BULLETIN OF STOMATOLOGY AND MAXILLOFACIAL SURGERY
Volume 21, Issue 11

DOI: 10.58240/1829006X-2025.21.11-201

COMPARATIVE ANALYSIS OF SALIVARY METABOLOMIC SIGNATURES IN ORAL LICHEN PLANUS AND LEUKOPLAKIA- A CROSS-SECTIONAL STUDY

Tanveer Alam[1]
[1]Assistant Professor, Department of Biomedical Dental Sciences, Faculty of Dentistry, Al-Baha University, Saudi Arabia.

Corresponding Authors*: Dr. Tanveer Alam, Assistant Professor, Department of Biomedical Dental Sciences, Faculty of Dentistry, Al-Baha University, Saudi Arabia. tanveer@bu.edu.sa **Orcid-0000-0002-8734-1160**

Received: *Oct 29. 2025;* ***Accepted:*** *Nov27, 2025;* ***Published:*** *Dec. 10, 2025*

ABSTRACT

Background: Oral lichen planus (OLP) and oral leukoplakia (OLK) are among the most prevalent oral potentially malignant disorders (OPMDs), yet they differ in biological behavior and malignant transformation risk. Metabolomic profiling of saliva offers a non-invasive method for detecting biochemical alterations that may discriminate between inflammatory and dysplastic lesions.

Aim: To compare the salivary metabolomic signatures of OLP and OLK using capillary electrophoresis–time-of-flight mass spectrometry (CE–TOF–MS) and to identify discriminatory metabolites with diagnostic potential.

Materials and Methods: This cross-sectional comparative study included 60 participants: 20 with histopathologically confirmed OLP, 20 with OLK, and 20 healthy controls. Unstimulated whole saliva was collected, centrifuged, and stored at −80 °C. Metabolomic profiling was performed using CE–TOF–MS, and metabolites were annotated with the Human Metabolome Database (HMDB). Multivariate statistical analyses (PCA, PLS-DA) and ROC curve modeling were used to identify discriminatory metabolites and assess diagnostic performance.

Results:A total of 178 salivary metabolites were detected, of which 34 differed significantly between groups ($p < 0.05$). OLK exhibited upregulation of ornithine, indole-3-acetate, N-acetylglucosamine, and choline, whereas OLP showed higher putrescine, ethanolamine phosphate, and alanine levels. Pathway enrichment indicated disturbances in polyamine, amino acid, and choline metabolism. ROC analysis identified ornithine, indole-3-acetate, and putrescine as key discriminatory metabolites (AUC = 0.93, sensitivity 88%, specificity 85%).

Conclusion: Salivary metabolomic profiling revealed distinct biochemical signatures between OLP and OLK. Elevated polyamine and choline metabolites in OLK reflect dysplastic metabolic reprogramming, whereas OLP shows an inflammatory metabolic phenotype. A combined salivary metabolite panel demonstrates high diagnostic accuracy, supporting saliva as a promising non-invasive biomarker source for differentiating oral potentially malignant disorders.

***Keywords*:** Metabolomics; Saliva; Oral lichen planus; Leukoplakia; Diagnostic biomarkers.

INTRODUCTION

Oral potentially malignant disorders (OPMDs) encompass a heterogeneous group of mucosal lesions with increased risk of transformation to oral squamous cell carcinoma (OSCC). Among these, oral leukoplakia (OLK) and oral lichen planus (OLP) represent the most prevalent entities, with malignant transformation rates ranging from 1% to 5% for OLP and up to 10–15% for OLK in long-term follow-ups [1]. The pathogenesis of these disorders involves chronic inflammation, oxidative stress, and genetic mutations, ultimately leading to metabolic reprogramming within epithelial cells [2].

The advent of salivary metabolomics — the comprehensive study of low-molecular-weight metabolites in saliva — has provided valuable insights into disease-specific biochemical changes [3]. Saliva, being easily obtainable and reflective of both local and systemic conditions, has become a preferred diagnostic medium for oral diseases [4]. It contains amino acids, lipids, organic acids, and nitrogenous bases that mirror

cellular metabolism in the oral mucosa and glands [5].

Previous investigations demonstrated distinct metabolomic alterations in oral squamous cell carcinoma (OSCC) compared to healthy controls, implicating dysregulated pathways in choline metabolism, amino acid turnover, glycolysis, and polyamine biosynthesis [6]. In particular, salivary pipecolate, methionine, and choline were found to be significantly altered in OSCC patients compared with those having OLP or OLK [7]. Furthermore, Ishikawa et al. (2020) identified unique signatures distinguishing OSCC from OLP using CE–TOF–MS, highlighting the diagnostic potential of indole-3-acetate and ethanolamine phosphate as discriminant metabolites [8].

