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Optimization of Oncogenic Risk Assessment in Patients with Oral Leukoplakia Associated with Type 2 Diabetes Mellitus: A Precision Diagnostic Approach

Keywords
oral leukoplakia; Type 2 diabetes mellitus; oral potentially malignant disorders; epithelial dysplasia; precision diagnostics; digital pathology; immunohistochemistry; oral squamous cell carcinoma.

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Abstract
Oral leukoplakia is the most common oral potentially malignant disorder (OPMD) and remains a significant precursor of oral squamous cell carcinoma (OSCC). Although histopathological grading is the current standard for evaluating malignant transformation, its predictive accuracy is limited, particularly in patients with Type 2 diabetes mellitus (T2DM), where chronic hyperglycemia, oxidative stress, persistent inflammation, and metabolic dysregulation may accelerate epithelial carcinogenesis. The present study aimed to optimize oncogenic risk assessment in patients with oral leukoplakia associated with T2DM through the development of an integrated precision diagnostic approach combining clinicopathological, metabolic, immunohistochemical, and digital pathological parameters. A prospective comparative observational study was conducted involving patients with oral leukoplakia with and without T2DM and healthy controls. Comprehensive clinical examination, histopathological grading, immunohistochemical evaluation of Ki-67, p53, p16, VEGF, and E-cadherin expression, glycemic assessment using fasting plasma glucose and glycated hemoglobin (HbA1c), inflammatory and oxidative stress biomarker analysis, digital pathology, morphometric image analysis, and artificial intelligence-assisted risk prediction were performed. Multivariate logistic regression, Cox proportional hazards modeling, receiver operating characteristic (ROC) analysis, and machine-learning algorithms were used to identify independent predictors of malignant transformation and to construct a personalized oncogenic risk model. The integrated diagnostic framework demonstrated superior predictive performance compared with conventional histopathological evaluation by more accurately stratifying patients according to their individualized risk profiles. Elevated HbA1c, severe epithelial dysplasia, increased Ki-67 and p53 expression, enhanced VEGF-mediated angiogenesis, reduced E-cadherin expression, and digital morphometric abnormalities collectively contributed to a significantly improved prediction of malignant progression. The proposed Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF) represents an original multidisciplinary model that integrates metabolic status, molecular biomarkers, digital pathology, and artificial intelligence into precision oral oncology. This approach has the potential to facilitate earlier diagnosis, improve individualized surveillance strategies, optimize therapeutic decision-making, and reduce the incidence of malignant transformation among high-risk patients with oral leukoplakia and Type 2 diabetes mellitus.

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1. INTRODUCTION

Oral leukoplakia (OL) is recognized as the most prevalent oral potentially malignant disorder (OPMD) and remains one of the greatest challenges in contemporary oral medicine due to its unpredictable biological behavior and variable risk of malignant transformation. According to the World Health Organization (WHO), oral leukoplakia is defined as a predominantly white plaque of questionable risk that cannot be clinically or histopathologically characterized as any other definable oral disease (WHO Collaborating Centre for Oral Cancer, 2022). Although many lesions remain stable for years, a considerable proportion progress to oral squamous cell carcinoma (OSCC), making early identification of high-risk lesions a priority in oral oncology (Warnakulasuriya et al., 2021).

Oral squamous cell carcinoma accounts for approximately 90–95% of all oral malignancies and represents a major global public health burden. More than 370,000 new cases of oral cancer and approximately 177,000 related deaths are reported annually worldwide, with incidence rates continuing to rise in many developing and transitional countries (Sung et al., 2024). Despite substantial improvements in surgery, radiotherapy, chemotherapy, and targeted therapies, the overall five-year survival rate remains approximately 50–60%, largely because many patients are diagnosed at advanced stages. Consequently, prevention of malignant transformation through early diagnosis and risk stratification of OPMDs has become a major focus of precision oral oncology.

Among all OPMDs, oral leukoplakia exhibits the greatest clinical heterogeneity. Lesions range from homogeneous white plaques with relatively low malignant potential to non-homogeneous lesions containing nodular, verrucous, or erythroplastic components that demonstrate substantially higher oncogenic risk. Histopathological evaluation of epithelial dysplasia remains the current gold standard for estimating malignant transformation risk; however, considerable interobserver variability and biological heterogeneity limit its predictive accuracy. Importantly, lesions with mild dysplasia occasionally undergo malignant transformation, whereas severe dysplasia may remain stable for prolonged periods, indicating that conventional histopathology alone cannot completely explain disease progression (Warnakulasuriya et al., 2021).

Current evidence suggests that oral carcinogenesis is driven by complex interactions among environmental exposures, chronic inflammation, host immune responses, genetic susceptibility, epigenetic alterations, and metabolic dysfunction. Traditional risk factors such as tobacco smoking, alcohol consumption, betel quid chewing, and persistent mucosal irritation remain important contributors to leukoplakia development. Nevertheless, increasing attention has shifted toward systemic diseases that may alter the biological behavior of OPMDs independently of these conventional carcinogenic exposures. Among these systemic conditions, Type 2 diabetes mellitus (T2DM) has emerged as one of the most important metabolic disorders associated with impaired oral health, delayed wound healing, chronic inflammation, and increased cancer susceptibility (American Diabetes Association [ADA], 2025).

T2DM currently affects more than 530 million adults worldwide, and its prevalence continues to increase because of population aging, obesity, sedentary lifestyles, and dietary transitions. In addition to well-recognized macrovascular and microvascular complications, diabetes profoundly influences oral tissues through persistent hyperglycemia, impaired immune function, oxidative stress, endothelial dysfunction, and altered epithelial regeneration. Patients with T2DM exhibit higher frequencies of periodontal disease, oral candidiasis, xerostomia, delayed postoperative healing, and several oral potentially malignant disorders, including leukoplakia (International Diabetes Federation [IDF], 2025).

The biological relationship between diabetes and carcinogenesis extends far beyond elevated blood glucose levels. Chronic hyperglycemia induces persistent metabolic stress that disrupts intracellular signaling pathways, promotes mitochondrial dysfunction, and enhances production of reactive oxygen species (ROS). Excessive ROS accumulation damages DNA, proteins, and cellular membranes while simultaneously activating oncogenic signaling pathways responsible for abnormal proliferation, impaired apoptosis, genomic instability, and malignant transformation. Consequently, diabetic tissues often exhibit a pro-carcinogenic microenvironment characterized by oxidative damage, chronic inflammation, and impaired tissue repair (Brownlee, 2022).

One of the principal molecular mechanisms linking diabetes with carcinogenesis involves activation of the advanced glycation end product–receptor for advanced glycation end products (AGE–RAGE) pathway. Persistent hyperglycemia accelerates non-enzymatic glycation of proteins, lipids, and nucleic acids, leading to progressive accumulation of AGEs within tissues. Binding of AGEs to RAGE receptors activates multiple intracellular signaling cascades, including NF-κB, MAPK, JAK/STAT, and PI3K/Akt pathways. These signaling networks stimulate production of pro-inflammatory cytokines, enhance oxidative stress, increase cellular proliferation, inhibit apoptosis, and promote angiogenesis. Collectively, these alterations create favorable conditions for epithelial dysplasia and malignant transformation (Schmidt et al., 2023).

Recent investigations indicate that the AGE–RAGE pathway may be particularly relevant in oral leukoplakia because oral epithelial cells are continuously exposed to oxidative stress, microbial biofilms, and mechanical irritation. Chronic activation of RAGE signaling enhances production of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β), and vascular endothelial growth factor (VEGF), thereby establishing a persistent inflammatory microenvironment conducive to carcinogenesis. Furthermore, AGE-mediated collagen cross-linking alters extracellular matrix composition and impairs epithelial-stromal interactions, potentially facilitating invasive tumor behavior.

Oxidative stress represents another critical mechanism connecting diabetes with oral carcinogenesis. Under

physiological conditions, antioxidant defense systems neutralize reactive oxygen species and maintain redox homeostasis. However, prolonged hyperglycemia overwhelms these protective mechanisms, resulting in oxidative DNA damage, lipid peroxidation, mitochondrial dysfunction, and activation of stress-responsive transcription factors. Oxidative stress not only promotes genetic mutations but also enhances epithelial proliferation, inhibits programmed cell death, and stimulates angiogenesis, thereby accelerating malignant progression. Several recent studies have demonstrated elevated oxidative stress biomarkers in patients with oral leukoplakia, particularly among individuals with poorly controlled diabetes (Liu et al., 2024).

Although accumulating evidence supports an association between diabetes and oral cancer, the precise biological mechanisms underlying malignant transformation of leukoplakia remain incompletely understood. Many previous studies have investigated either oral leukoplakia or diabetes independently, whereas relatively few have evaluated their combined influence using integrated clinicopathological, molecular, and digital diagnostic approaches. This gap has important clinical implications because diabetic patients may require different surveillance strategies and individualized risk prediction models than non-diabetic individuals.

The growing recognition of these limitations has shifted oral oncology toward precision diagnostics, in which clinicopathological findings are integrated with molecular biomarkers, metabolic characteristics, and advanced digital technologies. Rather than relying exclusively on conventional microscopic grading, precision diagnostic approaches seek to identify individualized biological signatures capable of accurately predicting malignant transformation. Such strategies are increasingly considered essential for improving early diagnosis, optimizing treatment selection, and reducing unnecessary interventions in low-risk patients while ensuring timely management of high-risk lesions.

The progression of oral leukoplakia from a benign hyperkeratotic lesion to invasive oral squamous cell carcinoma (OSCC) is increasingly recognized as a consequence of persistent chronic inflammation rather than solely the accumulation of random genetic mutations. Chronic inflammatory responses create a biologically permissive microenvironment characterized by continuous cytokine production, oxidative stress, extracellular matrix remodeling, and dysregulated epithelial regeneration. In patients with Type 2 diabetes mellitus (T2DM), this inflammatory milieu is further intensified by prolonged hyperglycemia, insulin resistance, and activation of the AGE–RAGE signaling pathway, leading to sustained production of pro-inflammatory mediators including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and transforming growth factor-beta (TGF-β). These cytokines stimulate epithelial proliferation, inhibit apoptosis, promote angiogenesis, and facilitate genomic instability, thereby accelerating malignant transformation (Hanahan, 2022; Schmidt et al., 2023).

Closely associated with chronic inflammation is immune dysregulation, which plays a fundamental role in determining whether dysplastic oral epithelial lesions remain stable or progress toward malignancy. Under physiological conditions, immune surveillance mediated by cytotoxic T lymphocytes, natural killer cells, macrophages, and dendritic cells eliminates genetically altered epithelial cells before malignant clones become established. However, chronic hyperglycemia impairs both innate and adaptive immune responses by reducing neutrophil chemotaxis, macrophage phagocytic activity, antigen presentation, and T-cell function. Simultaneously, prolonged inflammatory stimulation promotes the expansion of immunosuppressive regulatory T cells and myeloid-derived suppressor cells, allowing transformed epithelial cells to evade immune destruction. Consequently, diabetic patients may exhibit a diminished capacity to eliminate premalignant clones, thereby increasing the likelihood of malignant progression (Liu et al., 2024).

Among the histopathological features of oral leukoplakia, epithelial dysplasia remains the most important predictor of malignant transformation. Dysplasia reflects progressive architectural and cytological abnormalities, including loss of epithelial polarity, nuclear pleomorphism, increased nuclear-to-cytoplasmic ratio, abnormal mitotic figures, and disturbed maturation of keratinocytes. The World Health Organization currently recommends grading epithelial dysplasia as mild, moderate, or severe according to established histopathological criteria. Nevertheless, considerable interobserver variability and biological heterogeneity reduce the predictive value of dysplasia alone. Numerous studies have reported malignant transformation in lesions exhibiting only mild dysplasia, whereas some severely dysplastic lesions remain clinically stable for prolonged periods. These inconsistencies indicate that histopathological grading should be complemented by objective molecular biomarkers capable of improving individualized risk prediction (WHO Collaborating Centre for Oral Cancer, 2022).

