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Diagnostic Biomarkers of Oral Leukoplakia: A Brief Review

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ABSTRACT

Oral leukoplakia (OL) is the most prevalent OPMD and carries a significant risk of malignant transformation into OSCC. Although histopathology is the gold standard in diagnosis, it is usually not accurate in predicting the behavior of a lesion, and this aspect highlights the importance of supplementary molecular tools. This review examines the diagnostic potential of the emerging biomarkers, which are genetic, protein-based, epigenetic, salivary, and serum-derived, in evaluating OL progression. Salivary cytokines such as IL-6 and TNF-α, alongside microRNAs and enzymes like ALP and LDH, have shown strong diagnostic potential. Tissue-based markers, including loss of heterozygosity (LOH), DNA ploidy, podoplanin, p27, p53, and Ki-67, provide insights into genetic and proliferative changes associated with malignancy. The potential of these biomarkers is to facilitate more accurate diagnosis, treatment, and prognostic decisions, as well as complement prognostication by regular histology. Further testing and implementation into clinical practice are necessary to maximize OL management and early detection of oral cancer.

Keywords: *Oral leukoplakia, diagnostic biomarkers, malignant transformation, cytokines, epigenetics, histopathology*

1. INTRODUCTION

Oral cancer remains a significant global health burden, with late diagnosis being a key contributor to its high morbidity and mortality. Among the various OPMDs, OL is the most prevalent and carries a notable risk of malignant transformation into OSCC. OL has traditionally been diagnosed and treated based on clinical presentation and confirmed by histopathological examination. Nevertheless, histology is insufficient in predicting the biological behavior of these lesions and their risk of transformation in the future. Consequently, the need for more precise, objective, and non-invasive diagnostic tools has become increasingly important [1].

In recent years, molecular and cellular biomarkers have become applicable supplements to histological evaluation. Such biomarkers may facilitate early diagnosis of high-risk lesions, disease progression prediction, therapeutic effects monitoring, and even the personalization of interventions. Depending on saliva, serum, or tissue samples, this area has grown to encompass various types of biomarkers, including genetic, epigenetic, proteomic, immunologic, and transcriptomic signatures. The growth of cancer biology and the creation of technologies in the sphere of omics preconditioned the entry of these biomarkers into clinical practice.

2. Definition and Epidemiology of Oral Leukoplakia

Oral leukoplakia is clinically defined by the World Health Organization (WHO) as a "predominantly white lesion of the oral mucosa that cannot be characterized as any other definable disease" [2]. This definition underscores the diagnostic challenge, as OL is a diagnosis of exclusion, making objective diagnostic criteria critical for early identification and risk stratification.

Epidemiologically, OL affects approximately 1–5% of the global population, with a higher incidence in middle-aged and elderly individuals, particularly men. The condition is more prevalent in regions with high tobacco and alcohol consumption, including South Asia and parts of Eastern Europe. According to global surveillance studies, OL accounts for about 85% of all OPMDs, and its annual malignant transformation rate ranges from 1% to 3%, depending on clinical subtype, lesion site, and presence of epithelial dysplasia [3].

The clinical appearance of OL is heterogeneous, ranging from homogeneous, flat, well-demarcated white plaques to non-homogeneous types with nodular, verrucous, or speckled patterns. Non-homogeneous OL, particularly those located on high-risk sites such as the lateral tongue, floor of the mouth, and soft palate, are more likely to undergo malignant transformation [4].

3. Rationale for Biomarker-Based Diagnosis to Complement Histopathology

Histopathological examination, primarily based on hematoxylin and eosin (H&E) stained sections, remains the cornerstone for assessing epithelial dysplasia in OL. However, this method has inherent limitations. The assessment is often subjective, prone to inter-observer variability, and does not always correlate with biological behavior or transformation potential [5]. For instance, some lesions without significant histological dysplasia have been reported to transform into carcinoma, while others with moderate to severe dysplasia may remain stable for years.

For example, LOH at specific chromosomal loci (e.g., 3p, 9p) has been associated with increased risk of transformation. Similarly, DNA ploidy analysis and podoplanin expression have shown potential in stratifying lesions into high- and low-risk groups [6]. Salivary biomarkers, such as interleukin-6 (IL-6) and miR-21, are valuable tools for non-invasive screening, enabling real-time monitoring and early intervention [7].

Scope and significance of review.

This review discusses the dynamic biomarker of diagnostics in OL, the most common oral potentially malignant disease. It combines the results of the recent molecular and clinical research to outline salivary, serum, tissue-based, genetic, proteomic, and epigenetic markers that can be used to facilitate early diagnosis and risk prediction. The review critically analyzes the role of such biomarkers in supplementing traditional histopathology, improving the accuracy of diagnosis, and detecting high-risk lesions. The clinical relevance, non-invasive diagnostic methods, and possible incorporation of biomarkers into individual care paths are also stressed. This review integrates existing evidence to help researchers and clinicians use biomarker-based approaches to enhance the management of OL and the prevention of oral cancer.

