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Liquid Biopsy in Oral Cancer Diagnosis and Monitoring: A Narrative Review

Dr Charthad Snehal Bhanudas[1], Dr V Raj Kumar[2], Dr Kamble Saurabh Chandrakant[3], Dr Gaikwad Sweety Rajaram[1], Dr Rathod Vaibhav Sureshsingh[4]

[1](MDS Post Graduate Student, Department of oral and maxillofacial surgery, Jaipur Dental College, Jaipur, Rajasthan, India)

[2](Professor and Head, Department of oral and maxillofacial surgery, Jaipur Dental College, Jaipur, Rajasthan, India)

[3](Senior Lecturer, YMT Dental College and Hospital, Kharghar, Panvel, Maharashtra, India)

[4](Medical Officer, District Women Hospital, Akola, Maharashtra, India)

Abstract:
Liquid biopsy has emerged as a promising minimally invasive approach for the diagnosis and monitoring of oral cancer through the detection of tumour-derived biomarkers in biological fluids such as blood and saliva. Conventional tissue biopsy, although considered an essential diagnostic procedure, is invasive and may provide only a localized representation of the heterogeneous and dynamically evolving tumour. This narrative review discusses the principles, major biomarkers, clinical applications, advantages, limitations, and future perspectives of liquid biopsy in oral and maxillofacial oncology, with particular emphasis on oral squamous cell carcinoma. Circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), cell-free DNA (cfDNA), extracellular vesicles, exosomes, circulating proteins, and salivary biomarkers are reviewed for their potential roles in early detection, prognostic assessment, monitoring of treatment response, detection of recurrence, and identification of minimal residual disease. Liquid biopsy offers important advantages including minimal invasiveness, repeatability, and the potential for real-time assessment of tumour evolution and therapeutic response. However, challenges related to low biomarker concentrations, sensitivity and specificity, CTC isolation, pre-analytical variability, lack of standardized methodologies, cost, and clinical validation continue to restrict routine clinical implementation. Future integration of multi-analyte liquid biopsy platforms with artificial intelligence, multi-omics analysis, and advanced bioinformatics may improve diagnostic accuracy and support personalized cancer management. Overall, liquid biopsy represents a promising emerging approach that may complement conventional diagnostic methods and contribute to precision oncology in oral cancer.

Key Word*: Liquid biopsy, Oral cancer, Oral squamous cell carcinoma, Circulating tumour cells, Circulating tumour DNA, Exosomes.*

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I. Introduction

Generally speaking, cancer is described as a genetic illness caused by oncogenic mutations.[1] Tumor heterogeneity is now well understood thanks to recent technical developments in multi-omics platforms. Patients' cancer cells have quite different genetic backgrounds. Tumors' physiological states vary over time and are impacted by a number of variables, including as medication selection pressures, surrounding microenvironmental factors, and metabolic and homeostatic systems.[2] Patients with cancer experience treatment failure as a result of this intra-tumoral and interpatient heterogeneity.[1] As a result, accurately recording each person's tumor information is crucial for early detection, choosing treatment plans, forecasting the course of the illness, and predicting the efficacy of treatments. Traditional tissue samples have historically been used to diagnose and prognosticate certain cancers. Invasive and often uncomfortable for patients, tissue biopsies may not always adequately capture the heterogeneity of the tumor. Both of the accuracy of diagnosis and treatment planning may be impacted by these limitations.3 A state-of-the-art noninvasive diagnostic technique called liquid biopsy uses bodily liquids, including blood and saliva, to help detect, stage, and track cancers related to oral and maxillofacial surgery (OMFS).[3] By providing a real-time, minimally intrusive way to evaluate indicators produced from tumors that are present in bodily fluids like blood and saliva, a cutting-edge diagnostic technique known as liquid biopsy is transforming OMFS. Clinicians can get circulating biomarkers, such as circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and other molecular elements, using liquid biopsy.[4] CTCs have attracted a lot

