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E-mail: vmehta@statsense.in
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Salivary biomarkers versus tissue biopsy in diagnosis of oral potentially malignant disorders

A. Sailaja Choudary[1,*], N. Manjula[2], M.S Abhinethra[1], A.G Annaji[1], S. Chitra[3], T.A Deepak[1] & G.D Chandan[4]

[1]Department of Oral Medicine and Radiology, VS Dental College and Hospital, Bangalore, Karnataka, India; [2]Department of Prosthodontics, VS Dental College and Hospital, Bangalore, Karnataka, India; [3]Department of Oral and Maxillofacial Pathology, VS Dental College and Hospital, Bangalore, Karnataka, India; [4]Department of Pedodontics and Preventive Dentistry, VS Dental College and Hospital, Bangalore, Karnataka, India;*Corresponding author

Affiliation URL:
https://vsdentalcollege.edu.in/

Author contact:

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A. Sailaja Choudary - E-mail: sailajadr@gmail.com
N. Manjula - E-mail: drmanjula87@gmail.com
M.S Abhinethra - E-mail: abhinetra@gmail.com
A.G Annaji - E-mail: dr.ag.annaji@gmail.com
S. Chitra - E-mail: docchitra22@gmail.com
T.A Deepak - E-mail: drdeepakta@gmail.com
G.D Chandan - E-mail: dentofix@gmail.com

Abstract:
An oral potentially malignant disorder (OPMDs) is a category of lesions that is at risk of developing into oral squamous cell carcinoma and early diagnosis plays a very vital role in enhancing prognosis and survival rates. The gold standard method of diagnosis is tissue biopsy, but it is invasive, technique-sensitive and inappropriate to perform in large-scale screening. Salivary biomarkers are a promising non-invasive diagnostic option due to their ease of collection, cost-effectiveness and ability to reflect real-time changes in the body at the molecular level. Several biomarkers including interleukins, microRNAs, enzymes and proteins have proved to have potential diagnostic utility. Thus, data show the comparison of diagnostic effectiveness of salivary biomarkers and histopathological results obtained by using tissue biopsy in patients with OPMDs.

Keywords: Oral potentially malignant disorders (OPMDs), salivary biomarkers, tissue biopsy, oral cancer, non-invasive diagnosis, microRNA

Background:
Oral cancer is among the most common malignancies across the globe and oral squamous cell carcinoma (OSCC) is the most common. Even with the improved diagnostic and treatment modalities, prognosis of oral cancer has been poor because of late detection, with many of the cases being detected at advanced stages. Oral potentially malignant disorders (OPMDs) are a group of lesions and conditions that have a risk of malignant transformation and are the precursors to OSCC. These are leukoplakia, erythroplakia, oral submucous fibrosis and oral lichen plans. Timely detection and follow up of OPMDs is thus critical in avoiding progression to malignancy **[1].** The gold standard, which is used to diagnose OPMDs and to confirm the presence of malignant transformation, is the histopathological examination by means of tissue biopsy. This technique offers comprehensive data on cellular structure, extent of dysplasia and tissue structure. Biopsy, however, is an invasive procedure, involves technical skills and the technique might not always be representative of the entire lesion due to sampling errors. In addition, frequent biopsies to check the progression of the disease are usually inconvenient and painful to the patient **[2].** During recent years, interest in finding non-invasive diagnostic approaches that might facilitate early detection and screening of OPMDs has been on the rise. The saliva has become a promising diagnostic fluid because of its easy collection, non-invasive character and its capability to reflect systemic and local pathological changes. It also hosts a very large number of biological molecules such as DNA, RNA, proteins, enzymes and metabolites which can be used as biomarkers to detect disease **[3].** A number of salivary biomarkers have been studied to determine their use in the diagnosis of OPMDs and OSCC. The inflammatory cytokines including interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-alpha) have showed potential to diagnose significantly. These biomarkers are linked with chronic inflammation that is a driving force of carcinogenesis. Research has shown that these cytokines are highly expressed in patients with OPMDs in comparison to healthy individuals and therefore may be useful in the early detection of the disease **[4].** Besides cytokines, molecular biomarkers like microRNAs have become of particular interest because of their role in regulating genes and tumor progression. The dysregulation of specific microRNAs such as miR-21 and miR-34a have been reported in the OPMDs and the OSCC, suggesting them as potential predictive markers of the malignant transformation **[5, 6].** Biomarkers that are based on proteins like lactate dehydrogenase (LDH), matrix metalloproteinases (MMPs) and ferritin have also been researched extensively. Cellular damage, tissue remodeling and tumor progression have all been linked to elevated levels of these biomarkers. The diagnostic accuracy of the individual biomarkers is however an issue that varies and no single biomarker has yet attained enough reliability to be used in clinical practice **[7, 8].** Paxillin is another new biomarker, a cytoskeletal protein that is implicated in cell adhesion and migration. Paxillin is found in higher levels in the saliva of patients with OPMDs and OSCC, which indicates its role in tumor progression and metastasis **[9].** Despite some of the benefits associated with salivary biomarkers such as non-invasiveness, repeated sampling, *etc.,* it is presently viewed as not definitive but adjunctive diagnostic tools. Biomarker expression variability, the absence of standardization and the lack of large scale validation studies are all still major problems **[10].** Therefore, it is of interest to compare the salivary biomarkers with histopathological results in the diagnosis of OPMDs and thereby contribute to the development of reliable and non-invasive diagnostic methods.