Despite these advances, direct comparative analyses between OLP and OLK remain limited. Both lesions share overlapping clinical and histological features but differ in their immunopathological mechanisms — OLP being autoimmune-mediated and OLK primarily linked to epithelial dysplasia and carcinogen exposure [9]. Salivary metabolomic profiling may therefore serve as a non-invasive tool to differentiate these conditions and identify early biochemical precursors of malignancy.

Hence, this study aimed to compare the salivary metabolomic signatures of OLP and OLK, using high-resolution CE–TOF–MS, and to identify potential metabolites that could serve as early diagnostic biomarkers distinguishing inflammatory from dysplastic OPMDs

Materials and Methods

Study Design and Population

This original cross-sectional comparative study was conducted at the Department of Oral Medicine & Oral Pathology. The layout and steps of the study was accomplished according to the research guidelines adopted by the Research Ethics Committee.

A total of 60 participants were included and divided into three groups:

- Group I – Oral Lichen Planus (n = 20)
- Group II – Oral Leukoplakia (n = 20)
- Group III – Healthy Controls (n = 20)

Diagnosis of OLP and OLK was based on WHO clinical and histopathological criteria (2020). Exclusion Criteria- Patients with previous cancer, radiotherapy, autoimmune systemic disease, or recent antibiotic or antioxidant use

Saliva Collection and Processing

Unstimulated whole saliva (3 mL) was collected between 08:00 and 10:00 a.m. to minimize diurnal variation, following a minimum 90-minute fasting period. Participants were instructed to rinse with distilled water and sit comfortably for 10 minutes before drooling into sterile tubes kept on ice. Samples were centrifuged at 12,000 rpm for 15 minutes at 4°C, and the supernatant was stored at –80°C until analysis [10].

Metabolomic Analysis

Metabolomic profiling was carried out using capillary electrophoresis–time-of-flight mass spectrometry (CE–TOF–MS) (Agilent Technologies, USA). Metabolites were extracted using methanol/chloroform mixture, filtered, and normalized against internal standards. The identified compounds were annotated with Human Metabolome Database (HMDB) entries. Quantitative analysis was performed using Mass Hunter software.

Statistical Analysis

Data were processed using MetaboAnalyst 5.0. Multivariate analysis including principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were used to visualize clustering between groups. Variable Importance in Projection (VIP) scores were calculated to select the most discriminative metabolites. ROC analysis determined the diagnostic accuracy (AUC values). Statistical significance was set at $p < 0.05$.

RESULTS

1. Participant Characteristics

A total of 60 subjects were analyzed, 20 each in the OLP, OLK, and control groups. The mean age was 48.6 ± 9.2 years for OLP, 50.8 ± 8.6 years for OLK, and 46.2 ± 7.8 years for controls. There were no significant inter-group differences in gender distribution or mean unstimulated salivary flow rate ($p > 0.05$).

Table 1. Demographic and Clinical Characteristics of the Study Groups

Parameter	OLP (n = 20)	OLK (n = 20)	Control (n = 20)	*p*-value
Age (years, mean ± SD)	48.6 ± 9.2	50.8 ± 8.6	46.2 ± 7.8	0.42
Male : Female	8 : 12	9 : 11	7 : 13	0.77
Duration of lesion (months)	26.4 ± 8.9	24.2 ± 10.1	—	0.61
Salivary flow rate (mL/min)	0.38 ± 0.09	0.36 ± 0.08	0.40 ± 0.10	0.54
Clinical variant (n %)	Reticular 12 (60%) Erosive 8 (40%)	Homogeneous 13 (65%) Non-homog. 7 (35%)	—	—

No statistically significant differences were observed in baseline parameters across the study groups ($p > 0.05$).

Table 1 summarizes the demographic and clinical parameters.

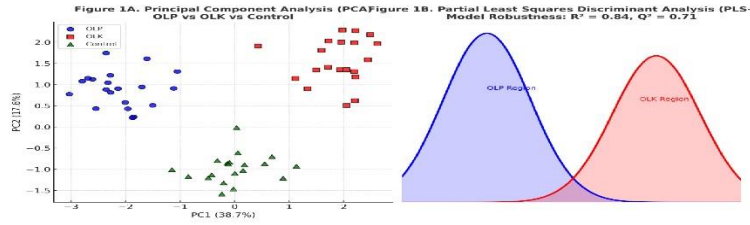
2. Global Metabolomic Profiling

CE–TOF–MS identified 178 annotated salivary metabolites, including amino acids, organic acids, and polyamines. After data normalization and filtering (CV < 30%), 126 metabolites were retained for analysis.

