Biochemical Markers of Oral Cancer

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***Abstract*—Early identification of oral squamous cell carcinoma (OSCC) is critical to improving patient outcomes, since early management correlates with clinical survival rates of over 85%. The carcinogenic cascade, which is fueled by major etiologies like tobacco use, chronic alcohol consumption, and human papillomavirus (HPV-16) infection, is primarily propelled by enhanced reactive oxygen species (ROS) and consequent lipid peroxidation. Traditional reactive techniques, which rely mainly on invasive tissue biopsies, are being replaced by proactive, multifluid molecular surveillance approaches. This research presents a highly authentic multi-omic framework for bridging the gap between benchtop molecular discoveries and point-of-care clinical diagnostics.**

Methodologically, this comprehensive approach uses high-throughput salivary proteomics via Ultra-Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) and Luminex multiplex assays to evaluate a core proteomic signature that includes matrix metalloproteinase-9 (MMP-9), interleukin-8 (IL-8), and chemerin, with diagnostic sensitivity of up to 94%. Concurrently, transcriptome surveillance using reverse transcription-quantitative PCR (RT-qPCR) identifies a high-fidelity seven-marker mRNA signature, including DUSP1 and OAZ1, that detects subclinical malignant changes before they become macroscopically visible. Serum glycomics uses advanced spectrophotometric analysis to offer precise quantifications of Total Sialic Acid (TSA) and Lipid-Bound Sialic Acid (LBSA) for real-time monitoring of systemic tumor load and clinical staging (TNM stages I–IV). Next-generation sequencing (NGS) of circulating tumor DNA (ctDNA) and exosomal microRNAs (miR-21, miR-184) allows for non-invasive deep genomic profiling and real-time tracking of occult primary tumors and therapeutic resistance. Finally, immunohistochemical (IHC) validation of tissue architecture using p53, Ki-67, and glucose transporter-1 (GLUT-1) confirms cellular proliferation indexes and the aggr. Combining these clinical pillars into a single multi-marker diagnostic panel significantly decreases false-positive "noise" from localized inflammatory conditions like as periodontitis, maximizing both sensitivity and specificity. Finally, switching from isolated single-marker testing to integrated multi-omic scores creates a comprehensive, high-resolution biochemical protocol that alters the paradigm of modern oral oncology from reactive clinical monitoring to precise, ultra-early treatment intercept.

***Index Terms*—Oral Squamous Cell Carcinoma (OSCC) , Liquid Biopsy ,Salivary Proteomics , Multi-Omic Integration , Serum Glycomics ,Transcriptomic Signature , Matrix Metalloproteinase-9 (MMP-9) , Sialic Acid , Warburg Effect , Circulating Tumor DNA (ctDNA) , Point-of-Care Diagnostics**

I. INTRODUCTION

- Oral cancer is a malignant growth in the mouth, most commonly oral squamous cell carcinoma (OSCC). Early detection is crucial, as survival rates exceed 85% when diagnosed early.
- Key Risk Factors: - Tobacco use (~75% of cases) - Heavy alcohol consumption - Human papillomavirus HPV-16) - Oral potentially malignant disorders (OPMDs), especially leukoplakia Tobacco increases DNA damage and reactive oxygen species (ROS), contributing to cancer development.
- Role of Oxidative Stress & Lipids: - ROS cause oxidative damage to DNA and cells - Lipids undergo lipid peroxidation, altering cell membranes - These changes promote malignant transformation.
- Importance of Biomarkers: Biomarkers from genes, proteins, metabolites, and microbiome help in: -
- Early detection - Predicting progression - Treatment decisions - Monitoring recurrence They can be detected noninvasively in saliva, serum, or tissues. Potential Tumor Markers: - β2-microglobulin -Total sialic acid - Lipid-bound sialic acid These markers show promise but require further research.

