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Salivary Metabolomics for Oral Precancerous Lesions: A Comprehensive Narrative Review

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Abstract

Oral submucous fibrosis (OSMF) is a chronic, potentially malignant disorder of the oral cavity, primarily associated with the consumption of areca nut products and other risk factors. Early and accurate diagnosis of OSMF is crucial to prevent its progression to oral cancer. In recent years, the field of metabolomics has gained momentum as a promising approach for disease detection and monitoring. Salivary metabolomics, a non-invasive and easily accessible diagnostic tool, has shown potential in identifying biomarkers associated with various oral diseases, including OSMF.

This review synthesizes current literature on the application of salivary metabolomics in the context of OSMF detection. The review encompasses a comprehensive analysis of studies conducted over the past decade, highlighting advancements in analytical techniques, metabolomic profiling, and identified biomarkers linked to OSMF progression. The primary objective of this review is to provide a critical assessment of the feasibility and reliability of salivary metabolomics as a diagnostic tool for OSMF, along with its potential to differentiate OSMF from other oral disorders.

In conclusion, salivary metabolomics holds great promise in revolutionizing OSMF detection through the identification of reliable biomarkers and the development of robust diagnostic models. However, challenges such as sample variability, validation of biomarkers, and standardization need to be addressed before its widespread clinical implementation. This review contributes to a comprehensive understanding of the current status, challenges, and future directions of salivary metabolomics in the realm of OSMF detection, emphasizing its potential impact on early intervention and improved patient outcomes.

Keywords: Oral Submucous Fibrosis, Salivary Metabolomics, Biomarkers, Metabolomic Profiling

1.Introduction

Oral cancer is the eleventh most common cancer worldwide & tobacco use is estimated to account for about 41% of oral cancer cases in men & 11% in women[1]. In India oral cancer accounts for almost 40-50% of the total diagnosed cancer cases and is one of the highest in the world[2,3,4]. In NE regions of India, the incidence of all sites of cancers in general and tobacco-related cancers in particular oral cancer has been reported to be very high.[5] Cancer is Latinized from the Greek word 'Karakinos', meaning crab, denoting how carcinoma extends

its claws like a crab into the adjacent tissues. The global burden of diseases is increased by the high rate of incidence and prevalence of cancer. Tobacco initially caused some precancerous lesions & conditions which is an intermediate clinical state with increased cancer risk. Leucoplakia, Erythroplakia, Oral Submucous Fibrosis, and Lichen Planus fall into this category

Oral squamous cell carcinoma (OSCC) (commonly termed oral cancer) is a type of cancer in the head and neck.[6 7] Cancers in the oral cavity, such as cancers of the lip, tongue, and mouth (WHO, ICD 10 classification: C 01–06) account for 48% of all head and neck cancers. Out of all these 90% are epithelial cell carcinomas.[8] The other oral sites of cancer include cheek linings, gingiva (gums), and palate (roof of the mouth). Oral cancer is a serious public health problem, with over 200,000 new cases reported annually worldwide, two-thirds of which occur in developing countries. The overall mortality rate for intra-oral cancer remains high at approximately 50%, even with modern medical services, probably due to the advanced stage of the disease at presentation.

In India oral cancer in men is the most common and fatal cancer.[9] It is the third most common cancer among Indian women.[10] The survival rate of patients with such cancers is 80% at 5 years when detected at early stages, 40% when regional spread, and less than 20% when there is metastasis.[11] Etiological studies show that the use of tobacco in any form, betel quid chewing, drinking of alcohol, and human papilloma virus (HPV) infection are the major causes of developing oral cancer.[12-16]. The potential oral premalignant or precancerous lesion is a morphologically altered condition that has a high risk of transforming into a malignant or cancerous form. The transformation occurs due to clinical, biochemical, and molecular alterations. [17,18] The most common oral premalignant lesions can be classified as (a) leukoplakia, (b) erythroplakia, (c) Oral Lichen Planus (OLP), and (d) oral submucous fibrosis (OSF). Lesions of long duration have a greater risk of malignant transformation. However, no premalignancy is guaranteed to eventually transform into cancer.[19]

Oral pre-malignant lesions

Leucoplakia clinically appears as a white patch or plaque in the buccal mucosa, gingiva, or floor of the mouth. About 20-50 % of leucoplakia biopsies may exhibit dysplasia and 40% of dysplastic changes were reported in the lesions located at the mouth floor.[18 20] Oral leukoplakia(OLP) has a malignant potential of 30%, with a transformation rate ranging from 0.3 to 17.5%.[21] Carcinomas arise from leucoplakia usually within 2-4 years. The average annual incidence of leucoplakia in Kerala is devastating (17 people per thousand)[22]. Erythroplakia at the oral mucosa appear as bright red, velvety patches or plaques. Though this type of lesion can appear anywhere in the oral cavity, major locations are the mouth floor, soft palate, ventral tongue, and tonsillar faucets. The prevalence of malignant transformation of this type of lesion ranges from 20- 68%.[23] OLP lesions clinically appear as white papules or white plaques generally with painful blisters. The presence of fine wavy keratotic lines was accepted as a sign of lichen planus. Major locations for OLP are buccal mucosa, tongue, and gingiva. About 2-8% of erosive OLP lesions are reported to transform into oral cancer.[24] The malignant potential of OLP is an ongoing controversial matter [25]. Determining its potential for malignant transformation is complicated by difficulties in diagnosis, differentiation from oral lichenoid lesions (OLLs), and the phenomenon of premalignant lesions exhibiting lichenoid characteristics

During oral submucous fibrosis, the mouth appears rigid and becomes difficult to open (i,e trismus). The most affected site is the buccal mucosa. Other parts of the oral cavity and pharynx may also get involved in the formation of this potential premalignant condition. Additional clinical features include inflammation and progressive fibrosis of the submucosal tissue.[26] Intolerance to spicy food and oral ulceration are common features of OSF.

