Review Article

BIOMARKERS FOR ASSESSING DISEASE PROGRESSION AND TREATMENT OUTCOMES OF ORAL SUBMUCOUS FIBROSIS

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Abstract:

Biomarkers for assessing disease progression and treatment outcomes of oral submucous fibrosis (OSMF) have gained significant attention in recent years. This review provides an overview of the current research on biomarkers in OSMF and their potential implications in clinical practice.

The review focuses on the different categories of biomarkers that have shown promise in assessing disease progression and treatment outcomes in OSMF. Epithelial dysplasia markers, including Ki-67, p53, and cyclin D1, are discussed in relation to dysplastic changes observed in OSMF. Inflammatory and immune markers, such as cytokines, chemokines, and inflammatory mediators, are explored for their role in OSMF progression. The dysregulation of extracellular matrix remodelling is highlighted, with a discussion on matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) as potential biomarkers.

Furthermore, the review delves into biomarkers for treatment response and outcome prediction. Collagen turnover markers, including pro-collagen type I and MMPs, are examined as indicators of collagen synthesis and degradation. Oxidative stress markers, such as malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx), are evaluated for their ability to reflect oxidative stress and antioxidant status. The review also touches upon the potential of epigenetic markers, such as DNA methylation and histone modifications, in predicting treatment response and prognosis in OSMF.

Additionally, the review explores the use of imaging techniques, including optical coherence tomography (OCT), autofluorescence imaging, and confocal microscopy, for assessing structural changes and biomarker expression in OSMF. It discusses the potential of molecular imaging modalities, such as positron emission tomography (PET) and molecular MRI, for visualizing specific biomarkers in OSMF. Moreover, the review highlights the importance of genomics approaches, including genomics, transcriptomics, proteomics, and metabolomics, in identifying novel biomarkers and unraveling molecular pathways in OSMF.

We emphasize the potential of biomarkers in improving patient care and management in OSMF. It acknowledges the challenges in biomarker validation, standardization, and cost-effectiveness for widespread clinical use. The review calls for further research in emerging areas, such as non-coding RNAs, microRNA panels, and multi-modal biomarker approaches, to enhance our understanding of OSMF and facilitate personalized treatment strategies.

Key-words: *Oral submucous fibrosis, biomarkers, disease progression, treatment outcomes, oral cancer, epithelial dysplasia markers, inflammatory markers*

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Introduction:

Oral submucous fibrosis (OSMF) is a chronic, progressive, and potentially malignant disorder characterized by fibrotic changes in the oral mucosa, resulting in restricted mouth opening, burning sensation, and difficulty in eating and speaking.[1] The condition predominantly affects the oral cavity and oropharynx, causing significant morbidity and impairing quality of life. OSMF has a high prevalence in certain regions of the world, particularly South Asia, including India, Sri Lanka, Bangladesh, and parts of Southeast Asia. It is more prevalent among individuals of Asian descent, although sporadic cases have been reported in other populations. The incidence and prevalence of OSMF vary across different geographical areas and ethnic groups, suggesting a complex interplay of genetic, environmental, and lifestyle factors.[2]

The exact etiology of OSMF remains unclear; however, it is thought to be multifactorial, involving various genetic and environmental factors. The primary risk factor associated with OSMF is the consumption of areca nut, which is commonly chewed with or without tobacco as a form of betel quid in many Asian countries. Areca nut contains alkaloids, flavonoids, and tannins, which have been implicated in the pathogenesis of OSMF. The habitual use of betel quid, along with other associated factors like tobacco and spices, increases the risk of developing OSMF. Other potential risk factors for OSMF include genetic predisposition, nutritional deficiencies, chronic irritation, immunologic factors and environmental factors.[3]

The diagnosis of OSMF is primarily based on clinical examination and the presence of characteristic signs and symptoms. The commonly accepted diagnostic criteria included are restricted mouth opening (interincisal distance less than normal), presence of palpable fibrous bands or bands seen during intraoral examination, presence of burning sensation, blanching of oral mucosa, and mucosal stiffness and / or absence of other conditions that may mimic the clinical features of OSMF, such as lichen planus, scleroderma, and chronic oral candidiasis. Histopathological confirmation, although not always necessary, can be obtained through biopsy to assess the extent of fibrosis and rule out malignancy.[4] It is important to note that the severity of OSMF can be graded based on the mouth opening range, with various classification systems available to assess the progression of the disease.

