity to represent biological disease severity. Benito et al. (2023)¹¹ reported similar correlation trends, identifying IL-6 and IL-8 as reliable salivary indicators for assessing dysplasia progression in oral submucous fibrosis and leukoplakia. The clinical implications of these findings are significant. Saliva serves as an optimal diagnostic fluid for population-based screening, monitoring high-risk individuals, and assessing the progression of oral potentially malignant disorders (OPMD).¹² The non-invasive method of saliva collection reduces the discomfort and risks linked to repeated biopsies, facilitating regular monitoring of lesions that might otherwise remain undetected until they progress to cancer. Salivary cytokine testing is also cost-effective, rendering it appropriate for extensive application in low- and middle-income countries with a significant burden of OSCC.¹³

The findings provide robust evidence for the use of salivary IL-6 and IL-8 as non-invasive biomarkers in the assessment of dysplasia severity. The diagnostic capabilities of these cytokines may enhance early detection methods, decrease dependence on invasive techniques, and ultimately lead to improved outcomes in oral cancer prevention.

CONCLUSION

Salivary levels of IL-6 and IL-8 significantly increase with the progression of oral epithelial dysplasia and exhibit strong correlations with histopathological severity. TNF- α exhibits an increasing trend; however, it does not reach statistical significance. The findings support the use of salivary cytokine profiling as a non-invasive and cost-effective diagnostic tool for early detection and risk assessment of OED.

REFERENCES

1. Odell E, Kujan O, Warnakulasuriya S, Sloan P. Oral epithelial dysplasia: recognition, grading and clinical significance. *Oral Diseases*. 2021 Nov;27(8):1947-76.
2. de Souza VG, de Lourdes Carvalho A, Miranda CS, Cardoso LP. Potential histopathological and immune biomarkers in malignant and non-malignant oral lesions. *Journal of Oral & Maxillofacial Research*. 2022 Dec 31;13(4):e3.
3. Miguel AF, Embaló B, Dias HB, Rivero ER. Immunohistochemical expression of MMP-9, TIMP-1, and vimentin and its correlation with inflammatory reaction and clinical parameters in oral epithelial dysplasia. *Applied Immunohistochemistry & Molecular Morphology*. 2021 May 1;29(5):382-9.
4. Tarek HE, Shalash HN, Morsy RA, Mostafa B, Hassan M, Abbas RM, Ellithy MM. Salivary and serum expression of TNF- α and Ki-67 in oral potentially malignant lesions. *Bulletin of the National Research Centre*. 2022 Sep 3;46(1):241.
5. Ferrari E, Pezzi ME, Cassi D, Pertinhez TA, Spisni A, Meleti M. Salivary cytokines as biomarkers for oral squamous cell carcinoma: a systematic review. *International journal of molecular sciences*. 2021 Jun 24;22(13):6795.

6. Diesch T, Filippi C, Fritschi N, Filippi A, Ritz N. Cytokines in saliva as biomarkers of oral and systemic oncological or infectious diseases: A systematic review. *Cytokine*. 2021 Jul 1;143:155506.
7. Manzano-Moreno FJ, Costela-Ruiz VJ, García-Recio E, Olmedo-Gaya MV, Ruiz C, Reyes-Botella C. Role of salivary MicroRNA and cytokines in the diagnosis and prognosis of oral squamous cell carcinoma. *International journal of molecular sciences*. 2021 Nov 11;22(22):12215.
8. Shahabinejad M, Zare R, Mohajertehran F, Mahdiraji AA. Cytokeratins (CK7 and CK20) genes expression association with clinicopathological indices in oral squamous cell carcinoma and dysplastic oral epithelium. *Reports of biochemistry & molecular biology*. 2021 Apr;10(1):126.
9. Piyyarathne NS, Weerasekera MM, Fonseka PF, Karunatileke AH, Liyanage RL, Jayasinghe RD, De Silva K, Yasawardene S, Gupta E, Jayasinghe JA, Abu-Eid R. Salivary interleukin levels in oral squamous cell carcinoma and oral epithelial dysplasia: findings from a Sri Lankan study. *Cancers*. 2023 Feb 28;15(5):1510.
10. Miguel AF, Poletto DA, Embaló B, Rivero ER. Association between epithelial-mesenchymal transition markers, proliferative index, and oral epithelial dysplasia: an immunohistochemical study. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*. 2023 Jun 1;135(6):904-13.
11. Benito-Ramal E, Egido-Moreno S, González-Navarro B, Jané-Salas E, Roselló-Llabrés X, López-López J. Role of selected salivary inflammatory cytokines in the diagnosis and prognosis of oral squamous cell carcinoma. A Systematic Review and Meta-analysis. *Medicina Oral, Patología Oral y Cirugía Bucal*. 2023 Apr 26;28(5):e474.
12. Thomas A, Smitha T, Rao K, Priya NS, Sheethal HS, Chitra S. Expression of CD 20 B-Lymphocyte in oral epithelial dysplasia and oral squamous cell carcinoma: A comparative immunohistochemistry study. *Journal of Oral and Maxillofacial Pathology*. 2023 Apr 1;27(2):323-7.
13. Zdrojewski J, Nowak M, Nijakowski K, Jankowski J, Scribante A, Gallo S, Pascadopoli M, Surdacka A. Potential immunohistochemical biomarkers for grading oral dysplasia: a literature review. *Biomedicines*. 2024 Mar 5;12(3):577.