- Biochemical markers for Oral Squamous Cell Carcinoma (OSCC)**,** detectable in saliva, serum, and tissue, are essential for early diagnosis and predicting patient outcomes. Key markers include pro-inflammatory cytokines like IL-6 and IL-8, which signal tumor progression; enzymes such as MMP-9 and LDH, which reflect tissue invasion and metabolic shifts; and proliferation markers like Ki67 and Cyclin D1, which indicate cellular aggressiveness. These markers provide a non-invasive or minimally invasive means to monitor the "Warburg effect" and total tumor burden, significantly improving survival rates when integrated into clinical screening.
- Biochemical markers for Oral Squamous Cell Carcinoma (OSCC) are essential diagnostic and prognostic tools found primarily in saliva and serum, offering a non-invasive alternative to traditional biopsy. Key markers include inflammatory cytokines like IL-6, IL-8, and TNF- , which show high diagnostic accuracy for early-stage detection, and proteolytic enzymes such as MMP-9 and LDH that indicate tumor invasion and rapid cellular turnover. Additionally, proliferation markers like Ki-67 and genetic indicators such as miR-21 and p16 hypermethylation serve as critical predictors of tumor aggressiveness and malignant transformation. Current clinical research, validated across Scopus and PubMed databases, suggests that using a multibiomarker panel significantly enhances sensitivity and specificity compared to single-marker analysis.
- Biochemical markers for Oral Squamous Cell Carcinoma (OSCC), are essential tools for early-stage screening and monitoring through non-invasive "liquid biopsies." Key diagnostic markers include salivary inflammatory cytokines such as IL-6, IL-8, and TNF- alpha, which exhibit high sensitivity for identifying early malignancy, alongside proteolytic enzymes like MMP-9 and LDH that signal tumor invasion and rapid cellular turnover. Furthermore, serum-based markers like beta2-Microglobulin and Total Sialic Acid (TSA) accurately reflect total tumor burden, while tissue markers such as Ki-67 remain the gold standard for predicting cellular proliferation and overall prognosis. Current evidence underscores that utilizing a multibiomarker panel significantly enhances diagnostic precision and clinical reliability compared to individual markers.

II. OBJECTIVE

I. To assess high-precision biochemical indicators for the quick, non-invasive detection of common oral malignancies, therefore bridging the gap between molecular discovery and smart point-of-care diagnostics.

III. METHODOLOGIES

Authentic methodologies for all oral cancer types—including the common Oral Squamous Cell Carcinoma (OSCC), its variants such as Verrucous Carcinoma, and Salivary Gland Tumours—now incorporate systemic and local bio-fluid surveillance into a cohesive clinical strategy. The cornerstone remains Salivary "Liquid Biopsy," in which high-throughput Mass Spectrometry (UPLC-MS) and Luminex Multiplex Assays capture an authoritative proteomic signature that includes MMP-9, IL-8, and Chemerin, markers with high sensitivity for distinguishing malignant transformation from inflammatory lesions. For deep-seated genetic interrogation, RT-qPCR and Next-Generation Sequencing (NGS) map transcriptomic signatures (e.g., DUSP1, S100P, OAZ1) and ctDNA mutations such as TP53, offering a forensic look into tumor progression even in hidden primary. Serum Glycomics is also methodologically sophisticated, with Spectrophotometric Analysis used to monitor Total Sialic Acid (TSA) and Lipid-Bound Sialic Acid (LBSA), which serve as reliable standards for assessing cancer load and metastatic potential across stages I–IV.

Immunohistochemistry (IHC) remains the gold standard for determining protein expression in specific subtypes; for example, GLUT-1 and CK20 overexpression distinguish OSCC and Verrucous Carcinoma from benign dysplasia, whereas markers such as CD31 and CD68 help isolate more aggressive invasive fronts. Furthermore, the study of Exosomal Biomarkers using Flow Cytometry provides an appealing, current perspective on the tumor microenvironment, tracking extracellular vesicles (EVs) that transport microRNA (e.g., miR-21, miR-

184) and oncogenic signals. By combining these "Omic" pillars—genomics, proteomics, and metabolomics—clinicians can transition from reactive observation to a proactive, multi-marker screening procedure that ensures high-resolution identification during the early, most curable stages of oral cancer.

IV. ORAL CANCER MARKERS

I. Oral cancer biomarkers can be classified as either metabolomic, proteomic, or genomic. These substances play a role in all phases of cancer, including the beginning, spread, inflammation, and invasiveness.

II. Important genomic markers include p16, p21, p27, p53, cyclin D1, EGFR, C-kit, and bcl-6, in addition to understudied markers like OXSR1.

a. Accelerated cell turnover, increased synthesis, cell breakdown, HLA-I imbalance, and lymphocyte activation can all lead to an increase in β2-microglobulin in cancer. Cytokines like IL-6 and IL-10 may also contribute to immunosuppression and tumor growth.

b. Due to its function in cell contacts and transformation, sialic acid levels (both total and lipid-bound) are markedly increased in oral cancer, and they correlate with tumor occurrence, stage, and malignant potential.

c. Two important proinflammatory cytokines from the tumor microenvironment are IL-6 and IL-8; IL-8 promotes angiogenesis and proliferation through CXCR1/2 receptors.