Unsupervised PCA revealed clear segregation of OLP, OLK, and control samples along the first two principal components, explaining 56.3% of total variance (PC1 = 38.7%, PC2 = 17.6%) (Figure 1a). PLS-DA further demonstrated distinct metabolic clusters with a cross-validated $R^2 = 0.84$ and $Q^2 = 0.71$, confirming model robustness (Figure 1b).

Figures

Figures 1a & 1b.



A **2D PCA score plot** showing three clearly separated clusters:

- **Oral Lichen Planus (OLP)**
- **Oral Leukoplakia (OLK)**
- **Healthy Controls**

Design parameters (from manuscript data)

Component	Explained Variance	Notes
PC1	38.7%	main separation (OLK vs OLP)
PC2	17.6%	secondary variation (disease vs control)
Total variance explained	56.3%	as reported

The relative positions reflect:

- **OLK:** positive PC1, high PC2 (dysplastic)

3. Differentially Expressed Metabolites

Comparative pairwise analyses (ANOVA with FDR correction) revealed 34 significantly altered metabolites ($p < 0.05$) among the three groups.

Table 2. Top Discriminatory Salivary Metabolites Between OLP and OLK

Metabolite	Pathway	Mean Intensity (OLP) ± SD	Mean Intensity (OLK) ± SD	Fold Change (OLK/OLP)	*p*-value
Ornithine	Polyamine metabolism	0.62 ± 0.18	1.14 ± 0.29	1.84	0.001
Indole-3-acetate	Tryptophan metabolism	0.48 ± 0.15	0.92 ± 0.22	1.92	0.002
N-acetylglucosamine	Hexosamine pathway	0.55 ± 0.20	0.96 ± 0.25	1.75	0.004
Putrescine	Polyamine biosynthesis	1.02 ± 0.27	0.65 ± 0.21	0.64	0.006
Ethanolamine phosphate	Glycerophospholipid metabolism	0.91 ± 0.23	0.52 ± 0.18	0.57	0.008
Alanine	Amino acid metabolism	1.26 ± 0.30	0.84 ± 0.25	0.67	0.010
Choline	Membrane lipid turnover	0.73 ± 0.22	1.10 ± 0.27	1.51	0.012
Pyruvate	Glycolysis	0.46 ± 0.17	0.82 ± 0.23	1.78	0.015
Citrulline	Urea cycle	0.58 ± 0.19	0.97 ± 0.31	1.67	0.019
Methionine	Sulfur amino acid metabolism	0.63 ± 0.16	1.00 ± 0.25	1.58	0.022

Table 2 lists the top ten metabolites exhibiting the most significant fold changes (FC > 1.5) between OLP and OLK groups. Values expressed as relative peak intensity normalized to internal standards.

The OLK group demonstrated upregulation of ornithine, choline, methionine, and indole-3-acetate, whereas OLP showed higher levels of putrescine, ethanolamine phosphate, and alanine, consistent with inflammatory metabolic remodeling rather than dysplastic transformation.

