



Salivary biomarkers of malignant transformation from proliferative verrucous leukoplakia to oral squamous cell carcinoma – narrative review

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Abstract

Introduction and Objective. Proliferative verrucous leukoplakia (PVL) is recognized as a distinct oral potentially malignant disorder (OPMD). It is characterized by progressive, persistent, multifocal oral leukoplakia that changes in clinical appearance and histopathological features over time, and has a marked propensity for malignant transformation. PVL occurs predominantly in older adults, shows a female predominance, and has a weaker association with tobacco and alcohol exposure than conventional oral leukoplakia.

Review Methods. This narrative review was based on a focused literature search in PubMed and Scopus for English-language articles published up to March 2025, using combinations of the terms ‘proliferative verrucous leukoplakia’, ‘PVL’, ‘oral leukoplakia’, ‘oral potentially malignant disorder’, ‘oral squamous cell carcinoma’, ‘saliva’, ‘salivary biomarkers’, ‘cytokines’, ‘proteomics’, ‘microbiome’, and ‘microRNA’.

Brief description of the state of knowledge. Most current knowledge comes from small PVL series combined with larger bodies of work on oral leukoplakia and established OSCC, which consistently show that inflammatory cytokines, proteomic changes, dysbiotic microbiome profiles, and deregulated microRNA networks are detectable in saliva.

Summary. PVL represents a high-risk, multi-focal OPMD in which current surveillance strategies rely on repeated biopsies that are invasive and may not reflect the full biological dynamics of the lesion field. Emerging data suggest that salivary biomarkers spanning inflammatory cytokines, multi-analyte protein panels, microbiome signatures, and microRNA profiles can capture aspects of PVL biology and its progression towards OSCC, but PVL-specific evidence is still limited and often extrapolated from leukoplakia or cancer cohorts.

Key words

biomarkers, microRNA, saliva, microbiome, oral squamous cell carcinoma, proliferative verrucous leukoplakia

INTRODUCTION

Proliferative verrucous leukoplakia (PVL) is recognized as a distinct oral potentially malignant disorder (OPMD). It is characterized by progressive, persistent, multifocal oral leukoplakia that changes in clinical appearance and histopathological features over time and has a marked propensity for malignant transformation [1–4]. PVL occurs predominantly in older adults, shows a female predominance, and has a weaker association with tobacco and alcohol exposure than conventional oral leukoplakia. The gingiva, alveolar mucosa, and buccal mucosa are frequently affected, often at multiple sites [1–3].

The clinicopathological spectrum includes homogeneous, flat, nodular, verrucous, and exophytic lesions. Histopathological findings may range from hyperkeratosis and oral epithelial dysplasia to verrucous carcinoma and conventional oral squamous cell carcinoma (OSCC); these findings should not be interpreted as a mandatory linear

sequence in an individual lesion [1, 4]. Meta-analyses have estimated malignant transformation in approximately 44–46% of patients, and multiple primary carcinomas may develop during follow-up [2, 3].

Current management relies on repeated clinical assessment, biopsies from clinically suspicious or changing sites, histopathological evaluation, treatment when indicated, and lifelong follow-up [1, 4]. Because repeated biopsies are invasive and may incompletely represent a multi-focal field, non-invasive adjuncts that could identify biological change, support risk stratification, and guide biopsy timing are invaluable. Saliva is an attractive source of biomarkers because its collection is simple, repeatable, and minimally invasive, and the sample contains proteins, nucleic acids, metabolites, extracellular vesicles, and microbial components [7, 13, 14].

REVIEW METHODS

A narrative literature review was performed using PubMed, Scopus, and Web of Science. The search strategy included combinations of the terms ‘proliferative verrucous