2. Salivary and Serum Biomarkers

2.1 Cytokines and Proteins

Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are pro-inflammatory cytokines widely researched in their role in inflammation-based carcinogenesis in Table 1. High salivary levels of these cytokines have demonstrated significant predictive capacity of the malignant conversion of OL. Meta-analysis and clinical reports show that the levels of IL-6 and TNF-alpha are highly elevated in OL patients with epithelial dysplasia compared to those with non-dysplastic lesions [8].

The serum protein biomarkers (lipid-bound sialic acid (LSA), total sialic acid (TSA), and C-reactive protein (CRP)) have also been explored. The high LSA and TSA in serum are associated with the severity of epithelial dysplasia, which indicates the possible role of LSA and TSA in systemic inflammation in high-risk cases of leukoplakia. In the same way, CRP is a well-known marker of acute-phase proteins whose elevation is a sign of chronic inflammatory activity that correlates with premalignant states [9].

2.2 MicroRNAs, ALP, and LDH

Small non-coding RNAs known as microRNAs (miRNAs) are of diagnostic accuracy in oral potentially malignant disorders. Specifically, miR-21, miR-184, and miR-145 have shown high sensitivity and specificity in differentiating dysplastic leukoplakia and normal mucosa. They are potentially good biomarkers of early OL detection because they are stable in saliva

and can be collected non-invasively [10].

Also, the release of enzyme markers, like alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), occurs in cellular turnover and necrosis. Researchers have found that high salivary ALP and LDH levels are directly related to the histological grade of leukoplakia's dysplasia. The enzymes offer a convenient chairside approach to assessing the severity of lesions and progression status [11].

2.3 Albumin and Oxidative Markers

Oxidative stress markers, such as albumin and different antioxidant enzymes (AOEs), also indicate the biological behavior of OL. Patients with OL have decreased serum albumin and an alteration of antioxidant profile, both indicative of systemic involvement. Moreover, the fact that these markers improve after curcumin treatment can help them become prognostic factors and therapeutic response monitoring tools [12, 13].

Table 1: Salivary and Serum Biomarkers for Diagnosis and Risk Assessment in Oral Leukoplakia

Biomarker	Type	Source	Function	Associated Finding	OL Relevance	Detection Method	Sensitivity	Specificity
IL-6	Cytokine	Saliva	Pro-inflammatory	Elevated in progressive lesions	Malignant transformation risk marker	ELISA	High	Moderate
TNF-α	Cytokine	Saliva	Immune modulation	Upregulated in dysplasia	Progression marker	ELISA	High	High
LSA	Protein	Serum	Stress marker	Elevated in OL with severe dysplasia	Inflammatory activity marker	Spectrophotometry	Moderate	Moderate
TSA	Protein	Serum	Total protein expression	Highly advanced leukoplakia	Disease severity indicator	Biochemical Assays	Moderate	Low
CRP	Acute-phase	Serum	Inflammation marker	Increase in high-risk lesions	General inflammation marker	Immunoturbidimetry	Moderate	Low
miR-21	microRNA	Saliva	Oncogenic regulatory RNA	Upregulated in dysplastic OL	Early diagnostic biomarker	qRT-PCR	High	High
ALP & LDH	Enzymes	Saliva	Cellular turnover, necrosis	Correlated with histological grade	Severity biomarker	Biochemical Assay	High	Moderate
Albumin + AOE	Protein/Enzyme	Serum	Oxidative stress and nutrition	Altered levels post-curcumin therapy in OL patients	Response to chemopreventive therapy	Spectrometry & ELISA	Variable	Context-specific

Table 2: Tissue-Based Biomarkers in Oral Leukoplakia and Their Diagnostic/Prognostic Significance

Biomarker	Category	Type	Detection Method	Clinical Finding	OL Relevance	Risk Prediction Accuracy
LOH at 3p	Genetic Alteration	DNA Loss	Microsatellite analysis	Found in dysplastic OL tissues	Early predictor of malignant potential	Moderate
LOH at 9p	Genetic Alteration	DNA Loss	PCR-based LOH assay	Observed in progressing lesions	Marker of genomic instability	Moderate
DNA Ploidy	Genetic Instability	Aneuploidy	Image cytometry	Aneuploid lesions are linked to higher dysplasia grades	Prognostic for cancer progression	High
Podoplanin (PDPN)	Protein Marker	Transmembrane protein	IHC	Overexpressed in dysplastic and OSCC tissues	Independent risk factor	High
p27	Protein Marker	Cell cycle regulator	IHC	Loss associated with aggressive phenotypes	Predictive of epithelial dysplasia	Moderate
p53	Tumor Suppressor	Nuclear protein	IHC	Overexpression in dysplastic epithelium	Marker of DNA damage response	Variable
Ki-67	Proliferation Marker	Nuclear protein	IHC	Expressed in basal/parabasal layers of OL	Indicates proliferative index	Moderate
CD3	Immune Marker	T-cell marker	IHC	Higher T-cell infiltration in early lesions	Immune surveillance role in OL	Limited
CD8	Immune Marker	Cytotoxic T-cell	IHC	Correlates with immune activation	Marker of anti-tumor response	Limited
EZH2 Methylation	Epigenetic Marker	Histone methyltransferase	MSP	Promoter hypermethylation in high-risk OL	Prognostic marker for progression	High
DAPK1 Methylation	Epigenetic Marker	Tumor suppressor gene	MSP	Frequently methylated in dysplastic tissues	Silencing is linked to transformation	Moderate
p16INK4a	Tumor Suppressor	Cell cycle regulator	IHC	Variable expression; loss in some progressive OL	Potential marker of early dysplasia	Low–Moderate