Diagnosis of Oral pre-malignant lesions

Usually, an oral examination under white light illumination followed by palpation is conducted for cancer screening. In such procedures, the experience of the examiner has a pivotal role, especially in early lesion development.[27] Surgical biopsy and histopathology are the diagnostic gold standard for a suspicious lesion. Biopsy together with the needed aftercare cannot be carried out easily for every patient. The patient is often

reluctant and at times fearful of such an invasive procedure. Additionally, the invasive procedure could cause wound-healing difficulties or aggravate the scarring in trismus patients. Hence, there is an urgent need to devise diagnostic tools that are practical, simple, non-invasive, inexpensive, painless, and can be easily performed in an outpatient set-up. [28, 29]

Various screening aids have been developed to improve and facilitate clinical examination for discriminating and identifying precancerous and cancerous oral lesions at the earliest stage. There are salivary proteomics, brush biopsy, and cytology with special adjuncts, such as DNA-image cytometry or evaluation of mRNA markers, immune cytology, and gene expression analysis as well as evaluating molecular markers.[30-35] Other optical-based adjuncts for better visualization of potentially dysplastic changes of the oral mucosa include iodine and toluidine blue staining as well as autofluorescence and spectroscopy.[36-39]

Optical coherence tomography (OCT) was first reported by Fujimoto et al. in 1991. OCT has been widely used in numerous clinical applications, including gastroenterology, ophthalmology, dermatology, and dentistry. OCT is a non-invasive, non-radiative optical diagnostic tool based on interferometers. Optical coherence tomography (OCT) has been proven to be a useful technique for oral disease diagnosis.[41].

Microfluidics is by definition suited for handling living cells (whose typical diameter is a few micrometers) in a three-dimensional, biologically relevant environment. This microfluidic chip accepts saliva samples, can be operated by minimally trained personnel, and can provide a diagnostic answer in an automated and timely fashion. The detection of oral pre-cancer (dysplastic) and cancer cells within the chip will take advantage of membrane-associated cell proteins that are singularly expressed on cell cancer cells. The measured profile is compared with archived gene transcription profiles to determine cancer type and stage.[42] In the 21st century, while blood remains the primary laboratory diagnostic specimen, it is no longer the sole one. Other biological fluids, such as saliva, have gained significant diagnostic importance. Saliva offers numerous collection advantages over blood due to its non-invasive nature, ease of sampling, and quick retrieval. This makes it an ideal diagnostic fluid, particularly in challenging cases involving children and the elderly, who might not readily cooperate. However, there are notable drawbacks associated with saliva samples. These include variations in marker levels between saliva and serum, potential alterations in salivary composition due to collection methods, and salivary flow dynamics. These factors gain significance in systemic conditions like Sjogren's syndrome or Cystic fibrosis, where the presence of enzymes can influence the concentration of specific diagnostic markers.

We find ourselves amidst the "emerging era of highly integrated precision diagnostics. where salivary diagnostics is a continually advancing field. It holds a pivotal role in diagnostics and aids clinicians in making informed treatment decisions. Whole saliva, a complex biofluid derived from both major and minor salivary glands, as well as sources like gingival crevicular fluid (GCF), expelled bronchial and nasal secretions, microorganisms such as bacteria, viruses, fungi, their by-products, and shed epithelial cells, constitutes a diverse array of cellular components. This renders saliva a rich reservoir of biomarkers, many of which are shared with blood, including hormones, antibodies, growth factors, enzymes, and microbial agents along with their products. **Figure 1**