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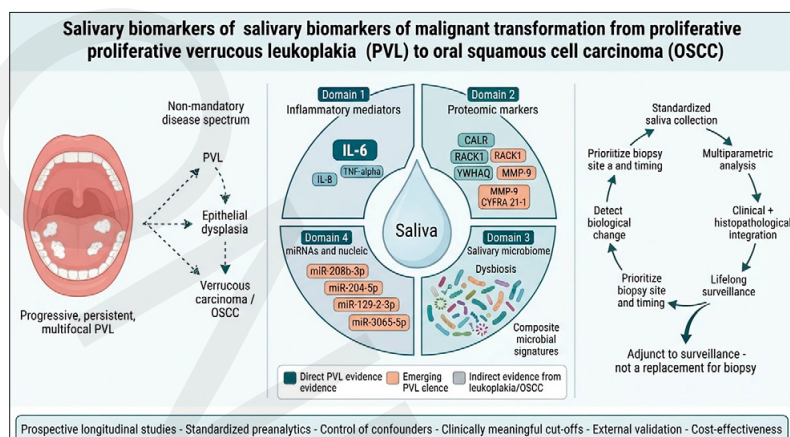


Figure 1. The initial visual concept was generated using OpenAI GPT-5.6 Sol and an AI image-generation tool. The figure was subsequently reviewed and edited by the authors, who take full responsibility for its content and accuracy

leukoplakia', 'oral potentially malignant disorder', 'oral squamous cell carcinoma', 'saliva', 'biomarker', 'microRNA' (abbreviated hereafter as 'miRNA'), 'proteomics', and 'microbiome'. Original studies, systematic reviews, meta-analyses, consensus reports, and relevant narrative reviews published in English were considered. Studies were included when they addressed saliva-based biomarkers, PVL, oral leukoplakia, or OSCC and were relevant to malignant transformation or disease monitoring. Publications without accessible full text or without relevance to the predefined biomarker domains were excluded.

The review summarizes evidence across four domains: inflammatory mediators, proteomic markers, salivary microbiome, and miRNAs, and other nucleic acids. Because PVL-specific salivary studies remain limited, evidence derived from oral leukoplakia and OSCC is clearly identified as indirect and hypothesis-generating.

Salivary inflammatory biomarkers

Interleukin-6 in PVL. Among inflammatory mediators, interleukin-6 (IL-6) has been evaluated directly involved in PVL. Bagán et al. measured serum and salivary IL-6 in 20 patients with PVL, 20 with OSCC, and 20 healthy controls. IL-6 concentrations were highest in OSCC, intermediate in PVL, and lowest in controls. Within the PVL group, salivary IL-6 was associated with the extent of the verrucous areas [5].

IL-6 is a pleiotropic cytokine involved in inflammation, angiogenesis, and tumour growth. Broader studies of oral leukoplakia and OSCC also report altered salivary inflammatory profiles, with IL-6, IL-8, and tumour necrosis factor alpha among the most frequently studied candidates [7, 8]. These findings support further evaluation of serial IL-6 measurements in PVL, but IL-6 lacks disease specificity and may be influenced by concurrent oral or systemic inflammation. It should therefore be investigated as one component of a biomarker panel rather than as a stand-alone diagnostic test.

Broader cytokine and protein panels. PVL-specific evidence for other salivary cytokines is sparse. Studies and reviews in OSCC have identified candidate proteins such as IL-8, matrix metalloproteinase-9 (MMP-9), cytokeratin 19 fragment (CYFRA 21-1), lactate dehydrogenase (LDH), and acute-phase proteins [7-9]. These OSCC-associated profiles may provide a malignant endpoint against which longitudinal changes in PVL can be compared; however,

their predictive value for PVL transformation has not yet been established.

Proteomic biomarkers

Proteomics in PVL. High-throughput proteomic analysis has begun to characterize molecular differences among PVL, conventional oral leukoplakia, and healthy oral mucosa. Arroyo et al. used liquid chromatography-tandem mass spectrometry to examine tissue and saliva samples and identified differential expression across pathways related to immune function, cell-cycle regulation, DNA regulation, and apoptosis. Calreticulin (CALR), receptor for activated C kinase 1 (RACK1), and 14-3-3 theta protein (YWHAQ) were highly expressed candidate proteins; immunohistochemical validation confirmed increased CALR expression in PVL compared with oral leukoplakia [6].

These findings indicate that PVL has a molecular profile distinct from conventional oral leukoplakia and provide candidates for targeted salivary validation. At present, however, no PVL proteomic panel has undergone sufficient external validation to support clinical use.