Recent advances in molecular carcinogenesis have substantially improved understanding of the biological mechanisms underlying malignant transformation. Oral carcinogenesis is now recognized as a multistep evolutionary process involving sequential accumulation of genetic mutations, epigenetic modifications, altered cell signaling pathways, metabolic reprogramming, and changes within the tumor microenvironment. These alterations affect cellular proliferation, apoptosis, angiogenesis, epithelial adhesion, and immune escape, ultimately leading to invasive carcinoma. Consequently, increasing emphasis has been placed on identifying molecular biomarkers capable of detecting these biological changes before irreversible malignant transformation occurs.

Among the most extensively investigated biomarkers is Ki-67, a nuclear protein expressed during active phases of the cell cycle but absent in resting cells. Ki-67 is widely regarded as one of the most reliable indicators of cellular proliferation. Numerous investigations have demonstrated progressive increases in Ki-67 expression from normal oral mucosa through epithelial dysplasia to invasive squamous cell carcinoma. Elevated proliferative activity is particularly evident in poorly controlled diabetic patients, suggesting that metabolic dysfunction

may accelerate epithelial turnover and dysplastic progression. Quantitative assessment of Ki-67 therefore provides valuable prognostic information beyond conventional histopathological grading (Warnakulasuriya et al., 2021).

Another critical biomarker is p53, often described as the "guardian of the genome." The p53 protein regulates DNA repair, cell-cycle arrest, and apoptosis following genomic injury. Mutation or functional inactivation of the TP53 gene represents one of the earliest and most frequent molecular events in oral carcinogenesis. Abnormal accumulation of p53 protein has been consistently associated with increasing dysplasia severity and greater risk of malignant transformation. Because oxidative stress and chronic inflammation increase DNA damage, diabetic patients may demonstrate earlier or more pronounced p53 abnormalities than non-diabetic individuals, further supporting the importance of integrating metabolic and molecular information into oncogenic risk assessment.

The cyclin-dependent kinase inhibitor p16 also plays an essential role in regulating cellular proliferation. Although p16 overexpression is traditionally associated with human papillomavirus-related malignancies, altered p16 expression has additionally been observed in oral leukoplakia and dysplastic lesions unrelated to HPV infection. Evaluation of p16 may therefore contribute to more accurate characterization of cell-cycle dysregulation, particularly when interpreted alongside Ki-67 and p53. Rather than functioning independently, these biomarkers collectively reflect complementary aspects of epithelial carcinogenesis.

Loss of E-cadherin expression represents another hallmark of malignant progression. E-cadherin is a transmembrane adhesion molecule responsible for maintaining epithelial architecture and intercellular cohesion. Reduced expression weakens epithelial integrity, promotes epithelial–mesenchymal transition (EMT), facilitates cellular migration, and enhances invasive potential. Progressive reduction of E-cadherin has been documented during transition from oral epithelial dysplasia to invasive squamous cell carcinoma, making it a valuable indicator of early malignant transformation.

Similarly, vascular endothelial growth factor (VEGF) is one of the principal regulators of tumor angiogenesis. Neoplastic progression depends upon adequate vascular supply to sustain proliferating cells. Increased VEGF expression stimulates endothelial proliferation, formation of new blood vessels, and enhanced nutrient delivery, thereby facilitating tumor growth and invasion. Diabetes-associated endothelial dysfunction and chronic inflammation may further modify VEGF activity, suggesting that angiogenic biomarkers should be incorporated into precision diagnostic models evaluating oncogenic risk.

Recent technological advances have transformed conventional pathology through the emergence of digital pathology. Whole-slide imaging, quantitative morphometric analysis, and artificial intelligence-assisted image interpretation permit objective assessment of epithelial thickness, nuclear morphology, mitotic activity, biomarker expression, and tissue architecture. Compared with conventional microscopy, digital pathology substantially reduces observer variability while improving reproducibility and quantitative precision. Integration of digital pathology with immunohistochemistry and clinical variables therefore represents an important step toward individualized oral cancer diagnostics (Bancroft & Gamble, 2021).

The rapid development of precision diagnostics has further expanded opportunities for personalized risk prediction. Rather than relying upon isolated clinical or histopathological parameters, precision diagnostic models combine clinicopathological findings, metabolic indicators, molecular biomarkers, and digital image analysis to generate individualized estimates of malignant transformation risk. Such approaches are particularly relevant for diabetic patients because metabolic control, inflammatory status, and molecular alterations collectively influence biological behavior of oral leukoplakia.

Despite substantial progress in oral oncology, important research gaps remain. Most published investigations have examined oral leukoplakia, epithelial dysplasia, molecular biomarkers, or diabetes independently. Very few studies have simultaneously integrate histopathological grading, glycemic control, oxidative stress, inflammatory biomarkers, immunohistochemical markers, and digital pathology within a single precision diagnostic framework. Furthermore, the interaction between chronic hyperglycemia and molecular carcinogenesis remains insufficiently characterized, particularly in relation to individualized prediction of malignant transformation.

Accordingly, the primary objective of the present study is to optimize oncogenic risk assessment in patients with oral leukoplakia associated with Type 2 diabetes mellitus by integrating clinicopathological characteristics, metabolic parameters, immunohistochemical biomarkers, and digital pathology into a comprehensive precision diagnostic model. The study further aims to identify independent predictors of malignant transformation, evaluate the prognostic performance of individual biomarkers, and develop an evidence-based framework capable of improving individualized clinical decision-making.

Based on current scientific evidence, the study is guided by the following hypotheses: (H1) poor glycemic control significantly increases the severity of epithelial dysplasia; (H2) oxidative stress and chronic inflammation contribute to increased proliferative activity; (H3) combined assessment of Ki-67, p53, p16, VEGF, and E-cadherin improves prediction of malignant transformation; (H4) digital pathology demonstrates greater diagnostic reproducibility than conventional microscopy; and (H5) integration of clinicopathological, metabolic, and molecular variables provides superior predictive accuracy compared with traditional histopathological evaluation alone.

The principal novelty of this study is the introduction of the Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF), an original multidisciplinary model integrating histopathological grading, diabetic metabolic status, molecular biomarkers, digital pathology, and artificial intelligence-assisted image analysis into a unified precision diagnostic system. Unlike previous

diagnostic approaches that evaluate these parameters separately, IPORAF provides individualized biological risk profiling capable of improving early detection of malignant transformation.

The scientific significance of the proposed framework extends beyond oral leukoplakia. By integrating molecular pathology, endocrinology, digital pathology, and precision oncology, this study contributes to the growing field of personalized medicine and establishes a foundation for future translational research aimed at preventing oral squamous cell carcinoma among metabolically vulnerable patient populations.

2. LITERATURE REVIEW

2.1 Oral Leukoplakia

Oral leukoplakia (OL) is recognized as the most prevalent oral potentially malignant disorder (OPMD) and represents one of the principal precursor lesions for oral squamous cell carcinoma (OSCC). The World Health Organization defines oral leukoplakia as a predominantly white plaque of questionable risk that cannot be clinically or histopathologically characterized as another specific oral disease. Despite this standardized definition, considerable heterogeneity exists in the clinical presentation, biological behavior, and malignant transformation potential of leukoplakia, making risk stratification one of the greatest challenges in oral medicine (WHO Collaborating Centre for Oral Cancer, 2022).

The reported prevalence of oral leukoplakia ranges between 1% and 4% worldwide, although higher rates have been documented in populations with widespread tobacco use, alcohol consumption, and betel quid chewing. Importantly, only a subset of leukoplakias undergo malignant transformation, with recent umbrella reviews estimating an average transformation rate of approximately 6%, whereas proliferative verrucous leukoplakia exhibits substantially higher rates approaching 50% . These findings indicate that leukoplakia should not be considered a single disease entity but rather a spectrum of biologically distinct lesions with variable oncogenic potential.

Traditionally, malignant risk assessment has relied primarily on clinical appearance and histopathological grading of epithelial dysplasia. Homogeneous lesions generally demonstrate lower transformation rates than non-homogeneous, nodular, verrucous, or erythroleukoplastic lesions. Nevertheless, several recent investigations have demonstrated that clinical phenotype alone provides insufficient prognostic accuracy because apparently low-risk lesions may progress to invasive carcinoma while clinically aggressive lesions occasionally remain stable for prolonged periods. Consequently, increasing emphasis has been placed on integrating molecular biomarkers and individualized biological risk factors into diagnostic algorithms (Gates et al., 2025).

Recent systematic reviews have further demonstrated that tissue biomarkers substantially improve prediction of malignant transformation when combined with histopathological evaluation. Biomarkers such as Ki-67, p53, DNA ploidy, podoplanin, and Bmi-1 have shown independent prognostic value, although considerable methodological heterogeneity continues to limit widespread clinical implementation.

2.2 Oral Potentially Malignant Disorders (OPMD)

Oral leukoplakia belongs to the broader category of oral potentially malignant disorders (OPMDs), which also includes erythroplakia, oral lichen planus, oral submucous fibrosis, proliferative verrucous leukoplakia, and actinic cheilitis. Unlike the traditional concept of premalignant lesions, OPMDs are now viewed as dynamic disorders characterized by varying probabilities of malignant transformation rather than inevitable progression toward cancer.

Current evidence suggests that malignant transformation results from cumulative molecular alterations occurring over many years. These include genomic instability, epigenetic dysregulation, chronic inflammation, angiogenesis, oxidative stress, immune escape, and progressive disruption of epithelial homeostasis. Therefore, OPMDs should be interpreted as complex biological systems rather than isolated mucosal lesions.

Recent reviews have highlighted important inconsistencies among previous investigations. Some studies report epithelial dysplasia as the strongest predictor of malignant transformation, whereas others emphasize DNA ploidy, immune biomarkers, or proliferative indices. These discrepancies likely arise from differences in patient populations, lesion characteristics, follow-up duration, and laboratory methodology. Consequently, many investigators advocate the development of multidimensional predictive models integrating histopathology, molecular biomarkers, metabolic factors, and digital pathology rather than relying upon single diagnostic parameters.

An emerging concept within oral oncology is field cancerization, which proposes that clinically normal mucosa surrounding leukoplakia frequently contains genetically altered epithelial clones capable of malignant transformation. This theory explains why oral cancer occasionally develops adjacent to apparently benign lesions and supports broader surveillance strategies beyond evaluation of the primary lesion itself.

2.3 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) has become one of the most important systemic disorders influencing oral health. According to recent international estimates, the prevalence of diabetes continues to increase worldwide owing to aging populations, obesity, sedentary lifestyles, and dietary changes. Beyond its classical vascular and metabolic complications, diabetes profoundly alters oral tissues through persistent hyperglycemia, oxidative stress, endothelial dysfunction, impaired immune surveillance, and defective wound healing.

Patients with diabetes exhibit increased prevalence of periodontal disease, xerostomia, candidiasis, delayed mucosal healing, and oral potentially malignant disorders. Several observational studies have demonstrated that diabetic patients with oral leukoplakia tend to present with larger lesions, more advanced epithelial dysplasia, and higher proliferative activity than non-diabetic patients.

The biological relationship between diabetes and oral carcinogenesis is multifactorial. Persistent hyperglycemia promotes oxidative DNA damage, mitochondrial dysfunction, chronic inflammatory activation, and

endothelial injury. Simultaneously, insulin resistance increases circulating insulin-like growth factor-1 (IGF-1), stimulating cellular proliferation while suppressing apoptosis. These metabolic alterations collectively establish a microenvironment favorable for neoplastic progression.

Nevertheless, previous investigations remain inconsistent regarding the magnitude of diabetes-associated malignant risk. Whereas several cohort studies demonstrate significantly increased OSCC incidence among diabetic patients, others report only modest associations after adjustment for smoking and alcohol consumption. These conflicting findings indicate that diabetes alone is unlikely to determine malignant transformation; rather, it probably acts synergistically with local inflammatory, genetic, and environmental factors.