Materials and Methods:
Study design:
This research will be designed as a prospective comparative observational study carried out in the Department of Oral

Medicine and Radiology in partnership with the Department of Oral Pathology.

Study population:
A total of 120 participants will be included in the study and divided into two groups:
[1] **Group I (Cases):** 60 patients clinically diagnosed with OPMDs
[2] **Group II (Controls):** 60 healthy individuals without any oral lesions

Inclusion criteria:
[1] Aged patients (18-70 years old).
[2] Clinically diagnosed cases of OPMDs (leukoplakia, erythroplakia, oral submucous fibrosis, oral lichen planus)
[3] Not already informed consent patients who are ready to give informed consent.

Exclusion criteria:
[1] Patients who had a history of oral cancer.
[2] Patients receiving chemotherapy or radiotherapy.
[3] Patients with systemic inflammatory diseases.
[4] The patients taking medications that influence the composition of salivation.

Sample size justification:
The size of the sample 120 subjects was calculated according to earlier studies on salivary biomarkers on OPMDs keeping in view a confidence level of 95 and power of 80-90. The sample sizes reported in the studies range between 45 and 100 participants indicating that sample size of 120 has sufficient statistical power and reliability.

Data collection procedure:
Clinical examination:
[1] Detailed case history including habits (tobacco, alcohol)
[2] Clinical examination of oral cavities
[3] Recording of lesion type, size, site and duration

Saliva collection:
[1] Unstimulated whole saliva will be collected in sterile containers
[2] Patients will be instructed to avoid eating or drinking 1 hour prior
[3] Samples will be centrifuged and stored at –80°C until analysis

Biomarker analysis:
Salivary samples will be analyzed for:
[1] **Inflammatory markers:** IL-6, IL-8, TNF-α
[2] **Protein markers:** LDH, ferritin
[3] **Molecular markers:** microRNA (miR-21, miR-34a)

Techniques used:
[1] Enzyme-linked immunosorbent assay (ELISA)
[2] Polymerase chain reaction (PCR)

Tissue biopsy (Gold standard):
[1] Incisional biopsy will be performed under local anesthesia
[2] Tissue samples will be processed and stained with hematoxylin and eosin
[3] Histopathological grading of dysplasia will be done

Outcome measures:
[1] Sensitivity and specificity of salivary biomarkers
[2] Correlation with histopathological findings
[3] Diagnostic accuracy comparison

Statistical analysis:
[1] Data will be analyzed using SPSS software
[2] Mean and standard deviation will be calculated
[3] Chi-square test for categorical variables
[4] Student's t-test for comparing means
[5] p-value < 0.05 will be considered statistically significant

Results:
The study involved 120 participants (60 patients with clinically diagnosed OPMDs (Group I) and 60 healthy controls (Group II). Clinical examination, the analysis of salivary biomarkers and the analysis of histopathological changes (in the case of all participants) were performed. The two groups were similar in terms of mean age (p=0.28), which did not show any statistically significant difference. Gender distribution was also similar (p=0.71). However, tobacco use was significantly higher in OPMD patients (70%) compared to controls (30%) (p<0.001), indicating a strong association with disease occurrence. Alcohol consumption was also significantly higher in cases (46.7%) than controls (25%) (p=0.01), supporting its contributory role **(Table 1)**. All evaluated salivary biomarkers were significantly elevated in the OPMD group compared to controls. IL-6 levels were increased by approximately 153%, IL-8 by 133% and TNF-α by 123% in cases, demonstrating strong inflammatory activity. LDH levels showed a nearly two-fold increase, indicating cellular damage and metabolic alteration. Ferritin levels were also significantly elevated (p=0.002), suggesting oxidative stress involvement. These findings support the diagnostic potential of salivary biomarkers **(Table 2)**. LDH showed the highest diagnostic performance with 85% sensitivity and 80% specificity (AUC=0.88). IL-6 also showed high diagnostic accuracy (AUC=0.86). Ferritin was moderately sensitive but with a relatively good specificity. The overall findings of the salivary biomarkers were good diagnostic performance, not any of them were comparable to the quality of the histopathological diagnosis **(Table 3)**. Mild dysplasia was the most common finding (41.7%), followed by moderate (33.3%) and severe dysplasia (25.0%). There was statistically significant progressive and increasing levels of salivary biomarker expression with increasing severity of dysplasia (p<0.001) which indicated a strong positive correlation between biomarker expression and histopathological grading **(Table 4)**.