Salivary protein markers in OSCC. In OSCC, salivary proteomic studies have identified inflammatory, cytoskeletal, metabolic, and tissue-remodelling proteins that can distinguish patients from healthy controls [7-9]. For PVL research, these markers can serve as comparators and candidate components of longitudinal panels. Their application to PVL should remain explicitly investigational until prospective studies demonstrate that baseline values or temporal changes predict malignant transformation.

Salivary microbiome

Microbiome profiles across oral leukoplakia, PVL, and OSCC. Intini et al. directly compared the salivary microbiome in patients with oral leukoplakia, PVL, and OSCC using 16S ribosomal RNA gene sequencing. Alpha-diversity measures did not differ significantly among groups, whereas beta-diversity distinguished oral leukoplakia from both PVL and OSCC; PVL and OSCC did not differ significantly in overall community structure [10].

These findings suggest that PVL may share elements of an OSCC-associated dysbiotic pattern before overt malignancy develops. Composite microbial signatures may ultimately be more informative than individual taxa, but longitudinal

cohorts are required to determine whether baseline or evolving microbiome profiles predict transformation. Potential confounders -including periodontal disease, antimicrobial exposure, smoking, diet, and saliva-collection procedures – also require standardized assessment.

MicroRNA and other nucleic acid biomarkers

miRNA signatures in oral leukoplakia and transformation. A microRNA (miRNA) is a small non-coding RNA that regulates gene expression post-transcriptionally. miRNAs are involved in oral carcinogenesis, and systematic reviews have identified differentially expressed miRNA signatures in oral leukoplakia and oral epithelial dysplasia that may be associated with progression to OSCC. Reported candidates include miR-208b-3p, miR-204-5p, miR-129-2-3p, and miR-3065-5p, although the evidence is heterogeneous and requires prospective validation [11].

Most transformation studies have analyzed tissue or blood rather than saliva. Salivary and other circulating miRNAs are nevertheless being investigated as non-invasive biomarkers for OSCC detection, staging, prognosis, and monitoring [12–14]. Differences in sample collection, RNA isolation, normalization, and analytical platforms currently limit comparison across studies.

Potential application to PVL. Direct evidence for salivary miRNAs in PVL is scarce. Candidate signatures identified in oral leukoplakia and OSCC should therefore be tested specifically in well-characterized PVL cohorts before they are used for risk stratification. Integration of miRNA data with inflammatory proteins, proteomic candidates, clinical features, histopathology, and microbiome profiles may provide a more robust representation of malignant risk than any single analyte [11–14].

Clinical implications and future directions. The evidence base is uneven across biomarker domains. IL-6 has direct PVL-specific salivary data [5], proteomic research has generated PVL candidate markers [6], and a recent study has compared the PVL salivary microbiome with oral leukoplakia and OSCC [10]. By contrast, the proposed use of many several protein markers and miRNA signatures in PVL is largely extrapolated from conventional oral leukoplakia or established OSCC [7–9, 11–14].

Future studies should use prospective, multicentre, longitudinal designs; contemporary clinical and histopathological criteria for PVL; prespecified clinical endpoints; and standardized saliva collection, storage, processing, and analysis. They should also control for oral inflammation and other relevant confounders, establish clinically meaningful cut-off values, compare biomarker panels with current surveillance, and perform independent external validation and cost-effectiveness assessment.

The most realistic objective is not to replace biopsy, but to develop an adjunctive liquid-biopsy approach that helps identify biological change across a multifocal field, prioritizes suspicious sites or visits for tissue sampling, and supports individualized surveillance. Multiparametric models combining biomarkers with clinical and histopathological findings are likely to be more informative than single-analyte tests.

Clinical relevance. Salivary biomarkers are not currently validated to replace oral examination, site-directed biopsy, or histopathological assessment in PVL. Their potential near-term role is as a repeatable adjunct that may help detect

biological change between visits and guide the timing and location of biopsy once predictive panels have been prospectively validated.

CONCLUSIONS

Salivary biomarkers are promising non-invasive adjuncts for monitoring PVL and investigating its transformation to OSCC. The strongest direct PVL-specific evidence currently concerns IL-6, with emerging evidence from proteomic and microbiome studies; miRNA evidence, however, remains largely indirect. Before implementation, candidate panels require standardized analytical methods and prospective, multicentre validation against clinically meaningful outcomes. Until then, careful clinical surveillance and appropriately targeted biopsy remain the standard of care.

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