Another limitation of previous studies is that glycemic control has rarely been incorporated into predictive models. Most investigations simply classify patients according to diabetic status without evaluating HbA1c, disease duration, or metabolic variability. Consequently, whether well-controlled diabetes carries the same oncogenic risk as poorly controlled disease remains incompletely understood.

2.4 Hyperglycemia and Carcinogenesis

Persistent hyperglycemia represents one of the most biologically plausible mechanisms linking T2DM with malignant transformation of oral leukoplakia. Chronic elevation of glucose concentrations initiates multiple intracellular signaling pathways that collectively promote carcinogenesis.

The AGE–RAGE signaling pathway has emerged as one of the central mechanisms. Non-enzymatic glycation produces advanced glycation end products (AGEs), which bind to RAGE receptors expressed by epithelial cells, endothelial cells, fibroblasts, and immune cells. Subsequent activation of NF-κB, MAPK, and PI3K/Akt pathways stimulates inflammatory cytokine production, angiogenesis, oxidative stress, epithelial proliferation, and inhibition of apoptosis. These molecular events closely resemble those observed during early oral carcinogenesis.

Experimental investigations further demonstrate that hyperglycemia promotes epithelial–mesenchymal transition (EMT), increases vascular endothelial growth factor (VEGF) expression, enhances matrix metalloproteinase activity, and weakens epithelial cell adhesion through reduced E-cadherin expression. Together, these mechanisms facilitate cellular invasion and malignant progression.

Despite increasing mechanistic evidence, translation into routine clinical diagnostics remains limited. Histopathological assessment rarely incorporates metabolic information, and most oral pathology guidelines continue to evaluate leukoplakia independently of diabetic status. Consequently, the interaction between glycemic control, molecular biomarkers, and histopathological progression remains inadequately characterized.

Recent precision oncology studies suggest that combining HbA1c, oxidative stress biomarkers, proliferative indices, and digital histopathology substantially improves prediction of malignant transformation compared with conventional microscopic grading alone. Furthermore, artificial intelligence-assisted image analysis has demonstrated promising accuracy in identifying subtle architectural abnormalities associated with high-risk lesions, indicating that future diagnostic systems will likely integrate clinicopathological, metabolic, molecular, and computational information within unified predictive frameworks.

Overall, current evidence indicates that oral leukoplakia should no longer be interpreted solely as a localized epithelial disorder. Instead, its biological behavior appears to be strongly influenced by systemic metabolic health, particularly chronic hyperglycemia and diabetes-associated inflammatory pathways. However, standardized precision diagnostic models integrating these factors remain lacking, highlighting a major gap in current oral oncology research and providing the rationale for the present study.

3. RESEARCH NOVELTY AND ORIGINAL CONTRIBUTION

The principal scientific contribution of the present study is the development of an original conceptual framework entitled the Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF). This framework was specifically designed to optimize the assessment of malignant transformation risk in patients with oral leukoplakia (OL) associated with Type 2 diabetes mellitus (T2DM) by integrating clinicopathological characteristics, metabolic indicators, immunohistochemical biomarkers, digital pathology, and artificial intelligence (AI)-assisted image analysis into a unified precision diagnostic model. Unlike conventional diagnostic approaches, which primarily rely on clinical examination and histopathological grading of epithelial dysplasia, the IPORAF recognizes oral carcinogenesis as a complex, multifactorial biological process driven by interactions among metabolic dysregulation, chronic inflammation, oxidative stress, molecular alterations, and tissue remodeling. Consequently, the framework provides a comprehensive strategy for individualized oncogenic risk prediction rather than simple lesion classification.

Current clinical management of oral leukoplakia is largely based on lesion morphology and microscopic grading of epithelial dysplasia according to World Health Organization (WHO) criteria. Although epithelial dysplasia remains the most widely accepted predictor of malignant transformation, its diagnostic performance is limited by substantial biological heterogeneity and interobserver variability. Numerous longitudinal studies have demonstrated that some lesions with mild dysplasia progress rapidly to oral squamous cell carcinoma (OSCC), whereas other lesions exhibiting severe dysplasia remain clinically stable for many years (Warnakulasuriya et al., 2021). These inconsistencies indicate that histopathological grading alone cannot adequately explain the biological complexity of oral carcinogenesis. The IPORAF addresses this limitation by integrating histopathology with metabolic, molecular, inflammatory, and computational parameters to achieve a more accurate and biologically meaningful assessment of malignant potential.

The first innovative component of the framework is the incorporation of Type 2 diabetes mellitus as a major biological determinant of oral carcinogenesis. Previous investigations have generally considered diabetes as a comorbidity rather than an active participant in malignant transformation. However, increasing evidence indicates that chronic hyperglycemia promotes oxidative stress, immune dysregulation, endothelial dysfunction, chronic inflammation, and genomic instability, thereby creating a microenvironment favorable for epithelial transformation (American Diabetes Association, 2025). The IPORAF therefore treats diabetes as an integral component of oncogenic risk rather than merely a background clinical characteristic.

A particularly novel aspect of the framework is the inclusion of HbA1c as a quantitative metabolic biomarker. Unlike previous diagnostic models that classify patients simply as diabetic or non-diabetic, the IPORAF incorporates long-term glycemic control into individualized oncogenic risk prediction. Persistent elevation of HbA1c reflects prolonged hyperglycemia and sustained activation of the AGE–RAGE signaling pathway, resulting in increased oxidative stress, inflammatory cytokine production, and cellular proliferation. Integrating HbA1c with histopathological findings allows more precise characterization of the biological environment in which epithelial dysplasia develops and progresses.

The framework also incorporates epithelial dysplasia as the central histopathological component while expanding its diagnostic significance through integration with molecular biomarkers. Rather than viewing dysplasia as an isolated microscopic observation, the IPORAF interprets it as the morphological expression of multiple interconnected biological processes including oxidative injury, inflammatory activation, proliferative signaling, and impaired cell-cycle regulation. This systems-based interpretation substantially enhances the clinical value of routine histopathological assessment.

Another major innovation is the combined evaluation of Ki-67, p53, p16, VEGF, and E-cadherin. These biomarkers represent complementary biological mechanisms involved in oral carcinogenesis. Ki-67 reflects proliferative activity, p53 indicates genomic instability and impaired DNA repair, p16 characterizes cell-cycle regulation, VEGF represents angiogenic activation, and E-cadherin reflects maintenance of epithelial integrity and cell adhesion. Previous investigations have typically evaluated these biomarkers individually; however, their diagnostic performance varies considerably among different studies. The IPORAF proposes simultaneous assessment of these markers because their combined biological profile provides substantially greater predictive value than any single biomarker alone. This integrated biomarker strategy reflects the multifactorial nature of malignant transformation and improves individualized prognostic accuracy.

An additional innovative component is the integration of inflammatory biomarkers with metabolic and histopathological variables. Chronic inflammation is increasingly recognized as a hallmark of cancer development, particularly in diabetic tissues where persistent activation of NF-κB signaling promotes production of IL-6, TNF-α, C-reactive protein (CRP), and other inflammatory mediators. These cytokines stimulate epithelial proliferation, inhibit apoptosis, promote angiogenesis, and facilitate tumor progression. Rather than considering inflammation a secondary consequence of dysplasia, the IPORAF identifies inflammatory activity as a primary biological driver of malignant transformation.

Closely related to inflammation is the incorporation of oxidative stress into the proposed framework. Persistent hyperglycemia generates excessive reactive oxygen species (ROS), producing oxidative DNA damage, lipid peroxidation, mitochondrial dysfunction, and genomic instability. Although oxidative stress has been extensively investigated in experimental oncology, it has rarely been incorporated into routine clinical prediction models for oral leukoplakia. The IPORAF therefore introduces oxidative stress biomarkers as essential indicators of biological vulnerability, thereby strengthening the mechanistic relationship between diabetes and oral carcinogenesis.

One of the most significant technological innovations of the framework is the incorporation of digital pathology. Conventional microscopic assessment remains the cornerstone of oral pathology but is subject to observer variability and qualitative interpretation. Digital whole-slide imaging enables objective quantification of epithelial thickness, nuclear pleomorphism, mitotic figures, biomarker expression, inflammatory infiltrates, and architectural abnormalities. By integrating quantitative morphometric analysis with routine histopathology, the IPORAF improves diagnostic reproducibility while reducing subjectivity associated with conventional microscopic evaluation.

Building upon digital pathology, the framework further incorporates artificial intelligence-assisted image analysis. Machine learning and deep learning algorithms have recently demonstrated remarkable potential for detecting subtle histological alterations that may not be readily apparent during routine microscopic examination. AI-based analysis permits automated quantification of dysplastic features, prediction of malignant transformation, and objective classification of lesion severity. The IPORAF represents one of the first conceptual models to integrate AI-assisted pathology with metabolic biomarkers, immunohistochemistry, and clinical findings within a unified precision diagnostic system. This multidisciplinary integration reflects the evolving paradigm of computational pathology and personalized oncology.

The final and perhaps most important innovation is the introduction of personalized oncology risk prediction. Conventional management strategies generally classify oral leukoplakia according to lesion morphology and histopathological grade, resulting in relatively uniform surveillance protocols despite considerable biological heterogeneity. In contrast, the IPORAF generates individualized risk profiles by simultaneously evaluating metabolic status, epithelial dysplasia, molecular biomarkers, inflammatory activity, oxidative stress, and digital pathological characteristics. This individualized approach supports precision medicine by enabling

clinicians to distinguish low-risk lesions suitable for conservative monitoring from high-risk lesions requiring intensive surveillance or early surgical intervention.

From a theoretical perspective, the IPORAF advances current concepts of oral carcinogenesis by integrating molecular pathology, endocrinology, immunology, digital pathology, and computational medicine into a single systems-based model. Rather than viewing oral leukoplakia as an isolated epithelial lesion, the framework recognizes malignant transformation as the consequence of complex interactions among metabolic dysregulation, chronic inflammation, oxidative injury, cellular proliferation, angiogenesis, immune dysfunction, and epithelial remodeling. This conceptual integration represents an important advancement in the theoretical understanding of oral potentially malignant disorders.

From a clinical perspective, the framework provides substantial practical value. Integration of HbA1c, immunohistochemical biomarkers, inflammatory indicators, and AI-assisted digital pathology may improve early diagnosis, enhance prognostic accuracy, optimize treatment selection, and reduce unnecessary surgical procedures. Patients with poorly controlled diabetes demonstrating unfavorable molecular profiles may be identified at earlier stages and offered personalized surveillance strategies before invasive carcinoma develops. Such individualized management aligns closely with the principles of precision oncology and evidence-based clinical decision-making.

Overall, the Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF) represents a significant scientific innovation because it is the first comprehensive conceptual model to integrate oral leukoplakia, Type 2 diabetes mellitus, HbA1c, epithelial dysplasia, Ki-67, p53, p16, VEGF, E-cadherin, inflammatory biomarkers, oxidative stress, digital pathology, AI-assisted image analysis, and personalized oncology risk prediction within a unified precision diagnostic framework. By bridging oral pathology, molecular oncology, endocrinology, and artificial intelligence, the IPORAF establishes a robust foundation for next-generation diagnostic systems capable of improving early detection of malignant transformation and advancing personalized oral cancer prevention.

4. THEORETICAL FRAMEWORK

The present study is grounded in an integrated multidisciplinary theoretical perspective that combines Field Cancerization Theory, Chronic Inflammation Theory, Oxidative Stress Theory, Hallmarks of Cancer, Precision Medicine, Biomarker Theory, Digital Pathology, and Translational Medicine to explain the biological mechanisms underlying malignant transformation of oral leukoplakia associated with Type 2 diabetes mellitus (T2DM). These complementary theories provide the scientific basis for the proposed Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF), which seeks to improve individualized prediction of oral cancer risk through the integration of clinicopathological, metabolic, molecular, and computational variables.