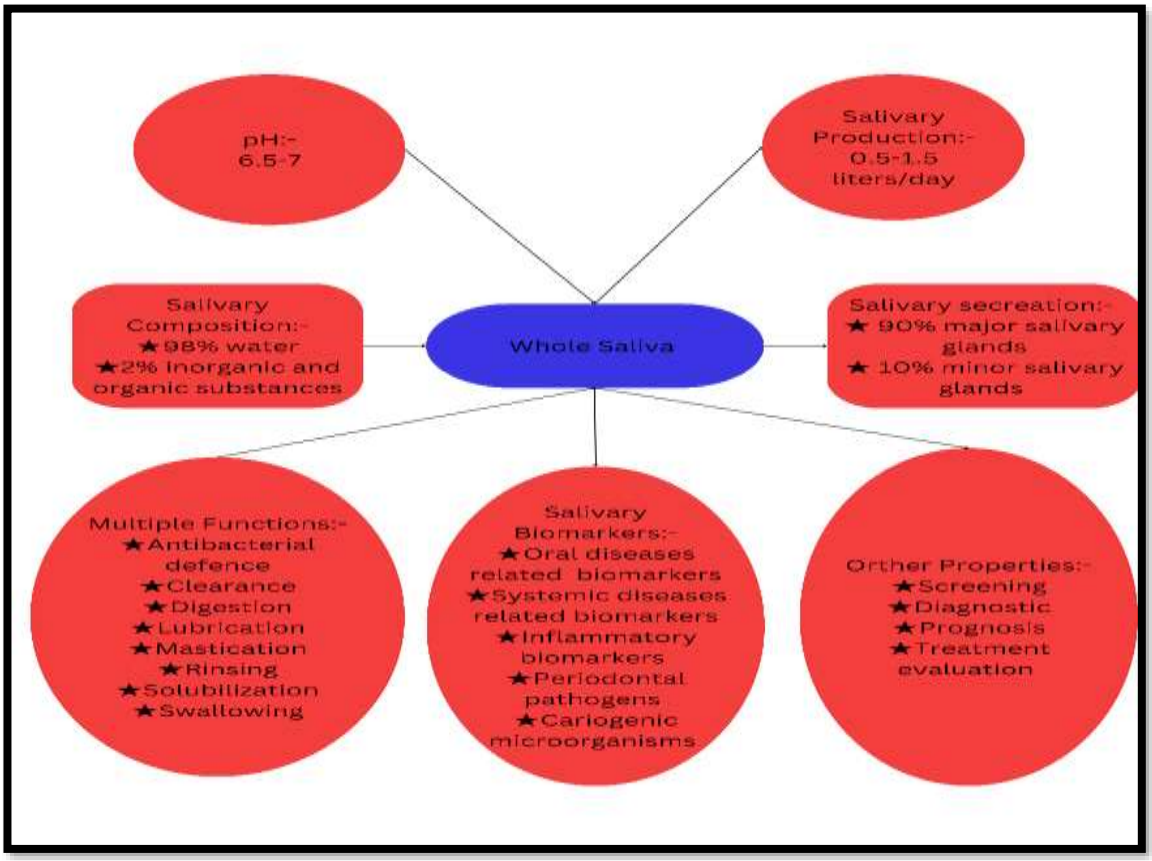

Figure 1: Properties of Saliva and their potency in the diagnosis of disease

Diagnostic Modalities for Early Detection of Oral Cancer

a) Vital tissue staining by Toluidine blue

Toluidine blue (tolonium chloride) is a metachromatic dye soluble in water and alcohol. It binds with DNA and is used as a vital stain. As malignant or dysplastic cells contain more amount of DNA than normal cells, this dye helps to distinguish the positive mucosal changes with dark blue staining. Though this test is highly sensitive (93.5 - 97.8 %) the limitation is its lower specificity (73.3- 92.9 %). The less specific nature gives rise to false positive results.[44] Toluidine blue has not been recommended as an adjunct in oral cancer screening in primary care but in high-risk populations only.[45]

b) Lugol's iodine staining

Italian Camillo Golgi introduced this stain. Lugol's Iodine solution is formed by two grams of iodine and four grams of potassium iodide in 100 cc of distilled water. After observation of the clinical features, 1% acetic acid is applied to the lesion for 20 Sec and then rinsed with water. Later, another photograph is taken following the application of Lugol's iodine at the lesion with a cotton bud for 10-20s.
Lesions showing brown stain are considered as positive while lesions without any retention of stain are considered as negative. The glycogen content present in the normal epithelium forms the basis of selective staining of the intact mucosa with Lugol's iodine. This selective staining helps in differentiating the inflammatory or carcinomatous epithelium from the normal epithelium where the glycogen content is low.

Staining with TB along with Lugol's iodine (Double staining technique) helps in differentiating the inflammatory lesions. This helps in the clinical determination of the degrees of differentiation of malignant lesions because poorly differentiated malignant lesions without glycogen content do not show Lugol's iodine retention. Pre-therapeutic assessment of the biological aggressiveness of the disease can also be made by this double staining technique. Depending on the retention of the dyes, the biopsy site can be determined. Double

staining technique is also used for high-risk patients and selecting biopsy sites for patients with wide-field cancers.

Brush biopsy or oral CDx test

In the brush biopsy procedure cells are collected from the oral epithelium, fixed with ethyl alcohol on a glass slide, stained with a modified "Papanicolaou test" and finally observed under the microscope. The test is easy to perform and painless. The "oral CDx test" is a commercial name after OraCDx Laboratories, Inc. Suffern, NY. This test has been reported to have well over 90% of sensitivity and specificity.[46] The test is effective for the diagnosis of already visible lesions, but its limitation is in detecting mucosal changes that are not easily visible with the naked eye.[47]

d) Exfoliative cytology

This is a simple, non-invasive, inexpensive technique for identifying biomarkers feasible to implement in a common laboratory and well accepted by patients.[48]. The Papanicolau (PAP) stain is considered the universal stain for exfoliative cytology that enables normal and pathological cytological features to be established in cytological smears. AgNOR staining of the nucleolus organizer regions (NORs) with silver salt (Ag), is also used to determine malignant changes in exfoliative cells. The number of AgNORs per nucleus correlates with the ribosomal RNA transcription rate, cell proliferation, and DNA ploidy. The greater the number of NORs, the higher the rate of replication of the ribosomes and cells.[49] AgNOR analysis is believed to be useful as a quantitative marker of incipient cellular alterations even before changes are identified histologically. The total number of AgNORs per nucleus could be a reliable marker for detecting cells with neoplastic characteristics; this method increases the sensitivity and specificity for distinguishing benign from malignant cells, decreasing the likelihood of false negatives or positives. Most importantly, AgNOR staining is a low-cost methodology and simple to perform.
Some studies suggest combining different methods for greater sensitivity in detecting incipient malignant changes and for application in prevention programs.[50]

e) Chemiluminescence

The ViziLite® system (VL; Zila Pharmaceuticals, Phoenix, AZ, USA) is a commercially available chemiluminescent device for oral cancer screening. Suspicious lesions appear distinctly white in this system. To reduce a high number of false positive cases, the manufacturer included toluidine blue (TB) (VLP; ViziLite Plus®) to the system. TB selectively binds to acidic tissue components of DNA and RNA and has shown to be retained in tissues with rapid cell proliferation[6,7].