of attention due to their dynamic representation of the heterogeneity of tumors and hematogenous dispersion since the 19th century, when they were discovered. In precision oncology, CTC-based liquid biopsy is a helpful technique. because it enables continuous, non-invasive monitoring of medication resistance, clonal selection, and tumor growth, in contrast to traditional tissue biopsies, which provide a static and constrained snapshot. The accuracy of CTC identification has increased recently thanks to advancements in methods including label-free detection, immunoaffinity enrichment, and microfluidic capture. CTC molecular research sheds light on how cancers develop, withstand therapy, and proliferate.[4] For patients with oral cancers like OSCC, these biomarkers provide vital information on the tumor's genetic and molecular profile, allowing for early identification, continuous monitoring, and customized care evaluation. Compared to traditional biopsies, this non-invasive method offers a number of benefits.[4] In certain situations, it also offers a more accessible and affordable option to tissue biopsy, which lessens the financial and practical costs of cancer diagnosis and follow-up therapy.[3] Liquid biopsy is a potentially ground-breaking method for enhancing oral cancer treatment outcomes in the setting of OMFS. The quality of cancer treatment at OMFS is expected to increase using liquid biopsy by facilitating more accurate diagnosis, improving the capacity to track tumor behaviors throughout time and providing useful information customize therapy for each patient.[2] Regulatory approvals, assay standardization, and pre-analytical variability remain major obstacles to Clinical application of liquid biopsy based on CTC despite these advancements.[5] Although CTCs continue to be Leading the way in liquid biopsy innovation, their diagnostic and prognostic utility is enhanced when combined with other circulating biomarkers including extracellular vehicles (EVs) and cell-free DNA (cfDNA).In addition to the functional and phenotypic information acquired from CTCs,[4] cfDNA analysis has shown significant promise in identifying genetic and epigenetic alterations unique to tumors and monitoring the least amount of residual disease.[6] Working together in multi-analyte liquid biopsy platforms, CTCs, cfDNA, and EVs have the potential to improve therapeutic decision-making, enhance cancer diagnostics, and spur the creation of individualized treatment plans..[4] An extensive review of liquid biopsy in maxillofacial and oral surgery is given in this article.

II. Material And Methods

A narrative overview of the research on circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and cell-free DNA (cfDNA) in oral and maxillofacial cancer was carried out. With an emphasis on recent developments, clinical utility, and present difficulties in CTC-based liquid biopsy, pertinent research addressing diagnostic, prognostic, monitoring, and precision oncology applications were reviewed.

III. The Principals and Techniques of Liquid Biopsy

By identifying molecular biomarkers that cancers release into physiological fluids, an innovative diagnostic method for oral and maxillofacial surgery (OMFS) is liquid biopsy.[3] Circulating tumor DNA (ctDNA), which is made up for obtaining DNA segments from cancer cells with mutations indicative of the genetic composition of the tumor; the primary tumor releases circulating tumor cells (CTCs) into the bloodstream, which may be a sign of metastatic activity; and microRNA (miRNA), which are tiny, non-coding RNAs having expression patterns that can be used to differentiate between benign and malignant tissues.[7] Biomarkers that may be examined without invasive tissue can be used to diagnose and treat OSCC, or oral squamous cell carcinoma, and other possibly cancerous oral diseases. Circulating extracellular vesicles (ctEVs), circulating tumor cells (CTCs), and circulating nucleic acids, including DNA and RNA, are the main elements of liquid biopsy and are crucial for revealing the molecular and genetic features of the tumor.[3] Clinicians can distinguish between diseased and non-cancerous tissues because to both genetic changes such as circulating tumor DNA (ctDNA)[8] mutations and epigenetic modifications unique to tumors, such as abnormal DNA methylation and promoter hypermethylation. Specialized biomarker panels that detect ctEVs, circulating RNA (ctRNA), DNA methylation patterns, and gene alterations have shown encouraging diagnostic and prognostic potential in OSCC. These molecular alterations are essential for early cancer identification.[9]