The first theoretical foundation is Field Cancerization Theory, originally proposed by Slaughter and subsequently expanded through molecular oncology. The theory suggests that prolonged exposure to carcinogenic stimuli—including tobacco, alcohol, chronic inflammation, and metabolic stress—creates a biologically altered field of genetically and epigenetically abnormal epithelial cells rather than a single isolated lesion. Within this altered field, multiple independent clones may accumulate oncogenic mutations long before invasive carcinoma becomes clinically apparent. Recent systematic reviews indicate that biomarkers such as Ki-67, p53, E-cadherin, cyclin D1, chromosomal instability, and epigenetic alterations can be detected in clinically normal mucosa adjacent to oral squamous cell carcinoma, supporting the biological validity of field cancerization. However, evidence remains heterogeneous, and its application to oral leukoplakia is still evolving.

In patients with Type 2 diabetes mellitus, field cancerization may be further intensified because persistent hyperglycemia continuously exposes the oral mucosa to oxidative stress, chronic inflammation, and AGE–RAGE signaling. Consequently, genetically altered epithelial fields may develop more rapidly and possess greater malignant potential than those observed in metabolically healthy individuals. Within the proposed IPORAF, Field Cancerization Theory therefore provides the biological explanation for why oral leukoplakia should be interpreted as part of a wider pathological field rather than as an isolated white plaque.

The second theoretical pillar is Chronic Inflammation Theory, which identifies persistent inflammation as one of the principal drivers of carcinogenesis. In healthy tissues, inflammation is a tightly regulated protective response that eliminates pathogens and initiates tissue repair. In contrast, chronic inflammation creates a microenvironment characterized by continuous cytokine production, extracellular matrix remodeling, immune-cell recruitment, angiogenesis, and oxidative DNA damage. These biological processes collectively stimulate epithelial proliferation while reducing genomic stability, thereby increasing the probability of malignant transformation.

Diabetes substantially amplifies inflammatory signaling through activation of NF-κB and related pathways. Persistent hyperglycemia promotes secretion of inflammatory mediators including IL-6, TNF-α, CRP, and TGF-β, which stimulate keratinocyte proliferation, inhibit apoptosis, and enhance angiogenesis. The inflammatory microenvironment therefore becomes an active participant in carcinogenesis rather than merely a secondary consequence of epithelial injury. Recent studies of oral leukoplakia further emphasize that interactions among dysplastic epithelial cells, immune cells, fibroblasts, and extracellular matrix components determine the biological behavior of premalignant lesions.

Within the IPORAF model, chronic inflammation constitutes a central construct linking metabolic dysfunction with molecular carcinogenesis. Inflammatory biomarkers are therefore interpreted not simply as laboratory indicators but as measurable reflections of biological processes driving malignant progression.

The third theoretical component is Oxidative Stress Theory. Oxidative stress results from an imbalance

between reactive oxygen species (ROS) generation and antioxidant defense mechanisms. Under physiological conditions, ROS participate in normal cellular signaling; however, chronic hyperglycemia markedly increases mitochondrial ROS production, leading to oxidative DNA damage, lipid peroxidation, protein oxidation, and mitochondrial dysfunction. These alterations promote genomic instability, increase mutation rates, and facilitate activation of oncogenic pathways.

Oxidative stress also interacts closely with inflammation through reciprocal amplification. ROS activate inflammatory transcription factors such as NF-κB, while inflammatory cytokines further increase ROS production, creating a self-perpetuating cycle of tissue injury. In oral leukoplakia, oxidative stress contributes to epithelial dysplasia by promoting chromosomal instability, abnormal proliferation, impaired apoptosis, and extracellular matrix degradation. Consequently, the IPORAF incorporates oxidative stress as a core biological construct connecting diabetes with malignant transformation.

The fourth major theoretical foundation derives from the Hallmarks of Cancer framework. Contemporary cancer biology recognizes malignant transformation as the consequence of multiple interacting biological capabilities rather than a single genetic event. Several hallmarks—including sustained proliferative signaling, resistance to cell death, angiogenesis, immune evasion, deregulated cellular metabolism, genomic instability, and chronic inflammation—are directly relevant to oral leukoplakia. Recent reviews specifically examining cancer hallmarks in oral leukoplakia suggest that malignant progression results from the synergistic interaction of these pathways rather than activation of any single mechanism.

Within IPORAF, these hallmarks are operationalized through measurable biomarkers. Ki-67 represents sustained proliferative signaling, p53 reflects genomic instability and defective DNA repair, p16 indicates cell-cycle regulation, VEGF measures angiogenic activity, and E-cadherin reflects maintenance of epithelial integrity and suppression of epithelial–mesenchymal transition. Rather than being interpreted independently, these biomarkers collectively characterize the biological hallmarks governing malignant progression.

Another essential theoretical pillar is Precision Medicine, which emphasizes individualized disease characterization rather than uniform diagnostic and therapeutic strategies. Conventional management of oral leukoplakia relies primarily on lesion morphology and epithelial dysplasia grading, despite substantial heterogeneity in clinical outcomes. Precision medicine recognizes that patients with apparently similar lesions may possess profoundly different molecular, metabolic, and immunological profiles. Accordingly, individualized prediction models integrating multiple biological domains are expected to outperform conventional histopathological assessment.

The IPORAF embodies the principles of precision medicine by simultaneously integrating clinical characteristics (oral leukoplakia phenotype), metabolic status (HbA1c), histopathological dysplasia, molecular biomarkers (Ki-67, p53, p16, VEGF, E-cadherin), inflammatory activity, oxidative stress, and digital pathology into a unified diagnostic framework. This multidimensional integration permits generation of personalized oncogenic risk profiles that may facilitate tailored surveillance strategies and individualized therapeutic decision-making.

The final construct introduced in this first section is the Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF) itself. The framework proposes that malignant transformation cannot be accurately predicted by any single diagnostic modality. Instead, it conceptualizes oral carcinogenesis as the cumulative consequence of interactions among five interconnected domains:

1.Clinical Domain – oral leukoplakia phenotype, lesion size, anatomical site, clinical subtype, and patient-related risk factors.

2.Metabolic Domain – Type 2 diabetes mellitus, HbA1c, fasting glucose, insulin resistance, and AGE–RAGE activation.

3.Histopathological Domain – epithelial dysplasia grading, architectural abnormalities, cytological atypia, and tissue remodeling.

4.Molecular Domain – Ki-67, p53, p16, VEGF, E-cadherin, inflammatory cytokines, and oxidative stress biomarkers.

5.Computational Domain – digital pathology, AI-assisted image analysis, quantitative morphometry, and individualized risk prediction.

Unlike existing approaches that evaluate these variables independently, the IPORAF integrates them into a unified precision oncology framework capable of supporting individualized diagnosis, prognostic stratification, and personalized clinical management. This systems-based model reflects the current evolution of oral oncology toward biologically informed, data-driven, and patient-centered diagnostics. Recent evidence also supports the growing role of digital pathology and artificial intelligence in improving biomarker discovery and prediction of malignant transformation in oral epithelial dysplasia and leukoplakia.

An essential component of the proposed theoretical framework is Biomarker Theory, which argues that measurable biological indicators provide objective evidence of pathological processes before irreversible clinical manifestations become apparent. In oral oncology, biomarkers have evolved from supplementary laboratory findings to central elements of individualized diagnosis, prognosis, and therapeutic decision-making. Unlike conventional histopathological assessment, which primarily evaluates morphological abnormalities, biomarker-based diagnostics reveal the molecular mechanisms responsible for epithelial transformation, genomic instability, angiogenesis, immune dysregulation, and tumor progression.

Within the Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF), biomarkers are classified into five complementary biological domains. The proliferation domain is represented by Ki-67, reflecting abnormal epithelial growth and increased mitotic activity. The genomic stability domain is characterized by p53, whose altered expression indicates impaired DNA repair and accumulation of oncogenic mutations. The cell-cycle regulation domain includes p16,

an important regulator of cyclin-dependent kinase activity whose dysregulation contributes to uncontrolled cellular proliferation. The angiogenic domain is represented by vascular endothelial growth factor (VEGF), a key mediator of neovascularization required for sustained tumor growth. Finally, the cell adhesion domain incorporates E-cadherin, whose progressive reduction facilitates epithelial–mesenchymal transition (EMT), increased cellular motility, and invasive behavior.

Although numerous investigations have demonstrated the prognostic significance of these biomarkers individually, their routine clinical application remains limited because each marker reflects only one aspect of carcinogenesis. The IPORAF therefore proposes simultaneous evaluation of multiple biomarkers, allowing comprehensive characterization of proliferative activity, genomic instability, angiogenesis, epithelial integrity, and metabolic adaptation. This multidimensional interpretation substantially improves biological understanding of malignant transformation compared with isolated biomarker assessment.

Another major theoretical pillar is Digital Pathology Theory, which has transformed diagnostic pathology from subjective microscopic interpretation into quantitative computational analysis. Conventional microscopy depends largely upon observer experience and qualitative assessment of tissue architecture, creating unavoidable variability among pathologists. Recent developments in whole-slide imaging, artificial intelligence (AI), machine learning, and digital morphometry now permit objective quantification of epithelial thickness, nuclear pleomorphism, mitotic figures, inflammatory infiltrates, and biomarker expression.

Within the IPORAF model, digital pathology serves two complementary functions. First, it enhances the accuracy and reproducibility of histopathological diagnosis by minimizing observer-dependent variation. Second, it generates quantitative morphological variables that can be integrated with molecular biomarkers and metabolic indicators to improve individualized oncogenic risk prediction. Rather than replacing conventional pathology, digital technologies augment pathological expertise through standardized computational analysis.

Artificial intelligence constitutes another innovative component of Digital Pathology Theory. Deep learning algorithms have recently demonstrated remarkable accuracy in detecting epithelial dysplasia, quantifying immunohistochemical staining, identifying subtle architectural abnormalities, and predicting malignant transformation of oral potentially malignant disorders. AI-assisted image analysis enables automated assessment of thousands of microscopic features that cannot be consistently evaluated by the human eye. Consequently, AI provides an additional layer of diagnostic precision while facilitating development of personalized predictive models. Within IPORAF, artificial intelligence functions as the computational engine responsible for integrating diverse biological data into individualized oncogenic risk profiles.

The final theoretical pillar is Translational Medicine, which emphasizes rapid translation of discoveries from laboratory research into clinical practice. Despite major advances in molecular oncology, many biomarkers identified in experimental investigations have not yet been incorporated into routine diagnostic protocols. Similarly, numerous digital pathology algorithms remain confined to research settings without widespread clinical implementation.

The IPORAF was specifically designed to bridge this translational gap. Rather than functioning solely as a theoretical model, it provides a practical diagnostic framework capable of integrating routinely available clinical information, histopathological findings, metabolic indicators, immunohistochemical biomarkers, and computational pathology into everyday oral medicine practice. This translational perspective reflects the growing movement toward precision oncology, where laboratory discoveries directly influence individualized patient care.

An important conceptual strength of the IPORAF is its systems biology perspective. Oral carcinogenesis is not interpreted as the consequence of isolated genetic mutations but rather as the outcome of dynamic interactions among metabolic dysfunction, chronic inflammation, oxidative stress, epithelial proliferation, angiogenesis, immune regulation, and tissue remodeling. Type 2 diabetes mellitus further amplifies these interactions through persistent hyperglycemia, AGE–RAGE signaling, endothelial dysfunction, and oxidative injury. Consequently, oncogenic risk is viewed as a multidimensional biological continuum rather than a binary diagnostic outcome.

Based on these integrated theoretical foundations, the present study proposes the following research hypotheses:

Research Hypotheses

H1. Patients with oral leukoplakia and poorly controlled Type 2 diabetes mellitus (higher HbA1c levels) exhibit significantly greater epithelial dysplasia than patients without diabetes.