f) Tissue fluorescence imaging (VELscope system)

Fluorescence spectroscopy is hoped to bridge the gap between clinical examination and invasive biopsy. Autofluorescence (AF) of tissues is produced by fluorophores occurring naturally in living cells on excitation with a suitable wavelength. The disease is indicated by changes in the concentration of the fluorophores as well as the light scattering and absorption properties of the tissues.[51]

Due to collagen matrix and elastin composition breakdown increased absorption and scattering of light reduces the autofluorescence signal. As a result, with intense blue light excitation (400-600 nm) dysplastic and malignant lesions will appear darker and normal mucosa will appear as pale green.[53] VELscope is useful in confirming the presence of oral leucoplakia, Erythroplakia and other oral mucosal disorders, but it is unable to discriminate high-risk from low-risk lesions.[38]

In this procedure, the patient's suspected oral lesions are rinsed with 1 % acetic acid solution for 30–60 seconds. This putatively removes debris and the surface glycoprotein barrier to allow penetration of the light. Under dimmed room light, the visually identified lesion is examined with a chemiluminescent system. Under this illumination (wavelength ranging from 430 to 580 nm) normal mucosa appears to be less blue or dark (negative

finding), while abnormal mucosa appears improved “aceto-white” (positive finding). After another swabbing with 1 % acetic solution, pharmaceutical-grade TB is applied to the lesion with a pre-soaked swab. Excess TB is removed with a 1 % acetic acid swab. Retention of TB is analysed for each lesion under standard incandescent light and data is recorded. However, this method is not completely reliable until additional definitive studies are performed. The VLP ancillary tool may help non-experienced clinicians to enhance their general awareness of screening for oral cancer.

5. Molecular Markers for Diagnosis of Oral Cancer

Saliva is used as a diagnostic fluid due to its non-invasive and inexpensive features. Developing knowledge in disease biomarkers can aid with high throughput assays like DNA microarray, mass spectrometry, and nanoscale sensors as a good diagnostic tool for premalignant oral lesion detection.[35] described DNA, RNA and protein as biomarkers present in saliva for early detection of oral cancer. Patients with oral cancer and oral potentially malignant lesions have been hypothesized to have elevated levels of specific chemokines in oral fluids that may be used as a marker for the early detection of malignant disease[56]. The chemokines are measured in saliva with robust methods including enzyme-linked immunosorbent assays (ELISA). Allelic loss on chromosome 9p, mitochondrial DNA mutation, p53 tumour-suppressor gene mutation, promoter hypermethylation, cyclin D1 amplification, presence of HPV etc. Presence of interleukin 8 (IL8), presence of IL1β, DUSP1 (Dual specificity protein phosphatase 1 plays an important role in the human cellular response to environmental stress and in the negative regulation of cellular proliferation**)**, H3F3A (a member of the histone H3 family; mutation of H3F3A are also linked to certain cancers), S100P (a member of S100 family of proteins which are involved in regulation of several cellular processes such as cell cycle progression and differentiation), etc. CD44, IL6, IL8, Defensin-1, SCC-Ag, serum tumour marker CA125, carcino antigen (CA19-9), carcinoembryonic antigen (CEA) etc. Further, the potential of S100A7 protein (also known as S100 calcium-binding protein A7 or psoriasin) overexpression has been hypothesized to serve as a biomarker for identifying dysplastic lesions at high risk of cancer development.[54]. Regular histopathological evaluation gives very limited information in terms of rate of proliferation, capacity for invasion and metastases, and development of resistance mechanisms to certain treatment agents. Biomarkers help in the early detection of cancer by providing valuable information about the status of a cell at any given point in time. As the cell transforms from non-diseased to neoplastic, distinct changes occur that could be potentially detected through the identification of the appropriate biomarkers. Proteomics is the study of expressed proteins, including the identification and elucidation of the structure–function relationship under normal or disease conditions, such as in cancer. It also provides an avenue to understand the interaction between the functional pathways of a cell and its microenvironment. Cancer proteomics encompasses the identification and quantitative analysis of differentially expressed proteins relative to healthy tissue counterparts at different stages of disease, from preneoplasia to neoplasia. Proteomic technologies can also be used to identify markers for cancer diagnosis, to monitor disease progression, and to identify therapeutic targets. It is valuable in the discovery of biomarkers because the proteome reflects both the intrinsic genetic program of the cell and the impact on its immediate environment of protein. At the protein level, distinct changes occur during the transformation of a healthy cell into a neoplastic cell, ranging from altered expression, differential protein modification, and changes in specific activity, to aberrant localization, all of which may affect cellular function. Identifying and understanding these changes are the underlying themes in cancer proteomics [55,56, 57]

The Human Salivary Metabolome and Oral Pathologies

The term "whole mouth saliva" (WMS) is employed in the field of oral sciences to describe the clear, transparent, watery fluid that results from the combination of parotid, submandibular, and sublingual saliva. It also contains contributions from minor salivary glands, gingival crevicular fluid, eukaryotic cells (including epithelial and leukocytic cells), food particles, microorganisms, and their byproducts. Primarily composed of water (constituting about 99% of its content), saliva is a predominantly aqueous solution. Additionally, it contains various constituents such as mucus, digestive enzymes, growth factors, cytokines, immunoglobulins, antibacterial peptides, salts, and low molecular weight metabolites[58]. The daily production of saliva in a healthy individual ranges from 0.75 to 1.5 litres, with higher output during waking hours. It also aids in oral digestion, safeguards the integrity of oral tissues and teeth, and provides defence against bacteria and viruses. These roles collectively contribute to oral homeostasis and overall quality of life [59 60].

