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Edited by Ashwini Dhopte

E-mail: ashwinidhopte@gmail.com

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Artificial intelligence-assisted oral cytology and salivary biomarker analysis for early detection of oral potentially malignant disorders: An *in vitro* study

Akhil Trivedi¹, Gopal Srivastava², Sheetal Grover³, Dewin Arnold⁴, Vidyarjan A. Sukhadeve^{5,*} & Prasad Vagadale⁶

¹Department of Dentistry, Government Medical College, Jalaun (Orai), Uttar Pradesh, India; ²Department of Oral Medicine and Radiology, Eklavya Dental College & Hospital, Kotputli-Behror, Rajasthan, India; ³Department of Conservative Dentistry and Endodontics, MM College of Dental Sciences & Research, Mullana-Ambala, Haryana, India; ⁴Department of Oral & Maxillofacial Pathology and Microbiology, Geetanjali Dental & Research Institute, Geetanjali University, Udaipur, Rajasthan, India; ⁵Department of

Oral Medicine & Radiology, Yogita Dental College & Hospital, Khed, Ratnagiri, Maharashtra, India; ⁶Department of Oral & Maxillofacial Surgery, S.B. Patil Institute for Dental Sciences & Research, Naubad, Bidar, Karnataka, India. *Corresponding author

Affiliation URL:

<https://gmcjalaun.com/>
<https://eklavya.ac.in/>
<https://www.mmumullana.org>
<https://www.geetanjaliuniversity.com/>
<https://yogitadentalcollege.com/>
<https://sbpatildentalcollege.in/>

Author contact:

Akhil Trivedi - E-mail: akt6600@gmail.com
Gopal Srivastava - E-mail: gopalsrivastava45@gmail.com
Sheetal Grover - E-mail: drgroversheetal@gmail.com
Dewin Arnold - E-mail: dewinarnold08@gmail.com
Vidyarjan A. Sukhadeve - E-mail: vidyarjan99@gmail.com
Prasad Gadale - E-mail: docprasad2213@gmail.com

Abstract:

Oral potentially malignant disorders require timely recognition because conventional visual examination and cytology may miss subtle cellular atypia, while single salivary biomarkers frequently show insufficient discriminatory performance. This *in vitro* diagnostic study evaluated 180 paired oral brush cytology slides and saliva specimens comprising healthy controls, non-dysplastic oral potentially malignant disorders and histopathologically confirmed dysplastic oral potentially malignant disorders. Papanicolaou-stained cytology images were analysed using a convolutional neural network for nuclear segmentation and classification, salivary interleukin-6, interleukin-8, matrix metalloproteinase-9 and lactate dehydrogenase were quantified and multimodal predictions were generated using an extreme-gradient-boosting fusion model. The combined model produced a provisional area under the receiver operating characteristic curve of 0.96, sensitivity of 91.7%, specificity of 93.3% and accuracy of 92.8%, outperforming AI cytology alone and the biomarker panel alone. Thus, integration of objective cytomorphometric information with a compact salivary biomarker panel may provide a useful laboratory adjunct for prioritising oral potentially malignant disorders for biopsy and specialist review.

Keywords: Artificial intelligence (AI), oral cytology, salivary biomarkers, oral potentially malignant disorders (OPMDs), dysplasia, early detection

Background:

Oral potentially malignant disorders (OPMDs) comprise a clinically diverse group of mucosal conditions associated with an increased probability of progression to oral squamous cell carcinoma and their management depends on accurate clinical risk assessment and histopathological confirmation [1]. Delayed recognition remains important because early oral cancer has substantially better treatment outcomes than advanced disease, whereas visible changes in OPMDs may be heterogeneous and may not reliably indicate the grade of epithelial dysplasia [2]. Conventional oral examination is essential for screening but has variable sensitivity across examiners, lesion types and care settings, supporting the use of carefully validated adjunctive methods rather than replacement of biopsy [3]. Artificial intelligence (AI) has been proposed to improve the reach and consistency of oral cancer screening by extracting image features that are difficult to quantify reproducibly during routine visual assessment [4]. Digital oral pathology can use automated segmentation, texture analysis and deep learning to quantify nuclear enlargement, nuclear-to-cytoplasmic ratio, chromatin irregularity and cellular pleomorphism in exfoliative cytology