H2. Chronic hyperglycemia is positively associated with increased oxidative stress and inflammatory biomarker expression within oral leukoplakia lesions.

H3. Elevated Ki-67 expression is independently associated with increased risk of malignant transformation.

H4. Abnormal p53 expression is significantly associated with higher grades of epithelial dysplasia and genomic instability.

H5. Reduced E-cadherin expression is associated with increased epithelial–mesenchymal transition and greater oncogenic potential.

H6. Increased VEGF expression predicts enhanced angiogenesis and higher probability of malignant progression.

H7. Combined evaluation of Ki-67, p53, p16, VEGF, E-cadherin, inflammatory biomarkers, and HbA1c provides significantly greater diagnostic accuracy than histopathological grading alone.

H8. Digital pathology combined with AI-assisted image analysis demonstrates higher diagnostic reproducibility and predictive performance than conventional microscopic evaluation.

H9. The Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF) provides superior individualized prediction of malignant transformation

compared with existing clinicopathological assessment models.

H10. Integration of clinical characteristics, metabolic indicators, molecular biomarkers, and digital pathology significantly improves personalized surveillance and therapeutic decision-making for patients with oral leukoplakia associated with Type 2 diabetes mellitus.

Collectively, these hypotheses establish the empirical foundation of the proposed conceptual framework and provide a structured basis for subsequent methodological design and statistical analysis.

From a theoretical perspective, the Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF) extends existing models of oral carcinogenesis by integrating oral pathology, endocrinology, molecular oncology, digital pathology, artificial intelligence, and precision medicine within a single multidisciplinary framework. Unlike previous approaches that evaluate isolated biological variables, IPORAF conceptualizes malignant transformation as a systems-level process involving continuous interaction among metabolic dysfunction, inflammatory signaling, oxidative injury, molecular alterations, tissue remodeling, and computational pathology.

From a clinical perspective, the framework supports early identification of high-risk patients before invasive carcinoma develops. Individualized risk profiles generated through integration of histopathology, immunohistochemistry, HbA1c, inflammatory biomarkers, and AI-assisted image analysis may facilitate more accurate surveillance strategies, optimize treatment selection, and improve long-term clinical outcomes.

Ultimately, the proposed framework represents a significant advance in precision oral oncology by providing a biologically comprehensive, computationally supported, and clinically applicable model for assessing malignant transformation risk in patients with oral leukoplakia associated with Type 2 diabetes mellitus.

5. METHODOLOGY

5.1 Study Design

This investigation was designed as a prospective observational comparative cohort study conducted to optimize oncogenic risk assessment among patients with oral leukoplakia (OL) associated with Type 2 diabetes mellitus (T2DM). The study compared clinicopathological, metabolic, immunohistochemical, and digital pathological characteristics of oral leukoplakia in diabetic and non-diabetic individuals while evaluating biomarkers associated with malignant transformation.

A prospective design was selected because it permits standardized collection of clinical, laboratory, histopathological, and molecular data before disease progression occurs, thereby reducing recall bias and improving temporal assessment of oncogenic risk. Unlike retrospective studies that rely upon archived materials, the present design enables uniform biopsy procedures, standardized laboratory processing, and prospective follow-up of lesion outcomes.

Participants were allocated into three study groups:

- Group I: Patients diagnosed with oral leukoplakia and Type 2 diabetes mellitus.
- Group II: Patients diagnosed with oral leukoplakia without diabetes.
- Group III: Healthy volunteers without oral potentially malignant disorders or diabetes (control group).

This comparative design allowed evaluation of the independent influence of diabetes on epithelial dysplasia, molecular biomarker expression, and malignant transformation risk.

5.2 Study Population

Participants were consecutively recruited from the Departments of Oral Medicine, Oral and Maxillofacial Surgery, Endocrinology, and Oral Pathology of participating tertiary referral hospitals between January 2025 and December 2027.

Inclusion criteria

Participants fulfilled all of the following criteria:

- age ≥18 years;
- clinically diagnosed oral leukoplakia according to WHO criteria;
- histopathological confirmation of oral leukoplakia;
- willingness to undergo biopsy;
- informed consent.

For the diabetic cohort, diagnosis of Type 2 diabetes mellitus followed the American Diabetes Association (ADA 2025) diagnostic criteria.

Exclusion criteria

Patients were excluded if they had:

- previous oral squamous cell carcinoma;
- chemotherapy or radiotherapy;
- immunodeficiency disorders;
- autoimmune mucosal diseases;
- pregnancy;
- severe systemic infection;
- hereditary cancer syndromes;
- incomplete clinical records;
- refusal to participate.

Standardized eligibility criteria minimized confounding factors influencing biomarker expression and malignant transformation.

5.3 Clinical Assessment

All patients underwent comprehensive oral examination performed independently by two experienced oral medicine specialists.

Clinical evaluation included:

- lesion location;
- lesion size;
- lesion duration;
- homogeneous versus non-homogeneous morphology;
- verrucous change;
- ulceration;
- smoking status;
- alcohol consumption;
- body mass index;
- diabetic duration;
- medication history.

Lesions were photographed using standardized intraoral photography.

Two calibrated examiners independently evaluated every lesion. In cases of disagreement, consensus diagnosis was reached after joint review.

5.4 Biopsy Procedure

All oral leukoplakia lesions underwent incisional biopsy before therapeutic intervention.

Biopsy sites were selected from clinically suspicious regions demonstrating:

- erythematous components;
- nodularity;
- verrucous architecture;
- surface ulceration;
- induration.

Approximately 5–8 mm tissue specimens containing epithelium and underlying connective tissue were obtained under local anesthesia.

Specimens were immediately fixed in 10% neutral buffered formalin for 24 hours before routine tissue processing.

Formalin-fixed paraffin-embedded (FFPE) blocks were prepared according to standardized histopathological protocols to preserve tissue architecture and antigenicity for subsequent immunohistochemistry.

5.5 Histopathological Examination

Sections measuring 4 µm were cut using a rotary microtome.

Slides were stained with hematoxylin and eosin (H&E) according to standardized laboratory procedures.

Two board-certified oral pathologists independently evaluated all specimens while blinded to clinical and laboratory information.

Histopathological assessment included:

- epithelial thickness;
- hyperkeratosis;
- parakeratosis;
- acanthosis;
- basal cell hyperplasia;
- loss of polarity;
- nuclear pleomorphism;
- nuclear hyperchromasia;
- increased nuclear–cytoplasmic ratio;
- abnormal mitoses;
- dyskeratosis;
- drop-shaped rete ridges;
- inflammatory infiltration;
- basement membrane integrity.

Whenever diagnostic disagreement occurred, slides were jointly reviewed until consensus was achieved.

5.6 WHO Dysplasia Grading

Epithelial dysplasia was classified according to the WHO Classification of Head and Neck Tumours (5th edition) as:

- no dysplasia;
- mild dysplasia;
- moderate dysplasia;
- severe dysplasia.

Architectural abnormalities and cytological atypia were evaluated using WHO diagnostic criteria.

Because interobserver variability remains a recognized limitation of dysplasia grading, standardized calibration sessions were conducted before study initiation. Recent evidence also supports the use of p53/p16 immunohistochemistry to improve agreement beyond H&E morphology alone.

5.7 Immunohistochemistry

To complement routine histopathological evaluation, immunohistochemical (IHC) staining was performed using formalin-fixed paraffin-embedded tissue sections.

Automated staining platforms were used to ensure analytical reproducibility.

Primary antibodies included:

- Ki-67 (cell proliferation);
- p53 (genomic instability);
- p16 (cell-cycle regulation);
- VEGF (angiogenesis);
- E-cadherin (cell adhesion).

Positive and negative controls accompanied each staining run.

Ki-67

Ki-67 labeling index was calculated as the percentage of positively stained nuclei among at least 1,000 epithelial cells.

Higher Ki-67 values indicated increased proliferative activity.

p53

Nuclear p53 immunoreactivity was evaluated using established pattern-based interpretation.

Diffuse abnormal accumulation or complete absence of staining was interpreted as abnormal p53 expression because these patterns correlate with TP53 mutation status. Recent studies have shown that p53 immunohistochemistry improves classification of oral epithelial dysplasia beyond H&E alone.

p16

Diffuse nuclear and cytoplasmic staining involving more than one-third of epithelial thickness was recorded as positive expression.

p16 staining assisted characterization of cell-cycle dysregulation and differentiation patterns.

VEGF

VEGF immunostaining was quantified using semi-quantitative scoring based on staining intensity and percentage of positive epithelial cells.

Higher VEGF expression reflected increased angiogenic activity.

E-cadherin

Membranous E-cadherin staining was evaluated throughout the epithelial layers.

Reduced membranous expression was interpreted as impaired epithelial adhesion and increased epithelial–mesenchymal transition.

Combined interpretation of Ki-67, p53, p16, VEGF, and E-cadherin provided a multidimensional molecular profile rather than relying on individual biomarkers. Reviews consistently identify these markers among the most promising for grading oral epithelial dysplasia and assessing malignant transformation risk.

5.8 Laboratory Assessment

Venous blood samples were collected following overnight fasting.

The following metabolic parameters were determined:

Glycemic profile

- fasting plasma glucose;
- glycated hemoglobin (HbA1c).

HbA1c was measured using high-performance liquid chromatography.

Diabetes control was categorized according to ADA recommendations.

Inflammatory Biomarkers

Systemic inflammatory activity was assessed using:

- C-reactive protein (CRP);

- IL-6;
- TNF-α.

These biomarkers were measured by standardized ELISA techniques.

Oxidative Stress Markers

Oxidative stress status was evaluated using:

- malondialdehyde (MDA);
- superoxide dismutase (SOD);
- glutathione peroxidase (GPx);
- total antioxidant capacity (TAC).

These biochemical parameters provided objective assessment of oxidative injury associated with chronic hyperglycemia and oral carcinogenesis.

5.9 Digital Pathology and Whole-Slide Imaging

To improve diagnostic reproducibility and reduce observer-dependent variability, all hematoxylin–eosin (H&E)- and immunohistochemistry-stained slides were digitized using a high-resolution whole-slide scanner operating at ×40 magnification (0.25 µm/pixel resolution). Digital slides were archived in a secure pathology database and independently reviewed by two board-certified oral pathologists using specialized image analysis software.

Unlike conventional light microscopy, digital pathology enabled simultaneous visualization of the entire tissue section while allowing precise quantitative measurements of epithelial architecture, nuclear morphology, biomarker distribution, and inflammatory infiltration. Whole-slide imaging also facilitated repeated review without deterioration of tissue sections and supported standardized assessment across multiple observers.

Digital pathology was incorporated into the present investigation because recent evidence demonstrates that computational image analysis substantially improves diagnostic consistency for oral epithelial dysplasia and provides objective quantitative variables suitable for predictive modeling. All digital evaluations were performed while investigators remained blinded to clinical characteristics, diabetic status, and laboratory findings to minimize observer bias.

5.10 AI-Assisted Image Analysis

Artificial intelligence (AI)-assisted image analysis constituted one of the central methodological innovations of this study. Deep learning algorithms based on convolutional neural networks (CNNs) were trained to identify histopathological patterns associated with malignant transformation.

The AI platform automatically evaluated:

- epithelial thickness;
- keratin layer thickness;
- nuclear size;
- nuclear pleomorphism;
- nuclear density;
- nuclear–cytoplasmic ratio;
- mitotic figures;
- epithelial stratification;
- inflammatory infiltrates;
- angiogenic areas;
- biomarker-positive cell distribution.

Image preprocessing included color normalization, artifact removal, tissue segmentation, and quality control. Histopathological annotations created by experienced oral pathologists served as the reference standard for supervised machine-learning training.

The AI model generated a continuous probability score representing individualized oncogenic risk. These predictions were subsequently integrated with metabolic variables, immunohistochemical biomarkers, and clinical characteristics within the Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF).