Human Salivary Metabolome Research and Its Limitations

Studies frequently concentrate on metabolite profiling in blood and urine, but there is little research on metabolic profiling in saliva. Saliva is used as an insightful diagnostic biofluid because of the well-established positive correlation between salivary and plasma metabolite levels (such as glucose, lactate, and pyruvate), as well as the fact that the proteomic and metabolomic alterations seen in saliva follow a similar pattern to the changes seen in blood [61]. Saliva has many advantages over blood collection, including ad libitum production, non-invasiveness, painlessness, relatively quick and inexpensive collection, minimal collector training, reduced anxiety when compared to blood collection, and a more child-friendly approach. As a result, saliva is the ideal, most useful, and widely accessible biofluid.

The term "metabolomics" was formed by combining "omics" and "metabolites," suggesting an analytical approach for measuring all metabolites. Nonetheless, due to the considerable diversity in the chemical structures of metabolites within biological samples, it is not feasible to employ a single method for comprehensive analysis. Consequently, a range of techniques has been devised, each with its own strengths and weaknesses. Multiple systems for separating and detecting metabolites have been applied to the examination of metabolites within saliva samples [62]. Table 1

Table 1. Summary of salivary metabolomics for OC diagnosis studies

Sample	Platform	Cohort Group	Molecules	Reference
Saliva	RPLC-MS and HILIC-MS *	Oral Squamous Cell Carcinoma (OSCC)/ Control	Propionylcholine, N-Acetyl-L-Phenylalanine, Sphinganine, Phytosphingosine, and S-carboxymethyl-L-cysteine	Wang et.al, 2014
Saliva	CE-TOFMS*	Oral Squamous Cell Carcinoma (OSCC)/ Control	Choline, p-hydroxyphenyl acetic acid, 2-hydroxy-4-methylvaleric acid, valine, 3-phenylacetic acid, leucine etc.	Oshima et. al,2017
Saliva	GC-MS*	Oral Squamous Cell Carcinoma (OSCC)/ Control	Malic acid, Maltose, Protocatechuic Acid, lactose, 2-ketoadipic, and catechol	Alves et. al,2021
Saliva	CPSI-MS*	OSCC from HC PML from HC OSC from PML	Putrescine,Cadaverine, Thymidine,	Song et.al 2020
Saliva	UPLC-QTOFMS*	OSCC from HC	Alanine,Lactic acid , Valine,Isoleucine,Leucine, Proline	Wei et.al, 2010
Saliva	CE-TOFMS*	OC from OLP	Indole-3-acetate and Ethanolamine phosphate	Ishikawa et. al,2020

*Reversed Phase Liquid Chromatography, Hydrophilic interaction liquid chromatography, capillary electrophoresis mass spectrometry, Gas Chromatography Mass Spectrometry, Conductive polymer spray Ionization Mass spectrometric, ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry, capillary electrophoresis mass spectrometry

The process of human saliva metabolomic profiling involves the development and integration of numerous analytical technologies. Mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (HNMR) are the two most well-known metabolite measurement techniques. Nuclear magnetic resonance (NMR) stands out as the most employed technique, frequently utilized in metabolomics. In comparison to mass spectrometry (MS), NMR exhibits superior reproducibility and requires minimal sample preparation across various sample types. The straightforward pre-treatment of saliva, a notably viscous fluid, adds to its appeal. This characteristic confers a distinct advantage by minimizing the potential for unexpected errors. NMR has played a pivotal role in discerning alterations in salivary metabolomic profiles, essentially metabolic signatures, enabling

differentiation between individuals with cancer and healthy counterparts (HCs). NMR has found application in diverse areas of salivary metabolomics research, including the identification of oral squamous cell carcinoma (OSCC), head and neck squamous cell carcinoma, and glioblastoma. Expanding beyond cancer, NMR has also been employed in the analysis of conditions such as hepatitis B infection, Parkinson's disease, and Alzheimer's disease.

Mass spectrometry (MS) is a prominent seems for detecting metabolites, offering high sensitivity. It requires only a small sample volume and can simultaneously identify and quantify hundreds of metabolites. Nevertheless, when directly introduced into MS, metabolites with identical m/z (mass-to-charge ratio) values, like leucine and isoleucine, cannot be differentiated. Hence, a separation mechanism is usually employed before MS analysis. Gas chromatography-mass spectrometry (GC-MS) facilitates the quantification of volatile compounds and non-volatile metabolite profiling through derivatization. This technique was utilized for scrutinizing samples from individuals with oral squamous cell carcinoma (OSCC). Liquid chromatography-mass spectrometry (LC-MS) has been extensively employed for both untargeted and targeted assessments of salivary metabolites. In untargeted investigations, hydrophilic metabolites like γ-aminobutyric acid, phenylalanine, valine, and lactic acid in saliva samples from oral cancer (OC) patients were examined. Various metabolites such as oligopeptides, phosphatidylcholine, and glycerophospholipids were assessed in saliva samples from breast cancer patients. In targeted studies, specific salivary biomarkers for OSCC such as choline, betaine, pipecolinic acid, and carnitine were quantified. Additionally, well-known breast cancer biomarkers, salivary polyamines, were also investigated. Capillary electrophoresis-mass spectrometry (CE-MS) was employed to profile hydrophilic metabolites in saliva samples from OC, breast cancer, and pancreatic cancer (PC) cases. [56 57 63]