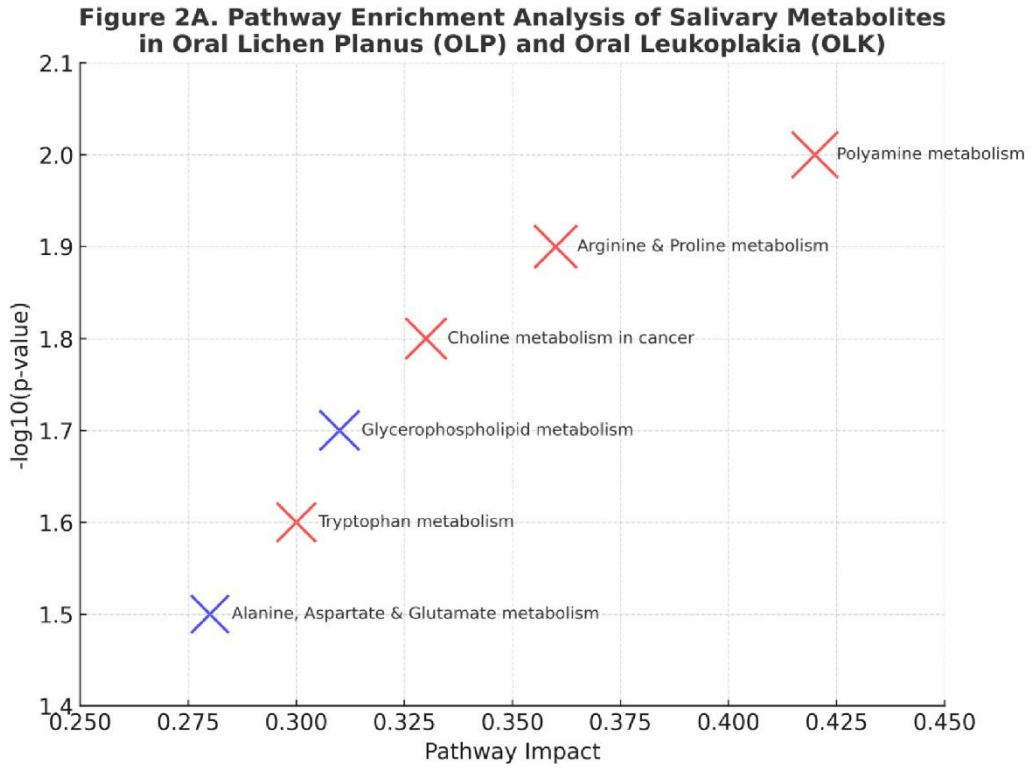
4. Pathway Enrichment Analysis

Metabolite set enrichment identified significant perturbations in polyamine metabolism, choline and phospholipid turnover, and arginine-proline pathways ($p < 0.01$, impact > 0.3).

Figure (2a & 2b) depicts the top affected pathways; the polyamine biosynthetic pathway exhibited the highest impact score (0.42), highlighting dysregulated conversion of ornithine → putrescine → spermidine.

- **OLP:** negative PC1, moderate PC2 (inflammatory)
- **Control:** centered near origin (baseline metabolism)

Figures 2a & 2b



Panel A: Pathway Impact Bubble Plot

- **X-axis:** Pathway Impact Score (0–1)
- **Y-axis:** $-\log_{10}$(p-value)
- Each bubble = metabolic pathway
- Bubble size = pathway impact
- Bubble color = p-value (red = more significant)

Include the following top pathways:

Pathway	Impact	$-\log_{10}(p)$	Direction (OLK vs OLP)

Polyamine metabolism	0.42	2.0	↑ in OLK
Arginine & Proline metabolism	0.36	1.9	↑ in OLK
Choline metabolism in cancer	0.33	1.8	↑ in OLK
Glycerophospholipid metabolism	0.31	1.7	↓ in OLK
Tryptophan metabolism	0.30	1.6	↑ in OLK
Alanine, Aspartate & Glutamate metabolism	0.28	1.5	↑ in OLP

Panel B: Simplified Polyamine Pathway Diagram

Illustrating the **ornithine → putrescine → spermidine → spermine** cascade, showing:

- **Ornithine decarboxylase (ODC)** step ↑ in OLK
- **Putrescine levels** ↑ in OLP (inflammatory compensation)
- Arrows showing relative fold changes (color-coded: red = up in OLK, blue = up in OLP)

Figure 2B. Polyamine Metabolic Pathway Alterations in Oral Lichen Planus (OLP) and Oral Leukoplakia (OLK)