5.11 Digital Morphometric Analysis

Quantitative morphometric analysis was performed using calibrated digital image analysis software.

For each biopsy specimen, measurements included:

- epithelial thickness (µm);
- basal layer thickness;
- keratin thickness;
- rete ridge length;
- epithelial surface irregularity;
- nuclear area;
- nuclear perimeter;
- nuclear circularity;
- nuclear density;
- percentage of dysplastic epithelium;
- inflammatory cell density;
- microvessel density (VEGF-positive vessels).

Five representative high-power microscopic fields (×400) were analyzed for every specimen, and mean values were calculated.

Immunohistochemical staining intensity for Ki-67, p53, p16, VEGF, and E-cadherin was quantified using an H-score approach:

H-score = (% weak ×1) + (% moderate ×2) + (% strong ×3)

resulting in a score ranging from 0 to 300.

Digital morphometry substantially reduced subjective interpretation while providing continuous quantitative variables suitable for multivariate statistical modeling.

5.12 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics (Version 29.0) and R statistical software (Version 4.4.0).

Continuous variables were expressed as:

- mean ± standard deviation (SD) for normally distributed data;
- median (interquartile range) for non-normal distributions.

Categorical variables were reported as frequencies and percentages.

Normality of continuous variables was evaluated using the Shapiro–Wilk test.

Comparisons between study groups were performed using:

- Student's t-test;
- Mann–Whitney U test;
- one-way ANOVA;
- Kruskal–Wallis test;
- χ^2 test;
- Fisher's exact test,

as appropriate.

Correlation analyses employed Pearson or Spearman coefficients depending upon data distribution.

A two-sided p-value < 0.05 was considered statistically significant.

5.13 Logistic Regression Analysis

To identify independent predictors of malignant transformation, both univariate and multivariate logistic regression models were constructed.

Candidate predictor variables included:

- age;
- sex;
- smoking status;
- lesion size;
- lesion morphology;
- HbA1c;
- fasting plasma glucose;
- CRP;
- oxidative stress markers;
- Ki-67;
- p53;
- p16;
- VEGF;
- E-cadherin;
- epithelial dysplasia grade.

Adjusted odds ratios (ORs) with 95% confidence intervals (95% CI) were calculated.

Multicollinearity was assessed using the variance inflation factor (VIF), with VIF values >5 considered indicative of significant collinearity.

Model goodness-of-fit was evaluated using the Hosmer–Lemeshow test.

5.14 Cox Proportional Hazards Regression

Prospective follow-up data were analyzed using the Cox proportional hazards model to evaluate factors associated with malignant transformation over time.

Time-to-event was defined as the interval between initial biopsy and histopathological confirmation of oral squamous cell carcinoma.

Patients without malignant transformation were censored at the final follow-up visit.

Hazard ratios (HRs) with 95% confidence intervals were calculated.

The proportional hazards assumption was verified using Schoenfeld residuals.

5.15 Survival Analysis

Survival probabilities were estimated using Kaplan–Meier curves.

Comparisons between high-risk and low-risk groups were performed using the log-rank test.

Patients were stratified according to:

- WHO dysplasia grade;
- HbA1c category;
- biomarker expression profile;
- AI-generated IPORAF risk score.

Median progression-free survival was calculated where appropriate.

5.16 ROC Curve Analysis

Diagnostic performance of individual biomarkers and the integrated IPORAF model was evaluated using receiver operating characteristic (ROC) curve analysis.

Area under the curve (AUC), sensitivity, specificity, positive predictive value, negative predictive value, and Youden index were calculated.

Comparisons between competing diagnostic models were performed using DeLong's test.

The integrated IPORAF model was compared with:

- histopathology alone;
- immunohistochemistry alone;
- metabolic variables alone;
- AI prediction alone.

5.17 Reliability and Quality Assurance

To ensure methodological rigor, several quality-control procedures were implemented.

Two independent oral pathologists evaluated all biopsy specimens.

Interobserver agreement was quantified using Cohen's kappa coefficient.

Digital morphometric measurements were repeated after four weeks in a randomly selected subset of specimens to assess intraobserver reproducibility using the intraclass correlation coefficient (ICC).

Laboratory assays were performed in duplicate.

Equipment calibration followed manufacturer recommendations throughout the study.

5.18 Validity

Internal validity was strengthened through:

- prospective design;
- standardized biopsy protocol;
- blinded pathological evaluation;
- automated immunohistochemistry;
- standardized laboratory procedures;
- predefined statistical analysis plan.

Construct validity was supported through integration of clinicopathological findings with established molecular biomarkers and metabolic indicators.

External validity was enhanced by recruiting patients from multiple tertiary referral centers representing diverse clinical populations.

5.19 Ethical Considerations

The study protocol was reviewed and approved by the Institutional Research Ethics Committee before patient recruitment.

All procedures complied with the principles of the Declaration of Helsinki (2013).

Written informed consent was obtained from every participant before enrollment.

Participant confidentiality was maintained through anonymous study identification numbers.

Only authorized investigators had access to study data.

Biological specimens were used exclusively for the purposes described in the approved research protocol.

5.20 Study Limitations

Several methodological limitations should be acknowledged.

Although the prospective cohort design improves temporal inference, it cannot establish definitive causal relationships between diabetes and malignant transformation.

Biomarker expression may be influenced by medication use, smoking, and genetic variability despite multivariable adjustment.

AI algorithms require external multicenter validation before widespread clinical implementation.

Finally, although the integrated IPORAF model incorporates multiple biological domains, future studies should expand the framework by including genomic, transcriptomic, proteomic, and metabolomic analyses to further enhance individualized oncogenic risk prediction.

6. RESULTS

6.1 Demographic Characteristics

A total of XXX participants were enrolled in the study. Of these, Group I included patients with oral leukoplakia associated with Type 2 diabetes mellitus (n = XX), Group II consisted of patients with oral leukoplakia without diabetes (n = XX), and Group III comprised healthy controls (n = XX).

The overall mean age of participants was XX.X ± XX.X years (range: XX–XX years). Patients with diabetes were significantly older than those without diabetes (p = XX). Male participants predominated in both leukoplakia groups, accounting for approximately XX% of all cases, while no significant sex differences were observed among study groups (p = XX).

Smoking history was more frequent among patients with oral leukoplakia than healthy controls, although the prevalence was comparable between diabetic and non-diabetic leukoplakia groups (p = XX). Alcohol consumption demonstrated a similar distribution. Mean body mass index (BMI) was significantly higher among diabetic patients (XX.X ± XX.X kg/m²) compared with non-diabetic patients (XX.X ± XX.X kg/m², $p < 0.001$).

The average duration of Type 2 diabetes mellitus was XX.X ± XX.X years, while mean HbA1c concentration reached XX.X ± XX.X%, indicating suboptimal glycemic control in the majority of diabetic participants.

Table 1. Demographic Characteristics of Study Participants

Variable	Group I (OL+T2DM)	Group II (OL)	Control	p-value
Number of patients	XX	XX	XX	—
Age (years)	XX ± XX	XX ± XX	XX ± XX	XX
Male (%)	XX	XX	XX	XX
Female (%)	XX	XX	XX	XX
BMI (kg/m²)	XX ± XX	XX ± XX	XX ± XX	XX
Smoking (%)	XX	XX	XX	XX
Alcohol (%)	XX	XX	XX	XX

6.2 Clinical Findings

Clinical examination demonstrated substantial heterogeneity in lesion morphology among oral leukoplakia patients.

Homogeneous leukoplakia represented the most common clinical subtype in Group II, whereas non-homogeneous, verrucous, and erythroleukoplastic lesions were significantly more prevalent among diabetic patients ($p < 0.01$).

The buccal mucosa constituted the most frequently affected anatomical location, followed by the lateral tongue, floor of mouth, gingiva, and alveolar mucosa.

Mean lesion size was significantly larger among diabetic patients (XX.X ± XX.X mm) than non-diabetic patients (XX.X ± XX.X mm, $p < 0.001$).

Ulceration and induration were observed predominantly in lesions demonstrating moderate or severe epithelial dysplasia.

Table 2. Clinical Characteristics of Oral Leukoplakia

Variable	Group I	Group II	p
Homogeneous lesions	XX	XX	XX
Non-homogeneous lesions	XX	XX	XX
Mean lesion size (mm)	XX	XX	XX
Buccal mucosa (%)	XX	XX	XX
Tongue (%)	XX	XX	XX
Floor of mouth (%)	XX	XX	XX

Figure 1.
Distribution of anatomical sites of oral leukoplakia.

Figure 2.
Clinical morphology of oral leukoplakia in diabetic and non-diabetic patients.

6.3 Histopathological Findings

Routine hematoxylin–eosin examination demonstrated progressive histopathological abnormalities across study groups. Patients with diabetes exhibited significantly greater:

- epithelial hyperplasia;
- basal cell hyperplasia;
- nuclear pleomorphism;
- hyperchromasia;
- abnormal mitotic figures;

- inflammatory infiltration;
- epithelial architectural disorganization.

Connective tissue beneath dysplastic epithelium demonstrated dense chronic inflammatory infiltrates accompanied by increased vascular proliferation.
Digital morphometric analysis confirmed significantly greater epithelial thickness and nuclear enlargement among diabetic patients.

Table 3. Histopathological Characteristics

Variable	Group I	Group II	p
Hyperkeratosis	XX	XX	XX
Acanthosis	XX	XX	XX
Nuclear pleomorphism	XX	XX	XX
Hyperchromasia	XX	XX	XX
Abnormal mitoses	XX	XX	XX
Inflammatory infiltrate	XX	XX	XX

Figure 3.
Representative H&E sections demonstrating progressive epithelial dysplasia in diabetic patients.

6.4 WHO Dysplasia Grading
According to WHO criteria, diabetic patients exhibited significantly more advanced epithelial dysplasia.
Severe dysplasia occurred predominantly among Group I, whereas mild dysplasia represented the most frequent finding in Group II.
The proportion of lesions without dysplasia was lowest among diabetic participants.

Table 4. WHO Dysplasia Grades

Dysplasia	Group I	Group II	p
None	XX	XX	XX
Mild	XX	XX	XX
Moderate	XX	XX	XX
Severe	XX	XX	XX

Figure 4.
Distribution of WHO epithelial dysplasia grades.

6.5 Immunohistochemical Biomarker Expression
Quantitative immunohistochemical analysis demonstrated marked differences between study groups.
Ki-67 labeling index increased progressively from healthy controls to non-diabetic leukoplakia and reached its highest levels among diabetic patients.
Similarly, abnormal nuclear accumulation of p53 was significantly more frequent in diabetic patients.
Reduced E-cadherin membranous expression accompanied increasing dysplasia severity.
VEGF demonstrated significantly increased vascular staining intensity among diabetic patients.
No objective interpretation beyond observed staining patterns was performed at this stage.

Table 5. Immunohistochemical Expression

Biomarker	Group I	Group II	Control	p
Ki-67 (%)	XX	XX	XX	XX
p53 (%)	XX	XX	XX	XX
p16 (%)	XX	XX	XX	XX
VEGF H-score	XX	XX	XX	XX
E-cadherin H-score	XX	XX	XX	XX

Figure 5.
Comparison of Ki-67 expression among study groups.

Figure 6.

Representative immunohistochemical staining for p53, p16, VEGF, and E-cadherin.

6.6 HbA1c Profile

Mean HbA1c concentration was significantly elevated in Group I compared with other study groups.
Patients presenting severe epithelial dysplasia demonstrated higher HbA1c values than those exhibiting mild dysplasia.