2. TISSUE-BASED BIOMARKERS

3.1 Genetic Alterations: LOH and DNA Ploidy

Genetic alteration is one of the first and most determinant processes in the malignant evolution of OL. LOH, particularly chromosomal loci, is one of the most examined genetic abnormalities in Table 2. Notably, 20 percent of OSCC progression risk is linked to LOH at chromosomes 3p and 9p. Tumor suppressor genes located at these loci include FHIT (3p) and CDKN2A (9p), which, when inactivated, can lead to loss of regulation of cell cycle and apoptosis and thus induce carcinogenesis [14].

Besides LOH, DNA ploidy analysis, which quantifies and localizes nuclear DNA content, has also become an essential predictor of OL behavior. It has been shown that aneuploidy (abnormal DNA content) is more frequent in the high-grade dysplastic lesions and those that ultimately develop into carcinoma. Combining DNA ploidy with the histopathological grading increases the accuracy of predicting malignant transformation, resulting in a more extensive risk assessment [15].

3.2 Protein Markers: Podoplanin, p27, p53, Ki-67, CD3/CD8

Protein expression markers give vital information about cellular proliferation, immune response, and the activity of tumor suppressor genes. One is podoplanin (PDPN), a transmembrane glycoprotein overexpressed in dysplastic oral epithelium and early OSCC. It is involved in the migration and invasion of cells, and its expression has correlated positively with the risk of progression. Likewise, the loss of p27, a cyclin-dependent kinase inhibitor, is also associated with diminished cell cycle regulation and is found to be an independent risk factor of OL [16].

One of OL's most commonly analyzed biomarkers is the tumor suppressor protein p53. It is overexpressed frequently because of mutation, which indicates an imbalance in cell cycle checkpoints and DNA repair. Ki-67 is a nuclear proliferation antigen that estimates cell turnover and often increases in high-grade dysplasia. Although p53 and Ki-67 demonstrate possible risk stratification, the prognostic value of these markers needs additional longitudinal confirmation [17, 18].

OL has also been studied regarding immune cell markers in CD3 and CD8. Increased infiltration of CD3+ T-cells and CD8+ cytotoxic T lymphocytes shows an active immune surveillance mechanism, which could be protective. Their prognostic role is, however, still under research and requires better data using long-term studies [19, 20].

3.3 Epigenetic Markers

There is emerging evidence that epigenetic changes, especially DNA methylation, may prove to be non-invasive and predictive of malignant transformation of OL. EZH2 (Enhancer of Zeste Homolog 2) is a histone methyltransferase that functions in chromatin remodeling and is one candidate. The EZH2 is aberrantly hypermethylated in promoters and associated with the poor prognosis and elevated risk of transformation due to the silencing of the tumor suppressor genes and inducing dedifferentiation of cells [21-23].

Epigenetic biomarkers have particular potential as they could provide an early indication before the development of histological changes. With the evolution of molecular technologies, these markers might enter a panel of biomarkers with the ability to stratify the risk of OL comprehensively.

3. CONCLUSIONS

The oral potentially malignant disorder is most common. It is known as OL, but its clinical and histological heterogeneity makes diagnosing and stratifying the risk difficult. Although histopathology can be considered the gold standard of diagnosis, it is neither objective nor sensitive enough to predict the malignant transformation in most cases. A combination of molecular and cellular biomarkers (genetic, proteomic, immunologic, and epigenetic markers) can be a revolutionary technology that will enhance the accuracy of the diagnosis. Non-invasive real-time monitoring tools are salivary and serum-based markers, including IL-6, miR-21, ALP, and LDH. Markers based on tissues, such as LOH, DNA ploidy, podoplanin, p53, and EZH2 methylation, provide greater insight into lesion biology and risk of progression. Developing multi-omics technologies and AI-based models also enhances the possibility of biomarker-guided oral cancer prevention. With the development of research, biomarker panels that have been validated may help to improve early diagnosis and individual therapy, and minimize the burden of oral cancer worldwide by intervening in the high-risk OL lesions early. Collaborating on clinical trials and standardizing the testing procedures will be necessary to implement these findings in regular practice.

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