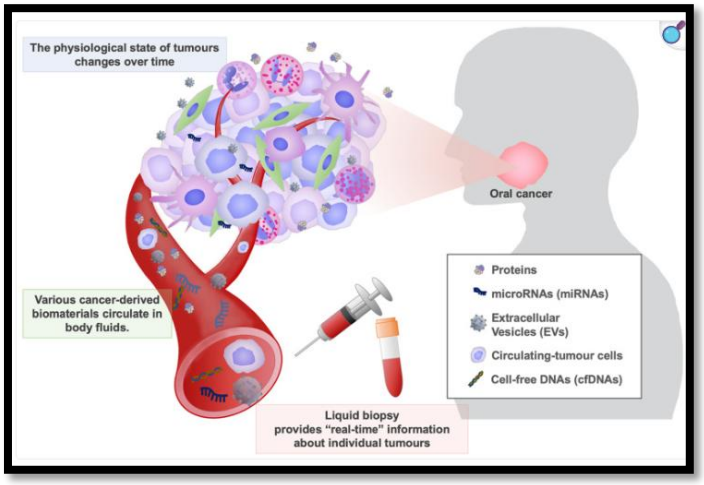

Figure 1: Liquid biopsy for oral cancer diagnosis.[2]

IV. Biomarkers

Table 1: Oral oncology biomarkers using liquid biopsies.[10]

Biomarker for Liquid Biopsy	An explanation	Clinical Utilization
Tumor cells in circulation (CTCs)	Tumor dynamics and metastatic potential are revealed by cancer cells released into the bloodstream.	Prognostic evaluation and early metastatic identification.
Nucleic acids without cells (cfNAs)	Tumor cells emit extracellular nucleic acids, such as microRNAs (miRNAs) and circulating tumor RNA (ctRNA).	Prognostic indicators that link patient outcomes with tumor aggressiveness.
Circulating tumor DNA (ctDNA)	Cancerous DNA fragments in the circulation that enable the non-invasive identification of genetic alterations.	Tracking the effectiveness of treatment and identifying any slight residual illness.
Exosomes	Tumor heterogeneity is reflected in small vesicles that cells release that include a range of biomolecules, such as proteins, DNA, and RNA.	Possibilities include early detection, tracking therapy response, and figuring out resistance mechanisms.
Tumor proteins in circulation	Tumor cells release proteins into the bloodstream, such as carcinoembryonic antigen (CEA) or cancer antigen 125 (CA-125), which are useful for diagnosis and prognosis.	Tracking the course of the illness, evaluating the effectiveness of treatment, and forecasting recurrence.

A. Circulating Tumor Cells (CTCs):

Among the mutational traits with tumoral clones discovered in the original tumor because they are cells released into the bloodstream by either distant metastatic lesions or the primary tumor.[11] Metastasis is the spread of original tumor cells by entering the circulation and moving to another location, where a fresh tumor develops.[12] They can move in bigger groups or on their own.[11] Most importantly, 90% of cancer patients die from it. Consequently, CTCs exhibiting a more robust epithelial character have been linked to a greater capacity for metastasis. Over the past few decades, a variety of antigen-dependent and antigen-independent methods have been created for CTC enrichment and detection.[11] Many Size, electrical charge, density, or deformability are used in CTC enrichment techniques.[13] These techniques, which collect CTCs according to their physical characteristics,

typically exhibit outstanding recovery effectiveness of above 80%. However, we must remember that not all CTCs such as tumor cells going through apoptosis or EMT are bigger than nucleated blood cells.[13]

Regardless of the isolation technique employed, it has been demonstrated that CTC measurement and genetic characterisation are helpful for prognosis, cancer recurrence detection, differential diagnosis, and real-time tumor development and treatment efficacy monitoring in many malignancies.[11] Gröbe et al.[14] used the CellSearch technology to examine CTC counting's predictive significance comparing their levels with clinicopathological, death, and recurrence in 110 patients with varying OSCC stages characteristics rates. They also looked at DTCs in the bone marrow of the iliac crest. Between 1 and 14 CTCs/7.5 mL, 12.5% of patients had CTCs and 20% had DTCs. Nodal status was significantly correlated with dispersed DTCs. and tumor size.