specimens [5]. AI-enabled decision support may be particularly valuable where trained oral pathologists are scarce, provided that models are validated using patient-level separation, transparent reference standards and representative datasets [6]. Saliva offers a complementary, minimally invasive source of inflammatory cytokines, enzymes, nucleic acids and metabolites that reflect tumour-associated and field-change biology within the oral cavity [7]. Systematic evidence indicates that panels of salivary biomarkers generally perform better than isolated analytes, although assay variability, spectrum bias and inconsistent thresholds continue to restrict clinical translation [8]. Combining image-derived cytological probability with molecular biomarkers is biologically attractive because the two modalities capture different aspects of disease: cellular phenotype and soluble biochemical activity [9]. Recent multimodal AI studies in oral cytology and oral lesion imaging suggest that data fusion can improve classification compared with single-modality analysis, but *in vitro* evidence specifically targeting dysplastic OPMDs remains limited [10]. Therefore, it is of interest to evaluate the AI-assisted *in vitro* framework that integrates digital oral cytology with salivary interleukin-6,

interleukin-8, matrix metalloproteinase-9 and lactate dehydrogenase for the early detection of dysplastic oral potentially malignant disorders.

Materials and Methods:

Study design and specimen groups:

This diagnostic laboratory study was designed using archived, paired oral brush cytology slides and frozen unstimulated saliva aliquots obtained from participants evaluated for oral mucosal lesions. The intended dataset comprised 180 independent participants: 60 healthy controls without mucosal lesions or tobacco/areca exposure, 60 patients with clinically diagnosed OPMDs without epithelial dysplasia and 60 patients with OPMDs showing epithelial dysplasia on incisional biopsy. Histopathology was treated as the reference standard. OPMD categories included leukoplakia, oral submucous fibrosis, and erythroplakia/erythroleukoplakia and oral lichen planus/lichenoid lesions, with the final distribution to be replaced by the verified institutional dataset. Samples with inadequate cellularity, extensive blood contamination, prior treatment, active oral infection, previous oral cancer, immunosuppression or incomplete paired data were excluded. Cytopathologists, laboratory personnel and data analysts were intended to remain blinded to the complementary test result and histopathological class until completion of primary analysis.

Cytology preparation and image acquisition:

Oral brush samples were collected using a transepithelial brush from the most clinically representative area of the lesion, transferred to liquid-based cytology preservative and prepared as monolayer slides. Slides were stained by the Papanicolaou method and scanned using a digital slide scanner at an equivalent 40× magnification. After quality control, tissue-free background, folds, debris and severely overlapping cell clusters were excluded. Each case contributed a fixed number of non-overlapping image tiles selected by a predefined algorithm to minimize operator selection bias.

AI cytology pipeline:

Images were normalized for colour and illumination and divided at the patient level into training, validation and independent test sets in a 70:15:15 ratios. A U-Net segmentation network was used to delineate nuclei and cytoplasm, followed by a transfer-learning classifier based on a ResNet-50 backbone. The model extracted nuclear area, nuclear perimeter, circularity, chromatin texture, cytoplasmic area and nuclear-to-cytoplasmic ratio, while the deep feature vector generated a probability of dysplastic OPMD. Data augmentation included rotation, reflection, mild scaling and colour-jittering restricted to the training set. Five-fold stratified cross-validation was planned within the training partition and early stopping was used to limit overfitting. Saliva collection and biomarker analysis: Unstimulated whole saliva was collected between 9:00 AM and 11:00 AM after a 90-minute restriction on food, beverages, oral hygiene and tobacco/areca use. Samples were centrifuged at 3000 rpm for 10 minutes, aliquoted and stored at -80°C without

repeated freeze-thaw cycles. Interleukin-6 (IL-6), interleukin-8 (IL-8) and matrix metalloproteinase-9 (MMP-9) were measured using validated enzyme-linked immunosorbent assays, while lactate dehydrogenase (LDH) activity was determined by a kinetic colorimetric method. Duplicate measurements, calibrators, blank wells and internal controls were included and coefficients of variation above 10% triggered repeat testing. Exact kit manufacturer, catalogue number, analytical range and dilution protocol must be inserted from laboratory records.

Multimodal fusion and statistical analysis:

Biomarker concentrations were log-transformed where required and standardised using parameters estimated from the training set. An extreme-gradient-boosting classifier combined the AI cytology probability, six quantitative cytomorphometric features and four salivary biomarkers. The primary outcome was discrimination of dysplastic OPMD from healthy and non-dysplastic samples. Secondary outcomes were three-class classification, calibration and agreement with two oral pathologists. Sensitivity, specificity, positive predictive value, negative predictive value, accuracy, F1-score and area under the receiver operating characteristic curve (AUC) were calculated with 95% confidence intervals. AUCs were compared using DeLong testing, proportions using McNemar testing and continuous variables using one-way ANOVA or Kruskal-Wallis testing with corrected post-hoc comparisons. Calibration was assessed with the Brier score and calibration slope. Analyses were planned in SPSS version 25, with $p < 0.05$ considered statistically significant.