Current Challenges in Disease Progression Assessment and Treatment Outcomes:

The current assessment of disease progression and treatment outcomes in oral submucous fibrosis faces challenges due to limitations of clinical assessment methods, difficulties in monitoring treatment response, and the need for reliable and objective biomarkers. Overcoming these challenges through the development and validation of robust biomarkers will enhance our understanding of OSMF, improve patient management, and facilitate the development of targeted therapeutic interventions. [5]

Clinical assessment of oral submucous fibrosis (OSMF) relies on subjective evaluation, which can be influenced by interobserver variability and may not accurately reflect disease severity and progression. Some of the limitations are difficulty in accurately measuring and quantifying fibrosis and stiffness of the oral mucosa, reliance on subjective measures such as mouth opening range, which may not capture the full extent of fibrotic changes and functional impairment and inability to assess subclinical changes and early stages of the disease, leading to delayed diagnosis and intervention.

Monitoring treatment response and assessing treatment outcomes in OSMF can be challenging because of lack of standardized and objective measures to assess treatment response, making it difficult to compare results across studies and interventions. Additionally, slow and gradual progression of fibrosis, requiring long-term follow-up to observe significant changes and evaluate treatment efficacy also is another challenge. Limited availability of validated tools and scoring systems to assess the functional outcomes and quality of life improvements following treatment further adds up to this

Hence, biomarkers play a crucial role in disease progression assessment and treatment outcome evaluation. In the context of OSMF, the development and validation of reliable and objective biomarkers are crucial for several reasons:

- Biomarkers can provide objective measures of disease severity, progression, and response to treatment, complementing clinical assessment methods.
- They can aid in early detection and diagnosis of OSMF, enabling timely intervention and prevention of disease progression.
- Biomarkers may help identify individuals at higher risk of malignant transformation and facilitate risk stratification for appropriate management.
- Reliable biomarkers can serve as surrogate endpoints in clinical trials, facilitating the evaluation of treatment efficacy and enabling the development of targeted therapies.
- Objective biomarkers can enhance the accuracy and consistency of disease monitoring, allowing for more precise prognostication and personalized treatment planning. [6]

Potential Biomarkers for Disease Progression Assessment in Oral Submucous Fibrosis (OSMF):

Epithelial Dysplasia Markers:

- Ki-67: Ki-67 is a marker of cell proliferation and is associated with dysplastic changes in OSMF. Elevated Ki-67 expression indicates increased cellular proliferation, which is often observed in the epithelial dysplasia associated with OSMF.
- p53: The tumor suppressor protein p53 plays a role in regulating cell growth and apoptosis. Mutations in the p53 gene are commonly found in dysplastic lesions and oral squamous cell carcinoma (OSCC), which can develop from OSMF. Therefore, p53 expression and mutations are potential markers for assessing disease progression and malignant transformation.
- Cyclin D1: Cyclin D1 is involved in cell cycle regulation and is frequently overexpressed in dysplastic lesions and OSCC. Elevated levels of cyclin D1 can indicate increased cell proliferation and the potential for disease progression in OSMF. [7]

Inflammatory and Immune Markers:

- Cytokines and Chemokines: Various pro-inflammatory cytokines and chemokines, such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and transforming growth factor-beta (TGF-β), have been implicated in the pathogenesis of OSMF. Increased levels of these inflammatory markers can indicate disease progression and the inflammatory response associated with fibrosis.
- Inflammatory Mediators: Other inflammatory mediators, such as prostaglandins, leukotrienes, and reactive oxygen species, may contribute to the fibrotic changes in OSMF. Monitoring the levels of these mediators can provide insights into disease progression and the underlying inflammatory processes. [8]

Extracellular Matrix Remodeling Markers:

- Matrix Metalloproteinases (MMPs): MMPs are enzymes involved in the breakdown of the extracellular matrix. Imbalance between MMPs and their inhibitors, such as tissue inhibitors of metalloproteinases (TIMPs), can lead to excessive collagen deposition and fibrosis. Dysregulation of MMPs and TIMPs may serve as biomarkers for assessing disease progression and the remodeling of the extracellular matrix in OSMF. [9]

These potential biomarkers provide valuable insights into the cellular and molecular changes occurring in OSMF. By evaluating their expression levels or activity, clinicians and researchers can assess disease progression, identify individuals at higher risk of malignant transformation, and potentially predict treatment outcomes.

Biomarkers for Treatment Response and Outcome Prediction in Oral Submucous Fibrosis (OSMF):

Collagen Turnover Markers:

- Pro-collagen Type I: Pro-collagen Type I is a precursor molecule of collagen, which is the main component of the fibrotic changes in OSMF. Monitoring the levels of pro-collagen

Type I can provide insights into collagen synthesis and turnover, indicating the progression or regression of fibrosis in response to treatment.

- Matrix Metalloproteinases (MMPs): MMPs, specifically those involved in collagen degradation, can serve as biomarkers for assessing treatment response. Decreased MMP activity may indicate reduced collagen degradation and fibrosis regression, whereas increased MMP activity may suggest ongoing fibrotic changes. [10]

Oxidative Stress Markers:

- Malondialdehyde (MDA): MDA is a byproduct of lipid peroxidation and serves as a marker of oxidative stress. Elevated levels of MDA indicate increased oxidative damage, which has been implicated in the pathogenesis of OSMF. Monitoring MDA levels can help evaluate the extent of oxidative stress and assess treatment response.
- Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx): SOD and GPx are antioxidant enzymes that counteract oxidative stress by scavenging free radicals and reducing oxidative damage. Monitoring the activity or levels of these enzymes can provide insights into the antioxidant status and the effectiveness of antioxidant-based therapies in OSMF.

Epigenetic Markers:

- DNA Methylation: DNA methylation is an epigenetic modification that regulates gene expression. Aberrant DNA methylation patterns have been observed in OSMF and may serve as potential biomarkers for treatment response and prognosis. Changes in DNA methylation status, particularly in genes involved in fibrosis and malignant transformation, can provide insights into the effectiveness of treatment and the risk of disease progression.
- Histone Modifications: Histone modifications, such as acetylation, methylation, and phosphorylation, influence gene expression and chromatin structure. Altered histone modification patterns have been associated with fibrosis-related gene expression changes. Assessing histone modification patterns can help predict treatment response and identify patients who are more likely to benefit from specific interventions. [11]

Imaging and Molecular Techniques for Biomarker Assessment in Oral Submucous Fibrosis (OSMF):

Imaging Techniques:

- Ultrasound Imaging: Ultrasound imaging can be used to assess the thickness and fibrotic changes in the oral mucosa. It provides real-time visualization of the affected tissues and can help in quantifying the extent of fibrosis.
- Magnetic Resonance Imaging (MRI): MRI can provide detailed anatomical images of the oral cavity and surrounding tissues. It can aid in evaluating the extent of fibrosis, identifying submucosal changes, and assessing disease progression.
- Optical Coherence Tomography (OCT): OCT is a non-invasive imaging technique that uses light waves to create high-resolution cross-sectional images of tissues. It can assist in visualizing the microstructural changes associated with OSMF, such as epithelial thickening, fibrosis, and submucosal alterations. [12]

Molecular Techniques:

- Gene Expression Profiling: Gene expression profiling techniques, such as microarray analysis or next-generation sequencing, can help identify differentially expressed genes associated with OSMF. By comparing gene expression patterns in OSMF patients before and after treatment, potential biomarkers for treatment response and outcome prediction can be identified.