B. Circulating Cell-Free DNA (cfDNA):

All cell types, including tumor and nonmalignant host cells, release cfDNA into the bloodstream and other biofluids from apoptotic or necrotic cells.[11] The highly fragmented cfDNA distinguishes between long (up to 21 kb) and short (70–200 bp) double-stranded DNA segments. Urine, cerebrospinal fluid, blood, saliva, plasma, and other body fluids contain it.[15] It has been demonstrated that features such overall dimensions, location, vascularity, cellular turnover, stage, and therapy responsiveness be connected to levels of ctDNA.[15] Due to cfDNA contains specific abnormalities associated with cancers and metastases, such includes copy-number variations, methylation changes, and single-nucleotide mutations, and is simpler to analyze than other biomarkers, it is currently the subject of intensive research. Crucially, tumor cell clonality has been shown to be reflected in ctDNA and represents the tumor genome from original tumors and metastases.[11]

cfDNA analyzes have been used in OSCCs in a number of research to date. The amount of DNA in 390 patients' plasma (150 OSCCs, 150 OSCCs after therapy, and 90 potentially malignant lesions) and 150 healthy controls was measured using spectrophotometry by Shukla et al.[16], but no discernible variations were found between the groups. This observation may be explained by the mouth mucosa's strong lymphatic drainage, which keeps cfDNA out of the blood. OSCC may also benefit greatly from cfDNA-based HPV detection. Mazurek et al.[17] used q-PCR to examine EGFR, KRAS, and HPV16/18 mutations were used to assess 200 head and neck squamous cell carcinomas' cfDNA levels. Compared to patients with other squamous cell carcinomas of the head and neck, oropharyngeal squamous cell carcinoma revealed greater levels of total cfDNA. Higher levels of cfDNA were discovered in individuals with more advanced tumor stages and lymph node involvement. Although KRAS and somatic EGFR mutations were not found, 14% of all patients tested positive for HPV, the majority of whom were HPV16-positive (96.4%). These findings demonstrated the potential use of HPV cfDNA testing for the early detection and monitoring of head and neck squamous cell carcinomas that are positive for HPV. Among the 93 patients, 30 had primary malignancies of the head and neck that were demonstrated to be HPV16-positive, while none had HPV18. With 29 patients exhibiting HPV16, the anatomic preference was for the oropharynx. It is interesting to note that 86% of plasma samples had HPV compared to 40% of salivary samples when the two were compared. HPV was measured in both fluids using digital PCR.

Additionally, cfDNA-based microsatellite instability analyzes have demonstrated potential for OSCC monitoring and prognosis. Hamana et al.[18] looked in OSCC patients' blood and tissue at three distinct periods, nine microsatellite markers were found. Because allelic imbalance patterns in blood were linked to the presence of imbalance of alleles in corresponding tumor tissue, this study demonstrated that the cfDNA reflected the features of the tumor. Samples from both preoperative (44%) and postoperative (20%) showed serum allelic abnormalities. People four weeks with an allelic imbalance following surgery experienced metastases, whereas individuals without this imbalance did not experience a return of the illness. IFNA at the most frequently altered chromosomal, 9p21, was present about 40% of individuals with a plasma allelic imbalance.

Lastly, it is possible to precisely evaluate aberrant DNA methylation patterns in cfDNA. The most reliable methylation indicators in biofluids from various malignancies that are far from the oral cavity are SHOX2 and SPEPT9.[11] Both indicators were found in a large prospective cohort of 649 individuals with head and neck cancer have been demonstrated to be helpful for tumor identification; Methylation was detected in 59% of patients with 96% specificity. It takes multiplexed assays to evaluate many mutations at once and address the problem of low amounts of cfDNA found in certain tumors due to tumor heterogeneity and evolution.[11] Last but not least, several methods for identifying ctDNA have been discovered; however, a standardized method is still absent, this is necessary for its clinical application and the requirement to lower the cost of analysis.[11]

C. Circulating tumor DNA (ctDNA):

The broken DNA from tumor cells that is present in blood and saliva is known as circulating tumor DNA, or ctDNA. Because it is viable in physiological fluids for less than two hours and its levels rise as the cancer spreads, it could be a helpful biomarker for individuals with OSCC stands for oral squamous cell carcinoma. Consequently, it is a trustworthy indicator of how the tumor load varies in real time during cancer treatment.[20] There are HPV strains 16 and 18, TP53, CDKN2A, NRAS, NOTCH 1, PIK3CA, and HRAS. Furthermore,

salivary DNA hypermethylation of genes such HOXA9, KIF1A, and EDNRB can be used for early diagnosis and detection.[20]