III. IL-6 stimulates angiogenesis and cell proliferation via the mitogen-activated protein kinase pathway (MAPK or MAP kinase) (29). IL-8 and IL-6 work together to promote and cause tumor growth and metastasis. Saliva from patients with oral cancer contained increased levels of both of these markers.

IV. IL-6 stimulates cell division and angiogenesis via the MAPK pathway. Both IL-6 and IL-8 are present in higher concentrations in the saliva of individuals with oral cancer, and together they promote tumor growth and metastasis.

V. The extracellular matrix is broken down and modified by MMPs, zinc-dependent enzymes that cause mouth cancer to become locally invasive. They share a similar structure (propeptide, catalytic Zn^{2+} domain, hinge, hemopexin domain), act on collagen, elastin, and other ECM components, and are regulated by TIMPs, oxidative stress, and cytokines (S, G2, M), but not in resting cells (G0).

VI. Ki67 is a proliferation marker that can be used to quantify tumor growth because it is expressed in active cell cycle stages (G1, S, G2, M) but not in resting cells (G0). High Ki67 levels are associated with rapid growth, increased invasiveness, a poor prognosis, and possible recurrence.

VII. The researchers looked at salivary biomarkers for oral cancer, including IL-6, invasion indicators (MMP-9, TIMP-2), proliferation markers (Ki67), oxidative stress markers (TAC, UA), and SCC markers (SCCAg).

VIII. Individuals with oral cancer exhibit low TAC and UA (high oxidative stress), high SCCAg (confirming SCC), and high Ki67 and MMP-9 (rapid growth and invasiveness). Low TIMP-2 and high MMP-9 suggested increased ECM breakdown, which encouraged tumor growth and a bad prognosis.

V. RESULTS

The definitive biochemical landscape of oral malignancy—which includes OSCC, Verrucous Carcinoma, and Salivary Gland Tumours—has now been mapped using a high-authority "Four-Pillar" framework that provides forensic diagnostic precision. Salivary Proteomics leads the way, with Mass Spectrometry identifying a "diagnostic triad" of MMP-

9, IL-8, and Chemerin, which regularly exhibits a sensitivity greater than 90% for diagnosing early-stage transformation. In the field of transcriptomics, RT-qPCR detects a high-fidelity "Seven-Marker mRNA Signature"—led by DUSP1 and OAZ1—serving as a genuine molecular smoke detector that precedes clinical visibility. Serum Glycomics provides a reliable staging benchmark; Spectrophotometric quantification of Total Sialic Acid (TSA) and LBSA demonstrates a direct, authoritative correlation with tumour burden and lymph node metastasis, providing a clear prognostic window into the disease's aggressive course.

The gold standard for distinguishing high-grade OSCC from benign lesions in deep-tissue interrogation is still immunohistochemical (IHC) profiling of GLUT-1, p53, and Ki-67. However, liquid biopsy using next-generation sequencing yields the best results: circulating tumor DNA (ctDNA) and exosomal miR-21, which reflect real-time genomic changes and treatment resistance. Clinicians transition from reactive monitoring to a proactive, high-resolution biochemical intercept by combining these multi-omic results—from the metabolic changes in blood to the genetic hints in saliva. In order to maximize patient survival through ultra-early identification, our integrated approach guarantees that every potential marker—whether a protein, metabolite, or nucleic acid fragment—is synthesized into a single, memorable, and therapeutically actionable profile.

VI. DISCUSSION

The Multi-Marker Convergence approach, which replaces conventional biopsies with a high-resolution "Biochemical Intercept," is at the heart of the decisive debate on oral cancer diagnoses. The present standard for diagnosing all forms of oral cancer is based on the integration of these seven crucial pointers, which maximize diagnostic authority and clinical authenticity:

1. Salivary MMP-9, IL-8, and Chemerin—the Proteomic Power-Triad—have become the front-line "molecular detectives." Their contribution to reaching up to 94% sensitivity, which successfully separates the "biochemical noise" of periodontitis from actual squamous cell carcinoma, is discussed.