- Proteomic Analysis: Proteomic analysis involves the identification and quantification of proteins in biological samples. By analyzing protein profiles, specific biomarkers associated with disease progression, treatment response, or malignant transformation can be identified in OSMF.
- Metabolomic Profiling: Metabolomics involves the comprehensive analysis of

metabolites in biological samples. Metabolomic profiling can provide insights into metabolic changes associated with OSMF, identify potential biomarkers, and assess treatment response. [13]

Immunohistochemistry (IHC):

- Immunohistochemistry is a technique that uses specific antibodies to detect and visualize proteins of interest in tissue sections. It can be used to assess the expression and localization of various biomarkers associated with OSMF, such as inflammatory markers, fibrosis-related proteins, or epithelial dysplasia markers. [14]

Optical Imaging Techniques for Assessing Structural Changes and Biomarker Expression in Oral Submucous Fibrosis (OSMF):

These optical imaging techniques offer non-invasive and real-time assessment of structural changes and biomarker expression in OSMF. They provide valuable insights into the pathophysiology of the disease, aid in early detection, and offer potential for personalized treatment approaches

Optical Coherence Tomography (OCT):

Optical coherence tomography is a non-invasive imaging technique that utilizes light waves to generate high-resolution, cross-sectional images of tissues. OCT provides detailed structural information of the oral mucosa and can be used to assess the following aspects of OSMF:

- Epithelial Thickness: OCT can measure the thickness of the oral epithelium, which is often increased in OSMF due to epithelial hyperplasia.
- Subepithelial Fibrosis: OCT allows visualization and quantification of the subepithelial fibrotic changes, providing valuable information about the extent and severity of fibrosis.
- Vascular Changes: OCT can detect alterations in the vascular network of the oral mucosa, such as dilated blood vessels or loss of capillary density, which may be associated with OSMF.
- Real-time Monitoring: OCT provides real-time imaging, allowing for longitudinal assessment of disease progression and treatment response. [15]

Autofluorescence Imaging:

Autofluorescence imaging is a non-invasive imaging technique that measures the intrinsic fluorescence emitted by endogenous fluorophores in tissues. It can provide insights into cellular and molecular changes associated with OSMF, including:

- Collagen Autofluorescence: Autofluorescence properties of collagen fibers can be altered in fibrotic conditions like OSMF. Quantitative analysis of collagen autofluorescence can potentially serve as a biomarker for assessing the severity of fibrosis.
- Cellular Changes: Autofluorescence imaging can detect changes in cellular metabolism and fluorophore distribution, providing information about cellular alterations associated with OSMF progression.
- Early Detection: Autofluorescence imaging may aid in the early detection of dysplastic or malignant changes by identifying abnormal fluorescence patterns indicative of precancerous alterations. [16]

Confocal Microscopy:

Confocal microscopy is a high-resolution imaging technique that provides detailed, real-time visualization of tissues at a cellular level. In the context of OSMF, confocal microscopy can be utilized to:

- Assess Epithelial Changes: Confocal microscopy allows direct visualization of the oral epithelium, enabling the detection of epithelial thickening, dysplasia, and cellular changes associated with OSMF.
- Biomarker Expression: By combining confocal microscopy with immunofluorescence staining, specific biomarkers associated with OSMF can be visualized and quantified at the cellular level, providing valuable insights into disease progression and treatment response. [17]

Molecular Imaging Modalities for Visualizing Specific Biomarkers in Oral Submucous Fibrosis (OSMF):

Positron Emission Tomography (PET):

PET is a molecular imaging technique that utilizes radioactive tracers to visualize and quantify specific molecular processes in tissues. It can be used to detect and monitor specific biomarkers associated with OSMF, including:

- Glucose Metabolism: OSMF is characterized by altered glucose metabolism, and PET imaging with the radiotracer. Fluorodeoxyglucose (FDG) can assess the metabolic activity of OSMF lesions. Increased FDG uptake may indicate areas of increased cellular proliferation and metabolic activity.
- Hypoxia: Hypoxia is a common feature in fibrotic and malignant tissues. PET imaging with hypoxia-specific radiotracers, such as fluoromisonidazole (FMISO), can visualize hypoxic areas within OSMF lesions, providing insights into the severity of tissue hypoxia and its potential role in disease progression.
- Integrin Expression: Integrins play a crucial role in cell adhesion, migration, and tissue remodeling. PET imaging with radiolabeled integrin-targeting probes can visualize integrin expression in OSMF lesions, allowing for the assessment of molecular changes associated with fibrosis and potential therapeutic targeting. [18]

Molecular MRI:

Molecular MRI techniques involve the use of contrast agents or specific imaging sequences to visualize molecular targets or biomarkers. In OSMF, molecular MRI can provide insights into the following aspects:

- Tissue Fibrosis: MRI techniques can be used to assess and quantify the extent of fibrosis in OSMF by detecting alterations in tissue water diffusion, relaxation times, or magnetization transfer properties. These parameters can be used as surrogate markers for fibrosis severity.
- Inflammation: Molecular MRI can visualize and quantify inflammatory processes associated with OSMF. Specific contrast agents or imaging sequences can target inflammatory mediators or cell populations, providing information about the inflammatory status and potential therapeutic response.
- Extracellular Matrix Remodeling: Molecular MRI techniques can be used to assess the dynamic changes in the extracellular matrix (ECM) associated with OSMF. Imaging probes targeting ECM components, such as collagen or fibronectin, can provide insights into ECM remodeling and fibrosis progression. [19]

Molecular imaging modalities, such as PET and molecular MRI, offer the ability to visualize specific biomarkers associated with OSMF. By providing molecular and functional information, these techniques can contribute to a deeper understanding of disease processes, facilitate early detection

Genomics Approaches for Identifying Novel Biomarkers and Unraveling Molecular Pathways in Oral Submucous Fibrosis (OSMF):

Genomics: Genomics involves the study of the entire genome, including the identification and characterization of genetic variations and their association with disease. In OSMF, genomics approaches can be used to:

- Genetic Association Studies: Genome-wide association studies (GWAS) and candidate gene studies can identify genetic variations associated with susceptibility to OSMF, disease progression, or treatment response.
- Single Nucleotide Polymorphisms (SNPs): SNPs are common genetic variations that can affect gene expression and protein function. Identifying OSMF-associated SNPs can provide insights into genetic factors contributing to disease development and progression.
- Copy Number Variations (CNVs): CNVs involve the duplication or deletion of large

genomic segments. Detecting CNVs associated with OSMF can uncover genomic regions and genes relevant to disease pathology.

Transcriptomics: Transcriptomics focuses on studying the entire transcriptome, including all RNA molecules present in a cell or tissue. Transcriptomics techniques, such as microarray analysis or RNA sequencing, can be used to:

- Gene Expression Profiling: Transcriptomics can identify differentially expressed genes between OSMF tissues and normal tissues, providing insights into molecular pathways involved in OSMF pathogenesis.
- Alternative Splicing: Transcriptomic analysis can identify alternative splicing events that result in different isoforms of genes associated with OSMF. These isoforms may have distinct functions or regulatory roles in the disease.
- Non-coding RNAs: Transcriptomics can uncover the expression patterns of non-coding RNAs, such as microRNAs and long non-coding RNAs, which play critical roles in gene regulation and may contribute to OSMF progression.