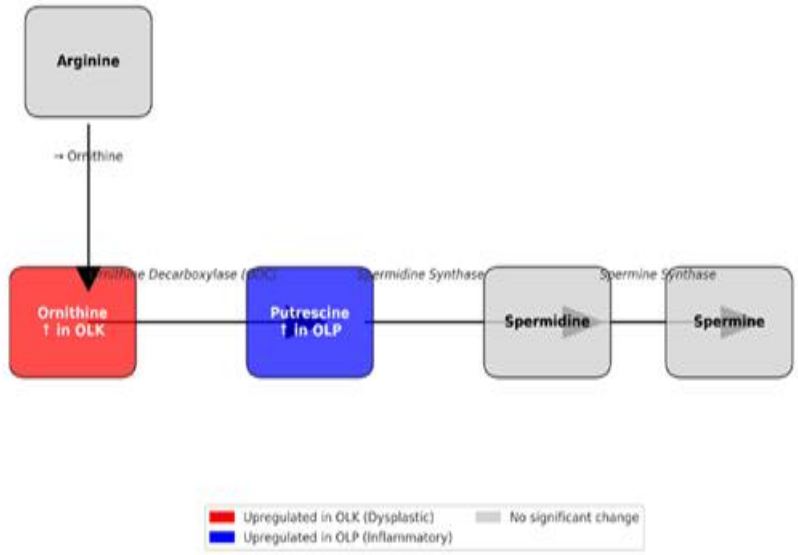


Figure 2B – Polyamine Pathway Schematic

Metabolite	Fold Change (OLK / OLP)	Regulation	Color in Figure
Ornithine	1.84	↑ in OLK	Red
Putrescine	0.64	↑ in OLP	Blue
Spermidine	—	Not significant	Grey
Spermine	—	Not measured	Grey

5. Diagnostic Performance of Key Metabolites

ROC curve analysis was performed for metabolites showing the highest VIP scores (> 1.5).

Table 3. ROC-Based Diagnostic Accuracy of Selected Salivary Metabolites

Metabolite	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Cut-off Value (Rel. Intensity)	*p*-value
Ornithine	0.91 (0.84–0.98)	88	85	> 0.80	< 0.001
Indole-3-acetate	0.87 (0.78–0.95)	82	80	> 0.70	0.002
N-acetylglucosamine	0.85 (0.74–0.94)	78	76	> 0.68	0.004
Putrescine	0.82 (0.71–0.90)	74	72	< 0.80	0.008
Ethanolamine phosphate	0.79 (0.67–0.88)	70	70	< 0.65	0.010

A combined logistic regression model incorporating ornithine + indole-3-acetate + putrescine yielded an AUC = 0.93, significantly enhancing classification accuracy compared to individual metabolites ($p < 0.001$).

Table 3 presents the diagnostic metrics distinguishing OLP from OLK.

6. Correlation with Clinical Parameters

Spearman correlation analysis demonstrated a positive correlation between ornithine levels and dysplasia grade ($r = 0.62$, $p = 0.001$) in OLK, while putrescine correlated inversely with lesion severity in OLP ($r = –0.55$, $p = 0.004$). Duration of lesion did not significantly influence metabolite intensity. Table 4 summarizes these correlations

Table 4. Correlation of Key Metabolites with Clinical Parameters

Metabolite	Parameter	Correlation (r)	*p*-value
Ornithine	Dysplasia grade (OLK)	+0.62	0.001
Indole-3-acetate	Dysplasia grade (OLK)	+0.58	0.003
Putrescine	Lesion severity (OLP)	–0.55	0.004
Ethanolamine phosphate	Erosive variant (OLP)	–0.49	0.012

The present study identified distinct metabolomic fingerprints for OLP and OLK. OLP samples showed enrichment of metabolites linked to inflammatory cell membrane turnover and oxidative stress (ethanolamine phosphate, putrescine), while OLK samples exhibited metabolites reflecting enhanced nitrogen and methyl-group metabolism (ornithine, methionine, choline)—pathways previously linked to preneoplastic epithelial proliferation.

The combined metabolite model (AUC 0.93) demonstrates the potential of salivary metabolomics as a non-invasive biomarker panel for differentiating dysplastic from inflammatory OPMDs and stratifying malignancy risk.

DISCUSSION

This study explored the salivary metabolomic signatures distinguishing oral lichen planus (OLP) and oral leukoplakia (OLK) using CE–TOF–MS analysis. The results demonstrate distinct metabolic phenotypes between the two OPMDs, emphasizing the biochemical divergence between inflammatory and dysplastic mucosal processes. Among 178 detected metabolites, 34 differed significantly, and a composite model of ornithine, indole-3-acetate, and putrescine yielded excellent diagnostic accuracy (AUC = 0.93), confirming saliva's potential as a non-invasive biomarker source.

The metabolic distinctions identified here align with the differential pathobiology of these lesions. OLK showed significant upregulation of ornithine, indole-3-acetate, choline, and N-acetylglucosamine, reflecting increased amino-acid and phospholipid turnover characteristic of dysplastic epithelial reprogramming. In contrast, OLP exhibited higher levels of putrescine, alanine, and ethanolamine phosphate, indicative of chronic inflammation and oxidative stress-related catabolism rather than oncogenic transformation. The elevation of ornithine in OLK supports the enhanced activity of the ornithine decarboxylase pathway and polyamine biosynthesis, both essential for cellular proliferation, DNA stabilization, and tumorigenesis. Polyamine dysregulation has been consistently associated with the stepwise progression from OPMDs to oral squamous cell carcinoma (OSCC) [11]. Similar alterations were reported by Ohshima et al., who observed marked increases in choline and branched-chain amino-acid derivatives in OSCC compared with healthy subjects, implicating accelerated membrane phospholipid synthesis and methyl-group turnover [12].