For ctDNA analysis, two different methods have been taken into consideration: Targeted approaches, such as PCR-based techniques like BEAMing and droplet digital PCR focuses on particular gene mutations or rearrangements in particular chromosomal areas that act as "hotspots" for variation in a specific type of tumor. On the other hand, untargeted techniques enable a more comprehensive analysis and tracking of the tumor genome, including data on chromosomal changes, copy number abnormalities, nucleotide changes, etc., without requiring any prior understanding of molecular changes. Nevertheless, 21 PCR techniques can only identify known mutations in specific genes. The usefulness of ctDNA analysis as a general diagnostic technique will be limited since patients without certain mutations will be excluded. Conversely, NGS methods examine the whole sequences of the target genes, encompassing a greater variety of mutations.[21] In 2018, Gale D. et al. created the InVisionTM liquid biopsy technique to determine 35 cancer-associated genes with a low frequency of Clinically significant somatic alterations in ctDNA. Improved Tam-SeqTM (eTAm-SeqTM) technology is used in this amplicon-based next-generation sequencing.[22]

D. Exosomes:

Exosomes are tiny membrane vesicles with a lipid bilayer membrane that are between 40 and 150 nm in size. The enriched surface of exosomes contains Heat shock proteins, integrins, tetraspanins, fusion and transport proteins, and elements of the endosomal sorting complexes necessary for transport complexes.[11] The presence of exosomes in the tumor microenvironment has been demonstrated, highlighting their importance in carcinogenesis, tumor invasion, and metastasis, due to their potential to either stimulate tumor growth or possess anticancer qualities.[23] A range of bioactive compounds derived from parent cells, such as long noncoding RNAs (lncRNAs), proteins, lipids, mRNAs, microRNAs (miRNAs), and genomic DNA, are found in exosomes. cDNA, and mitochondrial DNA (mtDNA), based on the type of cell, physiological conditions, and biogenesis method.[11] Blood, urine, tears, breast milk, lymph, bile, ascites, saliva, amniotic fluid, cerebral fluid, and other body fluids can all include significant amounts of exosomes secreted by various cell types under both normal and pathological circumstances.[11]

Exosomes have been shown to play a significant role in the OSCC tumor microenvironment by boosting OSCC formation is promoted by the signaling pathway of transforming growth factor-β (TGF-β) and drug resistance.[24] Tumor angiogenesis and metastasis in hypoxic environments have been linked to exosomes released by tumor cells.[25] Li and colleagues[26] discovered that HIF1α and HIF-2α were necessary for the exosomes from OSCC cells that are hypoxic to encourage invasion and migration of OSCC cells. In contrast to exosomes that have a typical oxygen concentration, higher amounts of miR-21, miR-205, and miR-148b were found in a miRNA expression profile of hypoxic tumor-derived exosomes. Their outcomes demonstrated a connection between hypoxic cell invasion and motility and levels of expression of exosomal miR-21. Furthermore, OSCC patients were demonstrated to have lymph node metastases and circulating exosomal miR-21 levels. Additionally, recent research indicates that tumor exosomes contribute significantly to immune suppression, which promotes the growth and spread of tumors. Tumor exosomes can actually interact with immune cells in the tumor microenvironment by sending immunostimulatory (antitumor) and immunoinhibitory (protumor) signals.[11]

E. Circulating tumor Protein:

Protein biomarkers have greatly enhanced the care of various cancers, improving follow-up and diagnostic techniques. Although a number of protein biomarkers have been identified, there are currently no proven biomarkers for oral cancer early detection, such as Carbohydrate antigen 19-9 (CA19.9), cancer antigen 125 (CA125), and carcinoembryonic antigen (CEA), etc. In an effort to better understand the molecular mechanisms behind oral cancer and find new potential protein biomarkers, an increasing number of research have concentrated on the proteomic analysis of various bodily fluids, including peripheral blood, serum, plasma, saliva, sputum, and urine.27 Recently, proteases such matrix metalloproteinases (MMPs) and inflammatory substances like C-reactive protein (CRP) and cytokines have been suggested as possible oral cancer salivary indicators.[27] Other research has concentrated on Cancer Antigen 125 (CA-125) and Cluster of Differentiation 44 (CD44), Mac-2 Binding Protein and other intracellular proteins, and elements of the cytoskeleton such as Tissue Polyoeotide-Specific Antigen (TPS) and CYFRA-21-1 demonstrate how these proteins might be interesting biomarkers for the early identification of oral cancers.[28]