Proteomics: Proteomics involves the comprehensive study of proteins expressed in a given sample. Proteomics techniques can:

- Identify Differentially Expressed Proteins: By comparing the protein profiles of OSMF tissues and normal tissues, proteomics can identify proteins that are upregulated or downregulated in OSMF. These proteins can serve as potential biomarkers or provide insights into molecular pathways involved in the disease.
- Post-translational Modifications (PTMs): Proteomics can detect PTMs, such as phosphorylation or acetylation, which can influence protein function and contribute to disease pathogenesis. Studying PTMs in OSMF can reveal novel regulatory mechanisms and potential therapeutic targets.

Metabolomics: Metabolomics aims to comprehensively analyze the metabolites present in a biological sample. Metabolomics techniques can:

- Identify Altered Metabolic Pathways: Metabolomics analysis can uncover dysregulated metabolic pathways in OSMF, providing insights into the metabolic alterations associated with disease progression.
- Biomarker Discovery: Metabolomics can identify specific metabolites that are significantly altered in OSMF, which can serve as potential biomarkers for disease diagnosis, prognosis, or treatment response.

Emerging Research and Future Directions in Oral Submucous Fibrosis (OSMF):

- Non-coding RNAs: Non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), have gained significant attention in OSMF research. These RNA molecules play crucial roles in gene regulation and can serve as potential biomarkers and therapeutic targets. Ongoing research is focusing on identifying dysregulated non-coding RNAs in OSMF and elucidating their functional roles in disease progression. Understanding the regulatory networks involving non-coding RNAs may provide new

insights into the molecular mechanisms of OSMF and open avenues for targeted interventions.

- MicroRNA Panels: MicroRNA panels are emerging as a promising approach for disease diagnosis, prognosis, and treatment response prediction. Rather than relying on individual microRNAs, combining multiple microRNAs into a panel can enhance diagnostic accuracy and provide more comprehensive information about disease status. Studies are exploring the use of microRNA panels to differentiate OSMF from other oral lesions, predict disease progression, and monitor treatment response. The development and validation of robust microRNA panels have the potential to improve clinical decision-making and patient management in OSMF.
- Multi-modal Biomarker Approaches: Integrating multiple biomarkers from different molecular levels (genomics, transcriptomics, proteomics, etc.) and imaging modalities can enhance the accuracy and reliability of disease assessment in OSMF. By combining information from various sources, such as genetic variations, gene expression profiles, protein signatures, and imaging features, a multi-modal biomarker approach can provide a comprehensive understanding of disease progression, treatment response, and prognosis.

These integrated approaches hold promise in identifying synergistic biomarker combinations and improving the precision of OSMF diagnosis, risk stratification, and therapeutic decision-making.

- Therapeutic Target Identification: A crucial area of future research in OSMF is the identification of therapeutic targets. Through comprehensive molecular profiling and systems biology approaches, researchers aim to identify key molecular pathways and targets involved in OSMF pathogenesis. This knowledge can guide the development of targeted therapies and personalized treatment strategies. Additionally, understanding the molecular mechanisms underlying disease progression and malignant transformation may lead to the discovery of novel therapeutic interventions that can halt or reverse the fibrotic process and prevent malignant progression.
- Precision Medicine and Therapeutic Strategies: The application of precision medicine approaches in OSMF is an area of growing interest. By considering individual patient characteristics, genetic variations, and molecular profiles, personalized treatment strategies can be developed. This may involve the use of targeted therapies, including novel drugs, gene therapies, or immunomodulatory agents. Additionally, the integration of biomarker-based monitoring systems and non-invasive imaging techniques can enable real-time assessment of treatment response and help tailor therapeutic interventions for improved outcomes. [20]

Conclusion:

In conclusion, the identification and utilization of biomarkers for assessing disease progression and treatment outcomes in oral submucous fibrosis (OSMF) hold great potential for enhancing patient management and improving clinical outcomes. OSMF is a complex and multifactorial condition, and the use of biomarkers can provide objective and quantitative measures to complement clinical assessment.

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