3. Conclusion

Before salivary metabolomics can find clinical application, several critical challenges need to be addressed. A fundamental step involves conducting a comprehensive validation study on a large scale to assess the accuracy and reliability of the identified biomarkers. Previous research in this field has primarily consisted of case-control studies, lacking broader validation. To establish the robustness of salivary metabolomics, a validation effort akin to the multi-institute evaluation conducted for an OSCC multigene expression test must be undertaken, spanning multiple countries. However, there are significant obstacles on this path. Achieving high reproducibility of metabolomic profiles across various human biofluid centres remains a formidable task. Similar to the recommended standardization of processes for blood and urine samples, a unified protocol is needed for handling saliva samples, encompassing aspects such as transfer and storage. Moreover, the real-world cohort exhibited a low prevalence of cancer cases, demanding extensive sample sizes to adequately cover OC patients. For successful validation, it's crucial to develop high-throughput, cost-effective assays that allow precise quantification of the identified biomarkers. A comprehensive evaluation of the holistic benefits of salivary-based screening, encompassing clinical and economic dimensions, is also imperative.

Future Prospects

In conclusion, the realm of salivary metabolomics holds significant promise as a burgeoning field of research. However, challenges abound in employing saliva metabolites as potential indicators of oral or systemic health issues, chiefly stemming from limited sample sizes in existing studies and the substantial hurdles involved in integrating these technologies into clinical diagnostics. To validate salivary biomarkers effectively, a shift from the "case-control study" approach to a "large-scale validation study" design is essential. Illuminating the underlying mechanisms of oral diseases could be achieved by concurrently exploring salivary metabolomics and microbiomics. The primary objective of profiling salivary metabolomics is not solely to contrast inflammatory statuses with those of healthy controls, but rather to differentiate between different types of inflammation. Salivary metabolomics has the potential to revolutionize our comprehension of pathogenesis, as well as to enhance the monitoring of diseases and the evaluation of treatment outcomes. Capturing the diverse panorama of an individual's saliva metabolome corresponds to the establishment of more personalized treatment strategies and follow-up protocols. In summation, the intricate nature of the oral cavity, influenced by numerous factors, underscores the complexity that may influence accurate research outcomes.

References

1. Stewart BW, Kleihus P.World Cancer Report.Lyon: WHO International Agency for Research on Cancer 2003;1-351.
2. Moore SR, Pierce AM, Wilson DF. 'Oral cancer'--the terminology dilemma. Oral Dis.2000, 6, 191-3.
3. Gupta PC, Ray CS. Smokeless tobacco and health in India and South Asia. Respiratory 2003, 8, 429-31.
4. Mehrotra R, Yadav S. Oral squamous cell carcinoma: etiology, pathogenesis and prognostic value of genomic alterations. Indian J Cancer 2006, 43, 60-6.
5. ICMR National Cancer Registry Programme – Consolidated Report of population-based cancer registries New Delhi, Dec 2006. 2001-2004. ICMR,
6. Epstein J B, Gorsky M, Cabay R J, Day T, Gonsalves W. (2008). Screening for and diagnosis of oral premalignant lesions and oropharyngeal squamous cell carcinoma: role of primary care physicians. Can Fam Physician. 6: 870-875.
7. Epstein J B, Silverman S Jr, Epstein J D, et al, (2008) Analysis of oral lesion biopsies identified and evaluated by visual examination, chemiluminescence and toluidine blue. Oral Oncol 44:538–544
8. Ram H, Sarkar J, Kumar H, et al (2011). Oral cancer: risk factors and molecular pathogenesis. J Maxillofac Oral Surg. 10: 132–137
9. Dikshit R, Gupta P C, Ramasundarahettige C, et al. (2012). Cancer mortality in India: A nationally representative survey. Lancet. 379:1807-16
10. Curado M P, Edwards B, Shin H R et al, (2007) eds. Cancer incidence in five countries, Vol. IX, IARC Scientific Publications No. 160, Lyon, IARC.
11. Diniz-Freitas M, García-García A, Crespo-Abelleira A, et al, (2004). Aplicaciones de la citología exfoliativa en el diagnóstico del cáncer oral. Med Oral. 9: 355–361.
12. Lee CH, Ko YC, Huang HL, Chao YY, et al. (2003). The precancer risk of betel quid chewing, tobacco use and alcohol consumption in oral leukoplakia and oral submucous fibrosis in southern Taiwan. Br J Cancer. 88:366-372
13. Sharma N, Fahmi N, (2014). Diagnostic Aids of Precancerous Oral Lesions. International journal of Scientific Study. 2: 77-80.
14. Steele T O, Meyers A (2011). Early detection of malignant lesions and oral cancer. Otolaryngol Clin North Am.44:221-229
15. Chin-Wan R C L, InoueL C-J, Kojun M S et al, (2005). J Hard Tissue Biol. 14: 291
16. Rousseau A, Badoual C. (2012). Head and Neck: Squamous cell carcinoma: an overview. Atlas of Genetics and Cytogenetics in Oncology and Haematology. 16: 145-155.
17. Guillaud M, Zhan, Po C, et al. (2008). Potential use of quantitative tissue phenotype to predict malignant risk for oral premalignant lesions. Cancer Res. 68: 3099–3107.
18. Ho P S, Chen P L, Warnakulasuriya S. et al. (2009). Malignant transformation of oral potentially malignant disorders in males: a retrospective cohort study. BMC Cancer. 9: 260–267.
19. Messadi D V. (2013). Diagnostic aids for detection of oral precancerous conditions. Int J Oral Sci. 5: 59-65.
20. Arduino P G, Surace A, Carbone M. et al. (2009). Outcome of oral dysplasia: a retrospective hospital-based study of 207 patients with a long follow-up. J Oral Pathol Med. 38: 540–544.
21. Scully C, Bagan J. (2009). Oral squamous cell carcinoma overview. Oral Oncol. 45: 301–308.
22. Rajendran R. (2004). Oral leukoplakia (leukokeratosis): Compilation of facts and figures. J Oral Maxillofac Pathol. 8, 58–68.
23. Villa A, Villa C, Abati S. (2011). Oral cancer and oral erythroplakia: an update and implication for clinicians
24. Sugerman P B, Savage N W, Walsh L J. et al. (2002). The pathogenesis of oral lichen planus. Crit Rev Oral Biol Med. 13: 350–365.
25. Barnes L, Eveson J W, Reichart P. et al. (2005). World health organization. Classification of tumours. Pathology and genetics of head and neck tumours. Lyon: IARC Press. 283–328.
26. Bhattacharyya, I., Chehal, H.K. (2011). White lesions. Otolaryngol Clin N Am. 44: 109–131.
27. Zadik Y, Orbach H, Panzok A, et al (2012) Evaluation of oral mucosal diseases: inter- and intra-observer analyses. J Oral Pathol Med 41:68–72
28. Gaikwad P, Hiremath S K, Singh S, (2013). Advancement in diagnostic aids for oral premalignant lesions: A review. J Dental Sci Oral Rehab. Jan-March: 11-15.