F. Salivary biomarkers for oral cancer:

The use of salivary biomarkers as a noninvasive cancer diagnosis method has increased. There is no greater source of genetic information than DNA, RNA, proteins, metabolites, and microbiota information that can be used for diagnostic purposes.[21] The metabolome, microbiome, transcriptome, micro-RNAs, and proteome have all previously been studied using salivary samples.[21] Hypermethylation of the HOXA9 and NID2 promoters in

tissue and salivary cells can serve as markers for the early detection of OSCC and prophylaxis.[29] The most common proteins in saliva include mucin, serum albumin, cystatin, proline-rich peptide, and alpha-amylase.[21] The saliva of OSCC patients has shown differential expression of a variety of free miRNAs in circulation that may represent new tumor markers for the detection of cancer and tracking.[21]

G. Surgical drain fluid as a novel liquid biopsy analyte:

Ramirez RJ et al. conducted a recent investigation[30] utilized Surgical drain fluid (SDF) containing HPV DNA to calculate the minimal residual disease following surgery. This novel liquid biopsy analyte offers a proximal, site-specific evaluation of locoregional MRD, in contrast to plasma. MRD detection of HPV + OPSCC is made possible by postoperative TaqMan qPCR of SDF. This sample shows a close relationship with aggressive pathologic traits, such nodal stage, and is significantly enriched compared to plasma. When making decisions on customized adjuvant radiotherapy SDF-based HPV quantification might be a useful and automated addition to traditional pathology if verified.

V. Advantages Over Conventional Tissue Biopsies

Regular, less invasive sampling to track tumor changes in real time, it removes the necessity of numerous surgical operations.[4] This method lessens patient discomfort and procedure risks. while improving adherence to procedures for disease surveillance. The ability of liquid biopsy, especially CTC analysis, to record tumor heterogeneity and clonal evolution over time is one of its main advantages.[31] Unlike static tissue biopsies that produce isolated data, liquid biopsies offer a dynamic, real-time evaluation of emergent resistance mechanisms and selection pressures caused by therapy.[4] The course of treatment might be adjusted in response to changes in CTC profiles prior to the appearance of clinical symptoms of progression. Additionally, by monitoring CTC levels and mutations in cfDNA, liquid biopsy improves early recurrence diagnosis and enables prompt clinical intervention.[32] It is a desirable choice for long-term monitoring across a variety of patient populations due to its affordability and scalability.[21,33]

Table 2: Comparative matrix of conventional tissue biopsy versus liquid biopsy across clinically relevant parameters.[34]

Parameter	Tissue Biopsy	Liquid Biopsy
Invasiveness	Core needle or surgical excision; requires anesthesia; procedural morbidity in ~3–8% of cases	Peripheral blood draw (7–10mL); minimally invasive; repeatable without clinical risk
Spatial heterogeneity capture	Single anatomical site sampled; metastatic sub-clones and spatially distinct driver mutations may be entirely missed	Plasma pool integrates shed biomolecules from all tumor foci simultaneously, providing a composite clonal landscape
Temporal resolution	Snapshot; serial re-biopsy is clinically impractical and ethically restricted in many protocols	Repeated sampling at weekly-to-monthly intervals permits real-time tracking of clonal dynamics and emerging resistance
Minimal residual Disease (MRD) detection	Not applicable; tissue is absent post-resection; imaging surrogates have limited sensitivity	Ultra-deep ctDNA sequencing detects MRD months before clinical relapse (sensitivity ~94% in GALAXY/DYNAMIC trials)
Repeatability	Severely limited; each procedure carries cumulative risk; regulatory and logistical barriers	Highly repeatable; no restriction on frequency; enables longitudinal multi-time-point study designs
Turn-around time	5–14 days (histopathology + molecular profiling); delays treatment initiation	3–7 days for standard NGS panels; rapid ddPCR assays available within 24–48 h for defined hotspot variants
Cost (approximate)	USD 1,500–5,000 per biopsy event including procedural and pathology costs	USD 300–2,500 depending on panel depth; costs declining with expanded use of tumor-naïve and methylation-based assays