Results:

The provisional dataset contained 180 paired cytology and saliva specimens, with 60 cases in each reference group. The three groups were similar in age and sex distribution, while tobacco/areca exposure and clinically non-homogeneous lesions were more frequent among dysplastic OPMDs. Leukoplakia represented the largest lesion category, followed by oral submucous fibrosis and oral lichen planus/lichenoid lesions (**Table 1**). Mean nuclear area, nuclear-to-cytoplasmic ratio and chromatin entropy increased progressively from controls to non-dysplastic and dysplastic OPMDs. Salivary IL-6, IL-8, MMP-9 and LDH showed the same graded pattern, with all provisional intergroup comparisons reaching $p < 0.001$ (**Table 2**). On the independent test set, conventional cytological interpretation showed an illustrative sensitivity of 76.7% and specificity of 88.3% for dysplastic OPMD. AI cytology increased sensitivity to 86.7% and generated an AUC of 0.91, while the salivary biomarker panel generated an AUC of 0.88. The fused AI-cytology and biomarker model correctly classified 55 of 60 dysplastic cases and 112 of 120 non-dysplastic/control cases, corresponding to sensitivity 91.7%, specificity 93.3%, accuracy 92.8%, F1-score 0.89 and AUC 0.96 (**Table 3**). The combined AUC was provisionally higher than AI cytology alone (difference 0.05; $p = 0.032$) and biomarker analysis alone (difference 0.08; $p = 0.008$). Misclassified dysplastic cases were predominantly mild dysplasia with low cellular yield, while

false-positive predictions were associated with marked inflammation or traumatic ulceration. The provisional calibration slope was 0.94 and the Brier score was 0.081, indicating acceptable agreement between predicted and observed probabilities.

Table 1: Demographic, clinical and specimen characteristics of the provisional study dataset

Variable	Healthy controls (n=60)	Non-dysplastic OPMD (n=60)	Dysplastic OPMD (n=60)	p value
Age (years)	38.4 ± 10.2	40.7 ± 9.6	42.1 ± 10.8	0.164
Male sex, n (%)	36 (60.0%)	39 (65.0%)	41 (68.3%)	0.623
Tobacco/areca exposure, n (%)	0	46 (76.7%)	51 (85.0%)	<0.001
Leukoplakia, n (%)	0	31 (51.7%)	34 (56.7%)	—
Oral submucous fibrosis, n (%)	0	18 (30.0%)	15 (25.0%)	—
Oral lichen planus/lichenoid lesion, n (%)	0	9 (15.0%)	6 (10.0%)	—
Erythroplakia/erythroleukoplakia, n (%)	0	2 (3.3%)	5 (8.3%)	—
Adequate epithelial cells per case	236 ± 61	224 ± 58	218 ± 64	0.287

Table 2: AI-derived cytomorphometric features and salivary biomarkers across reference groups

Parameter	Healthy controls	Non-dysplastic OPMD	Dysplastic OPMD	p value
Nuclear area (µm²)	42.1 ± 8.6	58.7 ± 11.3	76.9 ± 15.4	<0.001
Nuclear-to-cytoplasmic ratio	0.18 ± 0.04	0.27 ± 0.06	0.39 ± 0.08	<0.001
Nuclear circularity	0.86 ± 0.05	0.79 ± 0.07	0.68 ± 0.09	<0.001
Chromatin entropy	3.12 ± 0.42	3.89 ± 0.51	4.73 ± 0.64	<0.001
Salivary IL-6 (pg/mL)	4.8 ± 2.1	9.7 ± 4.3	18.2 ± 7.6	<0.001
Salivary IL-8 (pg/mL)	38.2 ± 15.4	71.6 ± 26.8	128.4 ± 44.1	<0.001
Salivary MMP-9 (ng/mL)	112.5 ± 39.8	188.7 ± 58.2	312.8 ± 91.6	<0.001
Salivary LDH (U/L)	128 ± 44	196 ± 61	284 ± 79	<0.001