29. Sharma N, Fahmi N, (2014). Diagnostic Aids of Precancerous Oral Lesions. International journal of Scientific Study. 2: 77-80.
30. Koch F P, Kunkel M, Biesterfeld S, et al, (2011) Diagnostic efficiency of differentiating small cancerous and precancerous lesions using mucosal brush smears of the oral cavity—a prospective and blinded study. Clin Oral Investig 15:763–769
31. Macaulay C, Poh C F, Guillaud M, et al, (2012) High throughput image cytometry for detection of suspicious lesions in the oral cavity. J Biomed Opt 17:086004–086001
32. Toyoshima T, Koch F, Kaemmerer P, et al (2009) Expression of cytokeratin 17 mRNA in oral squamous cell carcinoma cells obtained by brush biopsy: preliminary results. J Oral Pathol Med 38:530–534
33. Driemel O, Dahse R, Hakim SG, et al, (2007) Laminin-5 immunocytochemistry: a new tool for identifying dysplastic cells in oral brush biopsies. Cytopathology 18:348–355.
34. Zhang L, Rosin MP (2001) Loss of heterozygosity: a potential tool in management of oral premalignant lesions? J Oral Pathol Med 30:513–520
35. Markopoulos A K, Michailidou E Z, Tzimagiorgis G. (2010). Salivary markers for oral cancer detection. Open Dent J. 4: 172-178
36. Watanabe A, Taniguchi M, Tsujie H, et al, (2012) Clinical impact of iodine staining for diagnosis of carcinoma in situ in the floor of mouth, and decision of adequate surgical margin. Auris Nasus Larynx 39:193–197
37. Cancela-Rodriguez P, Cerero-Lapiedra R, Esparza-Gomez G, et al, (2011) The use of toluidine blue in the detection of pre-malignant and malignant oral lesions. J Oral Pathol Med 40:300–304
38. Awan K H, Morgan P R, Warnakulasuriya S. (2011). Evaluation of an autofluorescence based imaging system (VELscope^{TM}) in the detection of oral potentially malignant disorders and benign keratosis. Oral Oncol. 47: 274–277.
39. Mehrotra R, Gupta D K. (2011). Exciting new advances in oral cancer diagnosis: avenues to early detection. Head Neck Oncol. 3: 1-8.
40. K M K Masthan1, N Aravindha Babu1, Kailash Chandra Dash1, M Elumala. (2012) Advanced Diagnostic Aids in Oral Cancer. Asian Pacific J Cancer Prev, 13, 3573-3576.
41. Chaudhary R, Shah A, Shah DM, Singh S, Thakkar P, Goyal S. Advanced Diagnostic Aids in Detection of Oral Cancer. Int J Dent Med Res 2014;1(3):139-143.
42. Nigam P, Prasad K, Tak J, Gupta V, Sinha A, Bali R, Grewal P. Advanced Diagnostic Aids in Early Detection of Oral Cancer. J Adv Med Dent Scie Res 2014; 2:39-43.
43. Shwetha K N, Vanishri H C, Dominic A, Sowmya SV, Roopa R S. Recent Advances in Diagnosis of Oral Cancer. Journal of Dental & Oro-facial Research Vol 12 Issue 1 Jan 2016.
44. Su W W, Yen A M, Chiu S Y. et al. (2010). A community-based RCT for oral cancer screening with toluidine blue. J Dent Res. 89: 933–937.
45. Patton L L, Epstein J B, Kerr A R (2008) Adjunctive techniques for oral cancer examination and lesion diagnosis: a systematic review of the literature. J Am Dent Assoc 139:896–905.
46. Scheifele C, Schmidt-Westhausen A M, Dietrich T, Reichart A (2004). The sensitivity and specificity of the oral CDx technique: evaluation of 103 cases. Oral Oncol. 40:824-8.
47. Rick G M. (2003). Oral brush biopsy: the problem of false positives. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 96: 252–258.
48. Acha A, Ruesga MT, Rodríguez MJ, et al, (2005). Applications of the oral scraped (exfoliative) cytology in oral cancer and precancer. Med Oral Patol Oral Cir Bucal. 10: 95–102.
49. Chattopadhyay A, Ray J G, Caplan D J (2002). AGNOR count as objective marker for dysplastic features in oral leukoplakia. J Oral Pathol Med. 31: 512–517.
50. Yamashina M, Sato K, Tonogi M, et al, (2014). Evaluation of superficial oral squamous cell malignancy based on morphometry and immunoexpression of cytokeratin 13 and cytokeratin 17. Acta Cytol. 58: 67–75.
51. Jayanthi J L, Mallia R J, Shiny S T, et al. (2009). Discriminant analysis of autofluorescence spectra for classification of oral lesions in vivo. Lasers Surg. Med. 41: 345–352.
52. Balevi B. (2007). Evidence-based decision making: should the general dentist adopt the use of the VELscope for routine screening for oral cancer? J Can Dent Assoc. 73: 603–606.
53. Schwarz RA, Gao W, Redden W C. et al (2009). Noninvasive evaluation of oral lesions using depth-sensitive optical spectroscopy: simple device for the direct visualization of oral-cavity tissue fluorescence.