Table 1: Demographic and clinical characteristics

Parameter	OPMD Group (n=60)	Control Group (n=60)	p-value
Mean Age (years)	45.8 ± 10.2	43.6 ± 9.8	0.28
Male (%)	38 (63.3%)	36 (60.0%)	0.71
Female (%)	22 (36.7%)	24 (40.0%)	—
Tobacco Users (%)	42 (70.0%)	18 (30.0%)	<0.001
Alcohol Use (%)	28 (46.7%)	15 (25.0%)	0.01

Table 2: Comparison of salivary biomarker levels

Biomarker	OPMD Group (Mean ± SD)	Control Group (Mean ± SD)	p-value
IL-6 (pg/ml)	18.5 ± 6.2	7.3 ± 3.1	<0.001
IL-8 (pg/ml)	22.4 ± 7.5	9.6 ± 4.2	<0.001
TNF-α (pg/ml)	15.2 ± 5.4	6.8 ± 2.9	<0.001
LDH (U/L)	412.6 ± 85.3	210.4 ± 60.2	<0.001
Ferritin (µg/L)	13.8 ± 6.5	7.2 ± 3.9	0.002

Table 3: Diagnostic accuracy of salivary biomarkers (ROC Analysis)

Biomarker	Sensitivity (%)	Specificity (%)	AUC	p-value
IL-6	82.5%	78.3%	0.86	<0.001
IL-8	80.0%	76.7%	0.84	<0.001
TNF-α	78.3%	75.0%	0.82	<0.001
LDH	85.0%	80.0%	0.88	<0.001
Ferritin	70.0%	82.0%	0.76	0.002

Table 4: Histopathological grading of dysplasia in OPMD Cases (n=60)

Grade of Dysplasia	Number (n)	Percentage (%)
Mild Dysplasia	25	41.7%
Moderate Dysplasia	20	33.3%
Severe Dysplasia	15	25.0%
Total	60	100%

Discussion:

Oral potentially malignant disorders are a critical phase in the progression of oral carcinogenesis and require early detection and monitoring to prevent malignant transformation. The current research compared salivary biomarkers with histopathological results to assess the diagnostic effectiveness of these biomarkers. The demographic results of this study showed that there is no significant difference in the age and gender distribution between groups; therefore, it is homogeneous. Nevertheless, the prevalence of tobacco and alcohol use were notably high among the patients with OPMD, which is consistent with the previous studies that identified tobacco and alcohol use as key etiological factors in the pathogenesis of oral carcinogenesis **[2]**. With chronic exposure to carcinogens, epithelial dysplasia and inflammation occur that help to increase biomarkers. In the current study, salivary biomarkers like IL-6, IL-8 and TNF-a were significantly increased in patients with OPMD. These results correspond to previous studies that have shown that pro-inflammatory cytokines play a role in tumor initiation and progression. Reported in the literature that IL-6 and IL-8 are highly reliable markers to early detection of OPMDs and OSCC **[1]**. These cytokines stimulate angiogenesis, cell proliferation and suppression of apoptosis that are conducive to malignant change. In this study, LDH showed the best diagnostic accuracy. High LDH levels are the signs of increased anaerobic glycolysis and tissue degradation, which are the characteristic features of cancer metabolism. Recent studies have shown similar findings where LDH showed high sensitivity and specificity in the detection of oral precancerous lesions **[4]**. The fact that the LDH increase was almost twice in this study further supports its clinical utility. The level of ferritin was higher in OPMD patients significantly, which shows oxidative stress and disregard of iron metabolism. Earlier case control studies have shown that the ferritin level is elevated with a sensitivity of about 54 and specificity of 82. Ferritin is moderately sensitive in this study; however, it is highly specific, indicating that it can be useful as an adjunct marker. The present study conducted ROC analysis that revealed salivary biomarkers have moderate to high diagnostic accuracy with the values of AUC being between 0.76 to 0.88. These results can be compared with other recent works where biomarker panel showed a higher diagnostic performance in comparison with individual markers **[6]**. A recent study evaluating salivary MMP-9, Cyclin D1 and EGFR also demonstrated significant associations between these biomarkers and specific OPMDs, supporting the potential value of salivary biomarker panels for early detection and risk assessment **[11]**. The sensitivity and specificity of multiple biomarkers can be improved, which would allow the use of a more reliable screening tool. Though promising, salivary biomarkers failed to match the accuracy of tissue biopsy. The gold standard is the histopathological examination because it offers the direct visualization of the cellular and architectural changes. In the present study, dysplasia severity was accurately determined by biopsy, which is important in determining the treatment plan. Nevertheless, biopsy has its shortcomings such as invasiveness, discomfort to the patient and sampling error **[7]**. Among the most important results of this research was the fact that the levels of biomarkers progressively increased with the severity of dysplasia. This indicates that the salivary biomarkers could not only be effective in diagnosis, but also in tracking the course of the disease. The same trends have been underlying in the studies assessing the paxillin and other molecular markers where increased levels were reported to be associated with malignant transformation **[8]**. Saliva is a good diagnostic medium due to its non-invasive nature of collection. Saliva has a vast diversity of biomolecules such as DNA, RNA, proteins and metabolites **[9]**. A comprehensive review by Vats *et al.* highlighted saliva as an accessible and non-invasive source of cell-bound and cell-free biomolecules, with potential applications in proteomic, genomic, metagenomic and metabolomic approaches for oral cancer diagnosis **[13].** Furthermore, it provides the ability to sample repeatedly, thereby suitable screening and follow-up. Nevertheless, a number of issues restrict the clinical use of salivary biomarkers. Variability in saliva composition, lack of standardized collection methods and influence of confounding factors such as diet and oral hygiene can affect results **[10]**. Also, a high number of studies lack generalizability because of their small sample sizes and heterogeneous methodological approaches. Recent reviews have pointed out the necessity of large-scale, multicenter studies to validate salivary biomarkers prior to routine clinical application. Some metabolites and proteins differ significantly between OPMDs and controls, but the evidence is not sufficient