Multi-cancer applicability	Organ-specific; requires prior tumor localization; inaccessible sites (e.g., diffuse peritoneal disease) may preclude sampling	MCED assays screen many forms of cancer from a single blood sample; tissue-of-origin prediction via methylation (PATHFINDER, 2023)
Pre-analytical sensitivity	Tissue ischemia time, fixation artefacts (FFPE), and decalcification introduce molecular degradation	cfDNA half-life ~1–2 h in vivo; EDTA tube handling within 2–4 h critical; Streck BCT tubes extend window to 5–7 days
Clinical limitations	Sampling bias; cannot track emerging resistance; unsuitable for inaccessible or diffuse tumors; patient refusal rates 10–30%	Low ctDNA fraction in early-stage or low-volume disease; false positives from clonal hematopoiesis of indeterminate potential (CHIP); lack of inter-laboratory

VI. Challenges and Limitations of Liquid Biopsy Techniques

- **Sensitivity and Precision:** One of the primary issues with liquid biopsy is achieving a high enough degree of precision and sensitivity for the identification of biomarkers, especially in patients with low tumor load or early-stage disease. Technical heterogeneity in sample processing and background noise from sources other than tumors can both have an impact on the accuracy of results.[33,35]
- **Standardization:** Standardizing analytical techniques and liquid biopsy procedures is another significant issue. Conflicting results between labs and research could hinder the reproducibility and repeatability of findings because of differences in sample collection, processing methods, and data analysis pipelines.[35,36]
- **Cost-effectiveness:** Liquid biopsy examinations might not be frequently utilized in clinical practice because to their high cost, particularly if they include advanced technologies like digital PCR or next generation sequencing (NGS). Cost may limit access to liquid biopsy testing, particularly for people without adequate insurance or in settings with few resources.[33]
- Inconsistent tumor-derived material shed from all sites, little tumor DNA shed, and repeatability problems could restrict use.[21]
- Technically, CTC isolation can also be difficult.[21]
- The accuracy of these diagnostic processes is greatly influenced by the amount and quality of DNA extracted from bodily fluids.[21]
- The mutation may be missed if there is contamination, significant necrosis, or very little DNA available.[21]

VII. Future Perspectives of Liquid Biopsy

Three factors are driving the immediate future of liquid biopsy: the creation of trial infrastructure to collect the prospective data needed for regulatory approval and reimbursement; the development of multi-analyte testing platforms; and artificial intelligence in analysis. In order to identify biomarkers more precisely and at ultra-low concentrations, the goal of research is to increase liquid biopsy assays' sensitivity and specificity. The performance of liquid biopsy testing will be enhanced by technological advancements, such as the development of novel detection methods and the simplification of sample processing processes. A better knowledge of the biology of disease and how patients react to treatment may result from combining multi-omics data from metabolomics and proteomics, transcriptomics, and genomes. In order to completely utilize multi-omics data from liquid biopsy examination, the subsequent research will focus on creating comprehensive analytical frameworks and bioinformatics tools. Treatment choices based on patient genetic profiles could lead to a revolution in personalized medicine. Future research will focus on identifying biomarkers that are predictive of therapy response and resistance so that physicians can tailor treatment regimens to the specifics of each patient's situation.

VIII. Conclusion

A new minimally invasive method for diagnosing and tracking oral cancer is liquid biopsy. It offers useful molecular data from bodily fluids like saliva and blood. Prognosis, metastatic potential, and tumor dissemination can all be evaluated with the use of CTCs. Information about therapy response, minimal residual disease, and genetic changes can be obtained from ctDNA and cfDNA. Extracellular vesicles and exosomes provide further details about the development of tumors and treatment resistance. A promising non-invasive method for identifying and tracking oral cancer is salivary biomarkers. With less discomfort for the patient, liquid biopsy enables repeated sample and long-term evaluation of tumor development. However, low biomarker levels, expense, lack of standardization, and technical variability continue to be significant constraints. Prognostic and diagnostic accuracy may be enhanced by future integration with multi-omics techniques and artificial intelligence. All things considered, liquid biopsy offers a great deal of promise to enable individualized oral cancer treatment and supplement traditional biopsy.

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