Cancer. 115:1669–1679
54. Kaur J, Matta A, Kak I. (2014) S100A7 overexpression is a predictive marker for high risk of malignant transformation in oral dysplasia. International Journal of Cancer. 134:1379-1388.
55. Ardekani AM, Akhondi MM, Sadeghi MR. Application of Genomic and Proteomic Technologies to Early Detection of Cancer. Arch Iranian Med 2008; 11 (4): 427 – 34.
56. Rai V, Bose S, Mukherjee G, Shetty R, Perveen H, Mukherjee S, Das D, Chakraborty D, Sarkar R, Bhattacharjee D. (2023). Unraveling aberrant microenvironment in Oral Submucous Fibrosis. Journal of Population Therapeutics and Clinical Pharmacology, 30(17), 900–907.
57. Vertika Rai, Rashmi Mukherjee, Ananta K Ghosh, Aurobinda Routray, Chandan Chakraborty, "Omics" in oral cancer: New approaches for biomarker discovery, Archives of Oral Biology, 2018 87 15-34
58. Panneerselvam, K., Ishikawa, S., Krishnan, R., & Sugimoto, M. (2022). Salivary Metabolomics for Oral Cancer Detection: A Narrative Review. Metabolites, 12(5), 436.
59. Alapati, Susanth, et al. "Evaluation of Metabolomics as Diagnostic Targets in Oral Squamous Cell Carcinoma: A Systematic Review." Metabolites 13.8 (2023): 890.
60. Mali, Shrikant B. "Molecular screening of head neck cancer." Oral Oncology 144 (2023): 106481.
61. Grocholska, Paulina, Marta Kowalska, and Remigiusz Bąchor. "Qualitative and Quantitative Mass Spectrometry in Salivary Metabolomics and Proteomics." Metabolites 13.2 (2023): 155.
62. Rai, Vertika, et al. "Delineating metabolic dysfunction in cellular metabolism of oral submucous fibrosis using 1H nuclear magnetic resonance spectroscopy." Archives of oral biology 97 (2019): 102-108.
63. Bag, Swarnendu, et al. "Impact of spatial metabolomics on immune-microenvironment in oral cancer prognosis: a clinical report." Molecular and Cellular Biochemistry (2023): 1-9.
64. Wang, Q.; Gao, P.; Wang, X.; Duan, Y. The Early Diagnosis and Monitoring of Squamous Cell Carcinoma Via Saliva Metabolomics. Sci. Rep. 2014, 4, 6802
65. Ohshima, M.; Sugahara, K.; Kasahara, K.; Katakura, A. Metabolomic Analysis of the Saliva of Japanese Patients with Oral Squamous Cell Carcinoma. Oncol. Rep. 2017, 37, 2727–2734.
66. de Sá Alves, M.; de Sá Rodrigues, N.; Bandeira, C.M.; Chagas, J.F.S.; Pascoal, M.B.N.; Nepomuceno, G.; da Silva Martinho, H.; Alves, M.G.O.; Mendes, M.A.; Dias, M.; et al. Identification of Possible Salivary Metabolic Biomarkers and Altered Metabolic Pathways in South American Patients Diagnosed with Oral Squamous Cell Carcinoma. Metabolites 2021, 11, 650
67. Song, X.; Yang, X.; Narayanan, R.; Shankar, V.; Ethiraj, S.; Wang, X.; Duan, N.; Ni, Y.H.; Hu, Q.; Zare, R.N. Oral Squamous Cell Carcinoma Diagnosed from Saliva Metabolic Profiling. Proc. Natl. Acad. Sci. Usa 2020, 117, 16167–16173
68. Wei, J.; Xie, G.; Zhou, Z.; Shi, P.; Qiu, Y.; Zheng, X.; Chen, T.; Su, M.; Zhao, A.; Jia, W. Salivary Metabolite Signatures of Oral Cancer and Leukoplakia. Int. J. Cancer 2011, 129, 2207–2217
69. Ishikawa, S.; Sugimoto, M.; Edamatsu, K.; Sugano, A.; Kitabatake, K.; Iino, M. Discrimination of Oral Squamous Cell Carcinoma from Oral Lichen Planus by Salivary Metabolomics. Oral. Dis. 2020, 26, 35–42.