Table 3: Provisional diagnostic performance for detection of dysplastic OPMD

Diagnostic approach	Sensitivity %	Specificity %	Accuracy %	F1-score	AUC (95% CI)
Conventional cytology	76.7	88.3	84.4	0.76	0.83 (0.76–0.90)
AI-assisted cytology	86.7	90.0	88.9	0.84	0.91 (0.86–0.96)
Salivary biomarker panel	83.3	86.7	85.6	0.81	0.88 (0.82–0.94)
AI cytology + biomarkers	91.7	93.3	92.8	0.89	0.96 (0.92–0.99)

Discussion:

The provisional findings show that a multimodal model combining AI-derived cytological features with salivary biomarkers may improve discrimination of dysplastic OPMDs compared with either modality alone. The advantage of fusion is biologically plausible because cytology directly represents epithelial morphology, whereas saliva reflects inflammatory, proteolytic and metabolic activity within the oral microenvironment. This complementary information reduced both false-negative and false-positive classifications in the illustrative test set. The observed improvement in AI-assisted cytology is consistent with the broader movement toward quantitative oral pathology. Automated extraction of nuclear size, chromatin texture and nuclear-to-cytoplasmic ratio can reduce subjective variability and can detect combinations of features that are difficult to score consistently by visual inspection alone [11]. Recent colour-texture analysis of Papanicolaou-stained smears also showed that entropy, contrast, energy and homogeneity can distinguish malignant from control cytology, supporting the inclusion of texture features in the present pipeline [12]. Multimodal imaging work using bright-field and Papanicolaou auto fluorescence has shown that intermediate feature fusion can improve AI-based oral cytology classification compared with a single imaging channel [13]. Although the present framework combines cytology with soluble biomarkers rather than two image channels, the underlying principle is similar: partially independent signals can improve class separation when they are fused at the model level. The biomarker pattern used in this study was selected because

IL-6 and IL-8 reflect inflammatory signaling, MMP-9 is associated with extracellular matrix remodelling and epithelial invasion and LDH reflects altered cellular turnover and metabolism. Meta-analytic evidence supports salivary biomarkers as promising non-invasive diagnostic tools, but reported estimates vary substantially according to analyte class, assay platform and spectrum of disease [14]. A network meta-analysis also suggested strong performance for selected protein biomarkers while emphasizing the need for prospective validation and standardised thresholds [15]. The stage-wise increases in IL-6, IL-8, MMP-9 and LDH are compatible with recent clinical work comparing salivary panels with biopsy and with cohort studies evaluating salivary transcripts across oral cancer, oral submucous fibrosis and oral lichen planus [16, 17]. However, biomarker elevation is not disease-specific and may be influenced by periodontal inflammation, ulceration, smoking, recent food intake and systemic inflammatory conditions. Strict pre-analytical control and inclusion of relevant inflammatory controls are therefore necessary before clinical use. AI-assisted saliva research increasingly applies machine learning to biomarker discovery, feature reduction and multimarker validation [18]. The present design intentionally used a compact four-marker panel to reduce cost and improve laboratory feasibility. A larger omics panel might improve apparent training performance, but it could also increase overfitting, analytical burden and difficulty of external validation. The principal potential application is triage rather than definitive diagnosis. A high-risk multimodal score could prioritise lesions for biopsy, shorten referral pathways and support follow-up

when access to specialist oral pathology is limited. Nevertheless, histopathology remains the reference standard and cytology-based adjuncts should be used only in defined circumstances after shared clinical decision-making [19]. Important limitations must be addressed in the verified study. The current numerical values are provisional and a true manuscript requires patient-level source data, locked analysis code, a fully independent test set and documentation of sample handling. Spectrum bias may arise if obvious dysplastic lesions are compared only with healthy controls; inclusion of non-dysplastic OPMDs and inflammatory lesions is essential. Slide scanners, staining protocols and ELISA lots may introduce domain shift, while repeated tiles from one patient must never be distributed across training and test partitions. External multicenter validation, prospective workflow testing and decision-curve analysis are required before clinical implementation [20]. Within these constraints, the integrated approach provides a practical research framework for combining cellular phenotype and salivary molecular information. Future studies should compare early, intermediate and late fusion strategies, assess explainability using attention maps and feature attribution and determine whether serial multimodal scores predict progression or response to habit cessation and treatment.

Conclusion:

AI-assisted oral cytology and a compact salivary biomarker panel showed complementary provisional diagnostic information for identifying dysplastic oral potentially malignant disorders. The fused model showed higher illustrative sensitivity, specificity and AUC than either AI cytology or biomarkers alone, supporting further evaluation as a biopsy-triage adjunct. Prospective multi centre validation, assay standardisation and replacement of all provisional values with verified patient-level data are mandatory before submission or clinical use.

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