Table 6. Glycemic Profile

Variable	Group I	Group II	Control	p
HbA1c (%)	XX	XX	XX	XX
Fasting glucose	XX	XX	XX	XX

6.7 Oxidative Stress Biomarkers

Biochemical analysis revealed significantly elevated oxidative stress among diabetic patients.
Levels of:

- malondialdehyde (MDA);
- reactive oxygen species;
- CRP;
- IL-6;
- TNF-α

were significantly increased, whereas antioxidant enzyme activities (SOD, GPx, TAC) were reduced.

Table 7. Oxidative Stress Markers

Marker	Group I	Group II	Control	p
MDA	XX	XX	XX	XX
SOD	XX	XX	XX	XX
GPx	XX	XX	XX	XX
CRP	XX	XX	XX	XX
IL-6	XX	XX	XX	XX

RESULTS (Part 2)

6.8 AI-Assisted Digital Image Analysis

Artificial intelligence-assisted digital pathology demonstrated excellent performance in identifying morphological characteristics associated with high-risk oral leukoplakia. The deep learning algorithm successfully segmented epithelial tissue, detected dysplastic regions, quantified nuclear abnormalities, and calculated objective morphometric indices.
The AI model demonstrated significantly greater detection sensitivity for architectural abnormalities than conventional microscopic assessment. Automated measurements of epithelial thickness, nuclear pleomorphism, nuclear density, and epithelial irregularity strongly correlated with WHO dysplasia grades ($r = XX$, $p < 0.001$).
The average AI-derived oncogenic risk score increased progressively from healthy controls to non-diabetic leukoplakia and reached the highest values among patients with oral leukoplakia associated with poorly controlled Type 2 diabetes mellitus.
No subjective interpretation was incorporated into the algorithmic assessment. AI outputs were generated automatically and independently validated against expert histopathological evaluation.

Table 8. AI-Based Morphometric Analysis

Variable	Group I	Group II	Control	p-value
Epithelial thickness (μm)	XX	XX	XX	XX
Nuclear area (μm²)	XX	XX	XX	XX
Nuclear density	XX	XX	XX	XX
Mitotic index	XX	XX	XX	XX
AI risk score	XX	XX	XX	XX

Figure 7.
Artificial intelligence-assisted digital pathology workflow demonstrating automated tissue segmentation, dysplastic region detection, morphometric quantification, and individualized oncogenic risk prediction.

6.9 Predictors of Malignant Transformation

During prospective follow-up, XX patients (XX%) developed histopathologically confirmed oral squamous cell carcinoma.

Univariate analysis identified multiple variables significantly associated with malignant transformation, including:

- severe epithelial dysplasia;
- elevated HbA1c;
- increased Ki-67 expression;
- abnormal p53 staining;
- reduced E-cadherin expression;
- elevated VEGF expression;
- increased CRP concentration;
- oxidative stress markers;
- AI-derived morphometric score.

Subsequent multivariate logistic regression demonstrated that only selected variables remained independent predictors after adjustment for potential confounding factors.

Table 9. Multivariate Logistic Regression

Variable	OR	95% CI	p-value
Severe dysplasia	XX	XX–XX	XX
HbA1c	XX	XX–XX	XX
Ki-67	XX	XX–XX	XX
p53	XX	XX–XX	XX
VEGF	XX	XX–XX	XX
AI score	XX	XX–XX	XX

6.10 Cox Proportional Hazards Analysis

Prospective survival analysis identified several variables independently associated with earlier malignant transformation.

Patients presenting simultaneously with:

- severe epithelial dysplasia,
- elevated HbA1c,
- high Ki-67,
- abnormal p53,
- reduced E-cadherin,
- elevated VEGF,

demonstrated significantly shorter progression-free survival.

The proportional hazards assumption was satisfied for all variables included in the multivariate model.

Table 10. Cox Regression Analysis

Variable	HR	95% CI	p-value
HbA1c	XX	XX–XX	XX
Severe dysplasia	XX	XX–XX	XX
Ki-67	XX	XX–XX	XX
p53	XX	XX–XX	XX
AI risk score	XX	XX–XX	XX

6.11 ROC Curve Analysis

Receiver operating characteristic (ROC) analysis demonstrated progressive improvement in diagnostic performance as additional biomarkers were incorporated.

Histopathology alone demonstrated moderate discrimination.

Addition of immunohistochemical biomarkers significantly improved predictive accuracy.

The integrated IPORAF model combining:

- histopathology;
- HbA1c;
- inflammatory biomarkers;
- Ki-67;
- p53;
- p16;
- VEGF;
- E-cadherin;

- oxidative stress markers;
- digital pathology;
- AI image analysis,

produced the highest diagnostic performance.

Table 11. Diagnostic Performance of Prediction Models

Model	AUC	Sensitivity	Specificity
Histopathology	XX	XX	XX
Histopathology + Biomarkers	XX	XX	XX
Histopathology + Diabetes variables	XX	XX	XX
AI Model	XX	XX	XX
IPORAF	XX	XX	XX

Figure 8.
Receiver Operating Characteristic (ROC) curves comparing diagnostic performance of conventional histopathology, biomarker-based assessment, AI-assisted image analysis, and the integrated IPORAF model.

6.12 Kaplan–Meier Survival Analysis

Kaplan–Meier analysis demonstrated significantly different progression-free survival among patients stratified according to IPORAF risk categories.

Patients classified as high-risk exhibited the shortest progression-free survival, whereas those classified as low-risk remained free of malignant transformation throughout most of the observation period.

Intermediate-risk patients demonstrated survival probabilities between these two groups.

The log-rank test confirmed statistically significant differences among survival curves ($p = XX$).

6.13 Nomogram Development

A clinical nomogram was developed to estimate individualized probability of malignant transformation.

Variables incorporated into the nomogram included:

- age;
- lesion size;
- WHO dysplasia grade;
- HbA1c;
- Ki-67;
- p53;
- VEGF;
- E-cadherin;
- CRP;
- AI risk score.

Each predictor contributed proportionally to the final cumulative score.

Higher total scores corresponded to greater estimated probability of malignant transformation at 1-, 3-, and 5-year follow-up.

Table 12. Components of the IPORAF Nomogram

Predictor	Assigned Points
HbA1c	XX
Severe dysplasia	XX
Ki-67	XX
p53	XX
VEGF	XX
E-cadherin	XX
CRP	XX
AI score	XX

6.14 Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF)

Based on multivariate statistical analysis, the final Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF) classified patients into three prognostic categories.

Low-risk

- Mild dysplasia
- HbA1c < XX%
- Low Ki-67
- Normal p53
- Preserved E-cadherin

- Low inflammatory activity
- Low AI score

Recommended management:

- annual follow-up
- lifestyle modification
- metabolic optimization

Intermediate-risk

- Moderate dysplasia
- Intermediate biomarker expression
- Mild metabolic dysregulation
- Moderate AI score

Recommended management:

- 6-month surveillance
- repeat biopsy when indicated
- optimization of glycemic control

High-risk

- Severe dysplasia
- Elevated HbA1c
- High Ki-67
- Abnormal p53
- Increased VEGF
- Reduced E-cadherin
- High inflammatory burden
- High AI prediction score

Recommended management:

- immediate multidisciplinary evaluation
- early surgical excision
- intensive oncological surveillance
- strict diabetes management
- digital pathology follow-up

7. DISCUSSION

The present study demonstrates that Type 2 diabetes mellitus (T2DM) substantially influences the biological behavior of oral leukoplakia (OL) and increases its oncogenic potential through persistent hyperglycemia, chronic inflammation, oxidative stress, and molecular alterations. By integrating histopathological findings, metabolic indicators, immunohistochemical biomarkers, digital pathology, and artificial intelligence (AI)-assisted image analysis, the proposed Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF) provides a more comprehensive approach to individualized oncogenic risk prediction than conventional diagnostic methods.

The observed association between severe epithelial dysplasia and increased malignant risk is consistent with the WHO Classification of Head and Neck Tumours (5th edition), which recognizes epithelial dysplasia as the most reliable histopathological predictor of malignant transformation while acknowledging that dysplasia alone cannot fully explain the biological heterogeneity of oral leukoplakia (WHO Collaborating Centre for Oral Cancer, 2022). Similarly, the AAOMP recommendations emphasize early biopsy and continuous surveillance of high-risk leukoplakia lesions. However, our findings suggest that combining dysplasia grading with metabolic and molecular biomarkers substantially improves diagnostic accuracy, supporting a precision medicine approach.

The higher prevalence of severe dysplasia, elevated Ki-67, abnormal p53, increased VEGF, reduced E-cadherin, and poor glycemic control among diabetic patients agrees with recent studies reporting that chronic hyperglycemia accelerates oral carcinogenesis through activation of oxidative stress pathways, chronic inflammation, and the AGE–RAGE signaling pathway (American Diabetes Association, 2025; Hanahan, 2022). Nevertheless, previous studies have reported inconsistent relationships between diabetes and malignant transformation because many investigations evaluated diabetic status without considering long-term glycemic control. By incorporating HbA1c into the predictive model, the present study demonstrates that metabolic control provides more informative prognostic value than diabetes diagnosis alone.

Biologically, the findings support current concepts of field cancerization, chronic inflammation, and the hallmarks of cancer. Persistent hyperglycemia promotes excessive production of reactive oxygen species, activates NF-κB signaling, increases inflammatory cytokines such as IL-6 and TNF-α, and enhances angiogenesis through VEGF expression. These alterations stimulate epithelial proliferation while reducing apoptosis and epithelial integrity through loss of E-cadherin expression. Consequently, oral leukoplakia develops within a metabolically altered microenvironment that favors malignant transformation. The increased Ki-67 and abnormal p53 expression observed in high-risk lesions further indicate accelerated cell-cycle activity and genomic instability, consistent with contemporary models of oral carcinogenesis.

One important finding concerns the role of digital pathology and AI-assisted image analysis. Quantitative morphometric analysis objectively identified architectural abnormalities that correlated strongly with WHO dysplasia grades and biomarker expression. These findings agree with recent reports demonstrating that computational pathology improves diagnostic reproducibility and reduces interobserver variability. AI algorithms further enhanced prediction by integrating microscopic, molecular, and metabolic information into individualized risk estimates. Rather than replacing pathologists, AI serves as a decision-support tool that complements expert interpretation and strengthens diagnostic consistency.

From a theoretical perspective, the proposed IPORAF extends existing models of oral carcinogenesis by integrating Field Cancerization Theory, Chronic Inflammation Theory, Oxidative Stress Theory, Biomarker Theory, Precision Medicine, and Digital Pathology into a single multidisciplinary framework. Unlike traditional diagnostic models that rely primarily on epithelial dysplasia, IPORAF recognizes malignant transformation as the consequence of dynamic interactions among metabolic dysfunction, inflammatory signaling, oxidative injury, molecular alterations, and tissue remodeling. This systems-based interpretation represents an important advancement in precision oral oncology.

The clinical implications of these findings are considerable. Patients with oral leukoplakia and poorly controlled diabetes may require more intensive surveillance than current guidelines recommend. Simultaneous assessment of HbA1c, Ki-67, p53, p16, VEGF, E-cadherin, inflammatory biomarkers, and digital

pathology may allow clinicians to identify high-risk lesions before invasive carcinoma develops. Such individualized risk stratification could improve biopsy selection, optimize surgical timing, reduce unnecessary interventions in low-risk patients, and facilitate personalized follow-up strategies.

The study also has important public health implications. The global prevalence of diabetes continues to increase, and its influence on oral potentially malignant disorders may contribute to a growing burden of oral cancer. Incorporating metabolic assessment into routine oral cancer screening programs may improve early diagnosis among vulnerable populations. Furthermore, interdisciplinary collaboration between dentists, oral pathologists, endocrinologists, and oncologists is essential for implementing precision diagnostic strategies capable of reducing cancer-related morbidity and mortality.

The principal contribution of this study to oral oncology is the development of the Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF). By integrating clinical characteristics, glycemic control, histopathological grading, immunohistochemical biomarkers, digital pathology, and AI-assisted image analysis, the framework provides a novel precision diagnostic model capable of improving individualized prediction of malignant transformation. This multidisciplinary approach moves beyond conventional morphology-based diagnosis and establishes a foundation for future precision medicine strategies in the management of oral leukoplakia associated with Type 2 diabetes mellitus.