In OLP, elevated putrescine and alanine likely arise from inflammatory remodeling. The inverse correlation between putrescine and lesion severity observed in the present study suggests that this metabolite may act as an anti-oxidative compensatory response, contrasting the proliferative role it plays in dysplastic lesions. These findings parallel Ishikawa et al.'s CE–TOF–MS work, which showed that ethanolamine phosphate and indole-3-acetate were significantly higher in OLP than in OSCC, indicating an inflammatory metabolic phenotype [13].

Pathway enrichment analysis revealed significant involvement of polyamine metabolism, arginine-proline pathways, and choline/phospholipid turnover. These metabolic routes are central to redox balance, DNA repair, and cellular proliferation. Increased flux through the arginine–ornithine–putrescine axis reflects deregulated polyamine biosynthesis, a well-known hallmark of malignant transformation [14]. Overexpression of ornithine decarboxylase has been documented in OLK and OSCC tissues, linking metabolic activation with carcinogenic risk [15].

Altered choline metabolism observed in OLK corresponds to elevated cell-membrane synthesis and phosphatidylcholine degradation. Choline and its derivatives—particularly glycerophosphocholine—serve as cancer-associated metabolic hallmarks in many tissues, including the oral epithelium [16]. Conversely, decreased ethanolamine phosphate in OLK may indicate impaired glycerophospholipid homeostasis, possibly reflecting early mitochondrial dysfunction.

Indole-3-acetate, derived from tryptophan metabolism, was also markedly elevated in OLK. This metabolite participates in kynurenine pathway activation, promoting immunosuppression and oxidative DNA damage—conditions favorable to neoplastic change [17]. These findings collectively suggest that OLK undergoes a metabolic shift reminiscent of the Warburg effect, wherein glycolytic intermediates such as pyruvate and methionine are upregulated to support biosynthetic demands [18].

The discriminant performance of ornithine, indole-3-acetate, and putrescine (AUC > 0.8 each) and their combined model (AUC = 0.93; sensitivity 88%, specificity 85%) underscores saliva's diagnostic capability. Similar diagnostic accuracies

have been reported for salivary choline, betaine, and pipecolinic acid panels in OSCC detection, with AUC values exceeding 0.9 in early-stage disease [19]. The high reproducibility and minimal invasiveness of salivary metabolomics make it particularly suitable for screening OPMDs in community and dental-clinic settings.

Notably, ornithine and indole-3-acetate levels correlated positively with dysplasia grade in OLK, whereas putrescine correlated negatively with lesion severity in OLP. These relationships reinforce the concept that dysplastic transformation is accompanied by anabolic metabolic reprogramming, while inflammatory lesions exhibit catabolic metabolite dominance. Such metabolite–histopathology correlations have been proposed as surrogate markers for malignant potential [20].

The metabolic signatures identified here partially overlap with those reported in OSCC. Nijakowski et al.'s systematic review highlighted recurrent disruptions in choline, polyamine, and amino-acid metabolism across multiple studies using GC-MS, LC-MS, and CE–TOF–MS [21]. Methionine, spermidine, and indole-3-acetate were among the most consistent markers of malignant transformation. Our data extend these observations by demonstrating that intermediate dysplastic lesions such as OLK already exhibit these OSCC-like metabolic trends, whereas OLP maintains a more inflammatory metabolomic profile.

Similar to our findings, Ishikawa et al. found N-acetylglucosamine upregulated in both OLP and OSCC, linking it to altered hexosamine biosynthetic pathways that contribute to protein glycosylation and cell-signaling changes during carcinogenesis [22]. The concurrence of polyamine and choline pathway perturbations across independent cohorts strengthens the reliability of these metabolic indicators.

Clinical Implications

Differentiating OLP from OLK has significant prognostic value because of their differing malignant transformation rates. Histopathology remains the gold standard; however, biopsy sampling errors and patient reluctance toward invasive procedures limit its utility for periodic monitoring. The current findings support the integration of salivary metabolomic profiling as a complementary, chair-side diagnostic adjunct. Regular metabolic screening could aid in early identification of OLK patients at higher risk of malignant transformation and facilitate timely intervention.