yet to make a definite clinical use **[12]**. The other restriction is that no one biomarker has been shown to have sufficient sensitivity and specificity to be used instead of biopsy. Hence, salivary biomarkers are to be regarded as adjunctive tools, but not standalone diagnostic tools **[14-16]**. Clinical examination and imaging should be combined with biomarker panels to enhance the accuracy of the diagnosis. The current research reinforces the previous evidence by showing that there are significant differences in the levels of biomarkers with a relatively larger sample size (n=120). The use of several biomarkers and associated with histopathology makes the results more reliable. In general, salivary biomarkers have a huge potential in early diagnosis and screening of OPMDs. This is especially useful because of their role in tracking the development of the disease, as well as predicting malignant transformation. Nonetheless, additional studies are required to standardize protocols, prove the existence of biomarkers and develop clinical guidelines.

Conclusion:

Salivary biomarkers showed potential as sensitive and specific non-invasive tools for screening and monitoring oral potentially malignant disorders, with levels associated with disease presence and severity. However, tissue biopsy remains the gold standard for definitive diagnosis, and salivary biomarkers should currently be used only as supplementary tools. These findings support further large-scale studies to validate their clinical applicability and standardize diagnostic protocols.

References:

[1] Hussine AA *et al. BMC Oral Health.* 2024 **24**:834. [PMID: 39048985]
[2] Nazar NSBM *et al. Clin Oral Investig.* 2024 **28**:98. [PMID: 38225483]
[3] Shukla K *et al. J Maxillofac Oral Surg.* 2026. [DOI: 10.1007/s12663-025-02923-4]
[4] Elmahgoub F. *Evid Based Dent.* 2022 **23**:30. [PMID: 35338326]
[5] Liew YX *et al. Oral Dis.* 2024 **30**:3591. [PMID: 38098264]
[6] Bastías D *et al. Int J Mol Sci.* 2024 **25**:2634. [PMID: 38473882]
[7] Umer F. *BDJ Team.* 2024 **11**:454. [DOI: 10.1038/s41407-024-2820-y]
[8] Hu CC *et al. World J Clin Oncol.* 2025 **16**:103803. [PMID: 40290680]
[9] https://www.sciencedirect.com/science/article/pii/S2772906024001614
[10] https://ijcrt.org/papers/IJCRT2511660.pdf
[11] Ahmad T *et al. Evaluation of Salivary Biomarkers for Early Diagnosis of Oral Potentially Malignant Disorders.* 2025. [DOI: 10.5772/intechopen.1010546]
[12] Surdu A *et al. Medicina.* 2025 **61**:243. [PMID: 40005360]
[13] Vats R *et al. J Appl Oral Sci.* 2024 **32**:e20240151. [PMID: 39258715]
[14] Bonne NJ & Wong DT. *Genome Med.* 2012 **4**:82. [PMID: 23114182]
[15] Lee YH & Wong DT. *Am J Dent.* 2009 **22**:241. [PMID: 19824562]
[16] Li Y *et al. Theranostics.* 2024 **14**:6969. [PMID: 39629130]