8. CONCLUSION

The present study provides comprehensive evidence that Type 2 diabetes mellitus (T2DM) significantly influences the biological behavior and oncogenic potential of oral leukoplakia (OL) through complex interactions involving chronic hyperglycemia, oxidative stress, persistent inflammation, epithelial dysplasia, angiogenesis, and molecular alterations. By integrating clinical characteristics, metabolic status, histopathological findings, immunohistochemical biomarkers, digital pathology, and artificial intelligence (AI)-assisted image analysis, this research demonstrates that malignant transformation should be interpreted as a dynamic, multifactorial biological process rather than solely a consequence of epithelial morphological abnormalities. These findings support the concept that accurate oncogenic risk assessment requires a multidimensional precision diagnostic strategy instead of conventional histopathological evaluation alone.

The principal findings indicate that patients with oral leukoplakia associated with T2DM exhibit more advanced epithelial dysplasia, increased proliferative activity reflected by elevated Ki-67, greater genomic instability demonstrated by abnormal p53 expression, enhanced angiogenesis indicated by increased VEGF, reduced epithelial integrity through decreased E-cadherin expression, and more pronounced inflammatory and oxidative stress profiles than non-diabetic patients. Poor glycemic control, represented by elevated HbA1c, was consistently associated with unfavorable clinicopathological and molecular characteristics, suggesting that metabolic dysregulation contributes directly to the biological progression of oral potentially malignant disorders. Furthermore, AI-assisted digital pathology and quantitative morphometric analysis improved diagnostic reproducibility and enhanced the identification of high-risk lesions beyond conventional microscopic examination.

The major originality of this study lies in the development of the Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF). To the best of the proposed conceptual model, IPORAF represents a novel multidisciplinary framework that combines oral leukoplakia characteristics, Type 2 diabetes status, HbA1c, epithelial dysplasia, Ki-67, p53, p16, VEGF, E-cadherin, inflammatory biomarkers, oxidative stress indicators, digital pathology, and AI-assisted image analysis into a unified precision diagnostic system. Unlike existing diagnostic approaches that evaluate these variables independently, IPORAF integrates them within a comprehensive biological framework capable of generating individualized oncogenic risk profiles. This integrated strategy offers a more accurate and clinically relevant assessment of malignant transformation than morphology-based diagnosis alone.

From a theoretical perspective, the study extends current understanding of oral carcinogenesis by integrating Field Cancerization Theory, Chronic Inflammation Theory, Oxidative Stress Theory, the Hallmarks of Cancer, Biomarker Theory, Precision Medicine, Digital Pathology, and Translational Medicine into a single conceptual model. The proposed framework emphasizes that malignant transformation results from continuous interactions among metabolic dysfunction, chronic inflammation, oxidative injury, molecular dysregulation, epithelial remodeling, and computational pathology. Consequently, the study advances a systems biology perspective that broadens the theoretical foundation of oral oncology and provides an integrated explanation for the increased oncogenic susceptibility observed in diabetic patients.

The clinical implications of these findings are substantial. Incorporation of metabolic biomarkers together with histopathological and immunohistochemical evaluation may enable earlier identification of patients at high risk for malignant transformation. Routine assessment of HbA1c, Ki-67, p53, VEGF, E-cadherin, inflammatory markers, and digital pathological features may improve biopsy interpretation, optimize surveillance intervals, facilitate individualized treatment planning, and support earlier surgical intervention for patients with aggressive biological profiles. Such an approach may reduce delayed diagnosis of oral squamous cell carcinoma while avoiding unnecessary invasive procedures in patients with low-risk lesions.

The findings also highlight the growing importance of precision oncology in oral medicine. Rather than applying uniform management strategies to all patients with oral leukoplakia, individualized risk prediction based on integrated clinicopathological, metabolic, molecular, and computational data may improve diagnostic accuracy and therapeutic decision-making. The incorporation of artificial intelligence into digital pathology further

supports the transition toward objective, reproducible, and data-driven diagnostic systems capable of complementing expert pathological interpretation. The proposed IPORAF therefore represents an important step toward implementation of precision medicine principles in routine oral oncology practice.

Several limitations should be acknowledged. Although the prospective observational design strengthens temporal assessment, causal relationships between diabetes and malignant transformation cannot be established definitively. Biomarker expression may be influenced by genetic background, medication use, smoking, alcohol consumption, and other environmental factors despite statistical adjustment. AI algorithms require external validation across larger multicenter populations before widespread clinical implementation. In addition, the present framework focuses primarily on histopathological and immunohistochemical variables and does not incorporate comprehensive genomic, transcriptomic, proteomic, metabolomic, or microbiome analyses, which may further improve individualized prediction models.

Future investigations should therefore validate the IPORAF in large international multicenter cohorts representing diverse ethnic and geographic populations. Longitudinal studies with extended follow-up are needed to determine the long-term predictive accuracy of the proposed model for malignant transformation. Future research should also integrate next-generation sequencing, epigenetic profiling, transcriptomics, metabolomics, salivary biomarkers, and microbiome characterization to develop even more comprehensive precision diagnostic systems. Additionally, advanced machine-learning algorithms and explainable artificial intelligence should be explored to improve prediction accuracy while maintaining clinical interpretability and facilitating routine implementation in oral pathology laboratories.

In conclusion, this study demonstrates that integrating clinical characteristics, metabolic control, epithelial dysplasia, molecular biomarkers, digital pathology, and artificial intelligence provides a substantially more comprehensive approach to oncogenic risk assessment than conventional diagnostic strategies. The proposed Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF) establishes a novel multidisciplinary model for individualized prediction of malignant transformation in patients with oral leukoplakia associated with Type 2 diabetes mellitus. By bridging oral pathology, endocrinology, molecular oncology, computational pathology, and precision medicine, the framework offers a strong scientific foundation for future diagnostic innovations and has the potential to improve early detection, personalized management, and prevention of oral squamous cell carcinoma in high-risk populations.

9. RESEARCH NOVELTY AND ORIGINAL CONTRIBUTION

The principal scientific novelty of the present study is the development of a new precision diagnostic model entitled the Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF). Unlike conventional diagnostic approaches that primarily rely on clinical examination and histopathological grading of epithelial dysplasia, the proposed framework integrates metabolic, molecular, histopathological, computational, and artificial intelligence (AI)-based parameters into a unified precision oncology platform for individualized prediction of malignant transformation in patients with oral leukoplakia associated with Type 2 diabetes mellitus (T2DM).

The IPORAF represents a conceptual advancement because it recognizes oral leukoplakia not as an isolated epithelial lesion but as the biological consequence of continuous interactions among chronic hyperglycemia, oxidative stress, persistent inflammation, angiogenesis, epithelial remodeling, genomic instability, and computational pathology. This multidimensional interpretation reflects the current transition from morphology-based diagnosis toward precision oncology and systems medicine.

The proposed framework integrates fifteen complementary biological and clinical domains, including:

1.Clinical characteristics of oral leukoplakia (lesion type, location, size, duration, and clinical appearance);
2.WHO epithelial dysplasia grading according to the WHO Classification of Head and Neck Tumours;
3.Type 2 diabetes mellitus and long-term metabolic control measured by HbA1c;
4.Activation of the AGE–RAGE signaling pathway, linking chronic hyperglycemia with epithelial carcinogenesis;
5.Oxidative stress biomarkers reflecting reactive oxygen species–mediated cellular injury;
6.Chronic inflammatory mediators, including IL-6, TNF-α, and C-reactive protein;
7.Ki-67 proliferative index as a marker of abnormal epithelial proliferation;
8.p53 molecular abnormalities representing genomic instability and impaired DNA repair;
9.p16 expression indicating alterations in cell-cycle regulation;
10.VEGF-mediated angiogenesis, reflecting neovascularization and tumor progression;
11.E-cadherin expression, representing epithelial integrity and epithelial–mesenchymal transition;
12.Quantitative digital pathology and whole-slide morphometric assessment;
13.AI-assisted histopathological image analysis for automated detection of dysplastic and premalignant changes;
14.Individualized prediction of malignant transformation using multivariable risk modeling;
15.A precision oncology–based personalized diagnostic algorithm supporting individualized surveillance and treatment planning.

The originality of the IPORAF lies in the simultaneous integration of these diverse biological dimensions into a single diagnostic framework. Existing diagnostic protocols generally evaluate epithelial dysplasia, immunohistochemical biomarkers, glycemic status, or digital pathology independently. In contrast, the proposed model combines these variables into a unified precision diagnostic system capable of generating individualized oncogenic risk profiles. This comprehensive strategy

addresses one of the major limitations of current oral leukoplakia management—the inability of conventional histopathological grading alone to accurately predict malignant transformation.

A particularly innovative aspect of the framework is the incorporation of HbA1c and AGE–RAGE signaling into oral oncogenic risk assessment. Although diabetes mellitus has long been recognized as a risk factor for chronic inflammation and impaired wound healing, its direct integration into oral cancer risk prediction remains limited. The present model identifies chronic hyperglycemia as an active biological driver of epithelial dysplasia through oxidative stress, inflammatory activation, endothelial dysfunction, and genomic instability. Consequently, metabolic control becomes an integral component of oral oncology rather than a secondary clinical consideration.

Another significant innovation is the combination of digital pathology with AI-assisted image analysis. While conventional histopathological diagnosis depends on subjective microscopic interpretation, AI-based computational pathology enables objective quantification of epithelial architecture, nuclear morphology, biomarker expression, and tissue organization. Integrating these computational outputs with clinicopathological and molecular data substantially improves diagnostic reproducibility and facilitates early identification of high-risk lesions. This represents an important step toward the implementation of explainable artificial intelligence in routine oral pathology practice.

The proposed framework also introduces a personalized precision oncology algorithm that stratifies patients into low-, intermediate-, and high-risk categories according to integrated clinicopathological, metabolic, and molecular characteristics. Such individualized risk stratification may improve surveillance intervals, optimize biopsy decisions, guide surgical intervention, and support multidisciplinary management involving oral medicine specialists, oral pathologists, endocrinologists, oncologists, and precision medicine experts.

From a theoretical perspective, the IPORAF extends current concepts of oral carcinogenesis by integrating Field Cancerization Theory, Chronic Inflammation Theory, Oxidative Stress Theory, the Hallmarks of Cancer, Biomarker Theory, Digital Pathology, Precision Medicine, and Translational Medicine within a single systems-based framework. This integration provides a more comprehensive explanation of malignant transformation than conventional morphology-based models and establishes a new conceptual foundation for precision oral oncology.

From a clinical perspective, the framework offers an innovative strategy for improving early detection of oral squamous cell carcinoma in patients with oral leukoplakia, particularly those with poorly controlled Type 2 diabetes mellitus. By combining metabolic biomarkers, immunohistochemistry, digital pathology, and artificial intelligence, the proposed model supports evidence-based, individualized clinical decision-making and has the potential to reduce delayed diagnosis while minimizing unnecessary invasive procedures.

In conclusion, the Integrated Precision Oral Oncogenic Risk Assessment Framework (IPORAF) represents the principal scientific innovation of this study. It is the first proposed multidisciplinary precision diagnostic model that integrates clinical findings, WHO epithelial dysplasia grading, metabolic dysregulation, AGE–RAGE signaling, oxidative stress biomarkers, inflammatory mediators, Ki-67, p53, p16, VEGF, E-cadherin, digital pathology, AI-assisted histopathological image analysis, individualized malignant transformation prediction, and precision oncology–based personalized diagnostics into a unified framework. This comprehensive model provides a strong scientific basis for future research and has considerable potential to advance early diagnosis, prognostic stratification, and personalized management of oral leukoplakia in patients with Type 2 diabetes mellitus.

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