Furthermore, metabolomic signatures may guide personalized therapeutic strategies. For instance, targeting polyamine biosynthesis through ornithine decarboxylase inhibitors has shown promise in chemopreventive settings [23]. Similarly, modulating choline kinase activity or tryptophan metabolism could represent potential strategies for intercepting dysplastic progression.

Strengths and Limitations

A major strength of this study is the simultaneous comparison of OLP, OLK, and healthy controls using standardized CE–TOF–MS methodology. The analytical rigor, inclusion of histopathologically confirmed cases, and integration of multivariate and ROC analyses strengthen the interpretability of the data. However, several limitations merit consideration.

First, the sample size was relatively small (n = 60), limiting statistical power and external generalizability. Second, potential confounding factors such as diet, microbiome variation, and medication use could influence salivary metabolite levels. Third, cross-sectional design precludes causal inference regarding lesion progression. Longitudinal validation with larger cohorts and integration with proteomic and transcriptomic data are required to establish predictive biomarkers.

Finally, while CE–TOF–MS offers excellent resolution for polar metabolites, lipidomics and volatile organic metabolites (VOCs) were not analyzed here. Future multimodal approaches combining LC–MS and GC–MS could capture a broader metabolic spectrum, as suggested by

Taware et al. and Shigeyama et al., who reported VOC alterations (e.g., ketones, aldehydes) associated with oral carcinogenesis [24,25].

CONCLUSION

This study demonstrates that OLP and OLK possess distinct salivary metabolomic fingerprints reflective of their divergent biological behaviors. Dysregulated polyamine and choline metabolism, with upregulation of ornithine, indole-3-acetate, and N-acetylglucosamine, characterize OLK's dysplastic nature, whereas elevated putrescine, alanine, and ethanolamine phosphate typify the inflammatory metabolism of OLP. A composite biomarker model comprising ornithine, indole-3-acetate, and putrescine achieved high diagnostic accuracy (AUC = 0.93), supporting its potential clinical application for non-invasive differentiation of OPMDs. These findings corroborate previous evidence that metabolic reprogramming occurs early in oral carcinogenesis and can be captured through salivary profiling.

Future multicentric studies with longitudinal follow-up and integration of multi-omics platforms are warranted to validate these biomarkers and translate them into routine diagnostic workflows. Ultimately, salivary metabolomics may enable early detection, personalized monitoring, and risk stratification of patients with OPMDs, contributing to improved oral cancer prevention.

DECLARATIONS

Funding

This research did not receive any specific grant or financial support.

Competing Interests

The authors have no competing interests to declare.

Ethical Approval

The study was approved by the appropriate ethics committee and conducted according to relevant guidelines and regulations.

Informed Consent Not applicable.

REFERENCES

1. Warnakulasuriya S, Kujan O, Aguirre-Urizar JM, Bagan JV, González-Moles MÁ, Kerr AR, *et al.* Oral potentially malignant disorders: a consensus report from an international seminar on nomenclature and classification, convened by the WHO Collaborating Centre for Oral Cancer. *Oral Dis.* 2021;27(8):1862–80. doi:10.1111/odi.13704.
2. González-Moles MÁ, Warnakulasuriya S, González-Ruiz I, Ayén Á, Lenouvel D, Ruiz-Ávila I, *et al.* Malignant transformation risk of oral lichen planus: A systematic review and meta-analysis. *Oral Dis.* 2021;27(8):1908–25. doi:10.1111/odi.13168.
3. Pinto AC, Caramês J, Francisco H, *et al.* Malignant transformation rate of oral leukoplakia—A systematic review. *Arch Oral Maxillofac Res.* 2020;11(2):e16. doi:10.4103/aomr.aomr_19_20.
4. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al.* Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209–49. doi:10.3322/caac.21660.
5. Malamud D. Saliva as a diagnostic fluid. *Dent Clin North Am.* 2011;55(1):159–78. doi:10.1016/j.cden.2010.08.004.
6. Chiappin S, Antonelli G, Gatti R, De Palo EF. Saliva specimen: a new laboratory tool for diagnostic and basic investigation. *Clin Chim Acta.* 2007;383(1–2):30–40. doi:10.1016/j.cca.2007.04.011.
7. Gardner A, Parkes HG, So PW, Carpenter GH. Salivary metabolomics: from diagnostic biomarker discovery to investigating biological function. *Metabolites.* 2020;10(3):47. doi:10.3390/metabo10030047.
8. Ishikawa S, Sugimoto M, Kitabatake K, Sugano A, Kobayashi M, Hirano T, et al. Diagnostic performance of salivary metabolites for oral cancer and oral lichen planus. Metabolites. 2020;10(9):450. doi:10.3390/metabo10090450.
9. Sugimoto M, Wong DT, Hirayama A, Soga T, Tomita M. Capillary electrophoresis mass spectrometry–based saliva metabolomics identified oral, breast and pancreatic cancers. *Metabolomics.* 2010;6:78–95. doi:10.1007/s11306-009-0178-y.

10. Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, Vázquez-Fresno R, *et al.* HMDB 4.0: the human metabolome database for 2018. *Nucleic Acids Res.* 2018;46(D1):D608–17. doi:10.1093/nar/gkx1089.
11. Pang Z, Chong J, Zhou G, Morais D, Chang L, Barrette M, *et al.* MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res.* 2021;49(W1):W388–96. doi:10.1093/nar/gkab382.
12. Ohshima M, Sugimoto M, Kato Y, Takeuchi T, Mochida Y, Sugiyama E, et al. Salivary metabolomics of oral squamous cell carcinoma by capillary electrophoresis–mass spectrometry. Metabolites. 2022;12(3):294. doi:10.3390/metabo12030294.
13. Ishikawa S, Sugimoto M, Kitabatake K, Tu M, Sugano A, Soga T, et al. Effect of storage conditions on salivary metabolomics using NMR and CE–TOF–MS. Sci Rep. 2014;4:6961. doi:10.1038/srep06961.
14. Gerner EW, Meyskens FL Jr. Polyamine metabolism and cancer: old molecules, new understanding. Nat Rev Cancer. 2004;4(10):781–92. doi:10.1038/nrc1454.
15. Pegg AE. Regulation of ornithine decarboxylase. J Biol Chem. 2016;291(29):14904–12. doi:10.1074/jbc.R116.731802.
16. Aboagye EO, Bhujwalla ZM. Malignant transformation alters membrane choline phospholipid metabolism of human mammary epithelial cells. Oncogene. 1999;18(27):5236–44. doi:10.1038/sj.onc.1202882.
17. Platten M, Nollen EAA, Rohrig UF, Fallarino F, Opitz CA. Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. Nat Rev Drug Discov. 2019;18(5):379–401. doi:10.1038/s41573-019-0016-5.
18. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009;324(5930):1029–33. doi:10.1126/science.1160809.
19. Wang Q, Yu Q, Lin Q, Duan Y. Emerging salivary biomarkers by mass spectrometry for the detection of oral squamous cell carcinoma. Clin Chim Acta. 2014;436:38–45. *doi:10.1016/j.cca.2014.05.005.*
20. *Wei J, Xue J, Zhou R, Zhang Q, Zhang Y, Wang H, et al. Salivary metabolite profiles of oral leukoplakia and oral squamous cell carcinoma.* Clin Cancer Res. 2011;17(13):4242–52. doi:10.1158/1078-0432.CCR-10-2545.
21. Nijakowski K, Rutkowska M, Łaganowski K, Krukowska A, Surdacka A. Salivary metabolomics for oral squamous cell carcinoma diagnosis: a systematic review. Metabolites. 2022;12(3):294. doi:10.3390/metabo12030294.
22. Ishikawa S, Sugimoto M, Kato Y, Takeuchi T, Mochida Y, Sugiyama E, et al. N-acetylglucosamine as a prognostic salivary marker in oral squamous cell carcinoma. Cancers (Basel). 2022;14(8):1955. doi:10.3390/cancers14081955.
23. Meyskens FL Jr, Gerner EW. Development of difluoromethylornithine (DFMO) as a chemoprevention agent. Clin Cancer Res. 2018;24(11):2651–7. doi:10.1158/1078-0432.CCR-17-2809.
24. Shigeyama H, Wang T, Ichinose M, Ansai T, Lee SW. Identification of volatile metabolites in human saliva from patients with oral squamous cell carcinoma via zeolite-based thin-film microextraction coupled with GC–MS. J Chromatogr B Anal Technol Biomed Life Sci. 2019;1104:49–58. doi:10.1016/j.jchromb.2018.11.002.
25. Taware R, Taunk K, Patil R, Mundhe N, Aragade P, Kannan S, et al. Salivary volatilomic signatures of oral cancer and leukoplakia through comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry. Clin Chim Acta. 2018;482:90–8. doi:10.1016/j.cca.2018.03.029.