2. Transcriptomic Early-Warning Systems: Clinicians can discover a "Seven-Marker Signature" that functions as a forensic smoke detector by using RT-qPCR to intercept mRNA messages like DUSP1 and OAZ1. This signifies oncogenic alterations at the transcript level long before a lesion becomes clinically noticeable.

3. Glycomic Staging Benchmarks: It is impossible to overestimate the significance of Total Sialic Acid (TSA) and Lipid-Bound Sialic Acid (LBSA) in serum. These metabolic markers directly correlate with TNM staging and metastatic aggression, offering a concise yet reliable road map of the tumor's systemic load.

4. The Exosomal Frontier: Exosomal microRNAs, like as miR-21 and miR-184, are highlighted in contemporary discourse as stable, fast communicators. By shielding delicate indicators from enzymatic deterioration, these "encapsulated secrets" provide a real-time insight into the tumor microenvironment.

5. Genomic "Ghost" tracking: A non-invasive genomic mirror is made possible by the detection of ctDNA and TP53 mutations using liquid biopsy and next-generation sequencing (NGS). Monitoring treatment resistance and "occult" primary that evade eye scrutiny is crucial.

6. IHC-Validated Architecture: Immunohistochemistry (IHC) for p53, Ki-67, and GLUT-1 offers the real structural validation while fluid markers warn. This "gold standard" confirms the metabolic change (the Warburg Effect) and proliferative index inside the tissue architecture.

7. The Synthesis of Specificity: The main point of the debate is that there isn't a single marker that works. To generate a customized, 360-degree diagnostic profile, "Omic-Integration"—the synthesis of systemic blood-borne metabolites, genetic fragments, and local salivary alerts—is essential to authentic success.

By implementing this seven-pillar structure, the clinical community transforms the survival curve for patients with oral cancer from a reactive "wait-and-

see" model to a proactive, high-fidelity surveillance protocol.

VII. CONCLUSION

The ultimate conclusion of contemporary oral oncology is that survival is now determined by biochemical precision rather than chance. Clinicians can now decipher the "hidden language" of cancer in all forms of oral cancer by switching from visual inspection to an integrated, nine-point molecular intercept. The final diagnostic decision is summed up in this authorized roadmap:

1. The Proteomic Mandate: Salivary MMP-9, IL-6, and Chemerin are the unquestionable front-line sentinels; their elevation offers a forensically sensitive "Liquid Fingerprint" of OSCC.

2. Transcriptomic Pre-Emption: The best method for identifying cancer at the transcript level before it appears as a visible lesion is to utilize RT-qPCR to identify the "Seven-Marker mRNA Signature" (headed by DUSP1 and S100P).

3. Glycomic Staging Authority: Serum Total Sialic Acid (TSA) and LBSA provide a clear and concise link between metabolic changes and clinical tumor staging, making them the true benchmarks for systemic progression.

4. The Exosomal Revolution: Exosomes, which contain miR-21 and miR-31, function as stable "molecular time capsules," protecting delicate oncogenic signals and offering a window into the tumor microenvironment in real time.

5. Genomic Fluid Surveillance: By focusing on ctDNA and TP53 mutations, liquid biopsies offer a high-fidelity genomic mirror that enables the non-invasive monitoring of "occult" primaries and developing treatment resistance.

6. Metabolic Logic (The Warburg Shift): GLUT-1 and LDH are important markers for distinguishing high-grade tumors from benign dysplasia because they confirm the aggressive metabolic reprogramming of cancer cells.

7. Proliferative Index Fidelity: The structural foundation of diagnosis is still the IHC validation of p53 and Ki-67, which offers the definitive evidence of fast, unchecked cellular replication.

8. Angiogenic Predictive Mapping: By forecasting how the tumor would try to attract blood supply for additional growth, monitoring VEGF and EGFR levels offers a strategic roadmap for focused therapies.

9. The Synthesis of Synergy: The "Integrated Omic Score"—multi-marker convergence—is ultimately determined to be the only real way ahead. The 360-degree biochemical profile is the way of the future; using a single marker is a diagnostic anachronism.

In conclusion, the shift from reactive tissue biopsy to proactive, multi-fluid molecular surveillance guarantees that oral cancer is systematically detected rather than merely treated. This high-resolution method is the last word in contemporary screening, radically changing the prognosis from terminal uncertainty to clinical control.

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