Review Article

Liquid Biopsy to the Rescue of Oral Cancer Diagnostics- A Narrative Review

Abstract

Oral cancer is a malignant tumor that arises from the tissues of the oral cavity. It is the eighth most common cancer worldwide, with a high mortality rate due to late-stage diagnosis. Traditional histological investigation is the gold standard for oral cancer diagnosis, but it is invasive and requires tissue biopsy. Liquid biopsy, a non-invasive approach, has emerged as a promising diagnostic technique for oral cancer in recent years. This technique examines tumors and their microenvironment by utilizing biomaterials found in bodily fluids such as blood, saliva, breast milk, and urine. The molecular cargo in liquid biopsy of malignant tumors include circulating tumor DNA(ctDNA), Exosomes, Circulating tumour cells (CTC), Micro RNA(miRNA) and cell free DNA. The use of liquid biopsy as a noninvasive method for early cancer detection, molecular profiling, treatment response monitoring, and detecting minimal residual disease holds great promise. This paper provides an overview of liquid biopsy as a diagnostic technique for oral cancer, focusing on its advantages, molecular candidates, and clinical applications.

Keywords: *Liquid biopsy, Oral cancer, Circulating tumor cells, cfDNA, miRNA*

Deeksha Anil Gandhi[1], Afiya Memon[1], Supriya Kheur[1]*

[1]Department of Oral and Maxillofacial Pathology and Oral Microbiology, Dr. D.Y. Patil Vidyapeeth, Dr.D.Y.Patil Dental College and Hospital, Pimpri, Pune, India.

Introduction

Oral squamous cell carcinoma, or oral cancer, is the most prevalent type of head and neck cancer. It typically arises in the mouth's tongue, lips, and floor. Unfortunately, the global oral health status report on oral cancer reveals an alarming situation in South-East Asia.[1] India alone accounts for 36% of new cases and 42% of deaths globally. Although there have been many advancements in the treatment of oral cancer, the overall survival rate is still low. Thus, developing quick, precise, and noninvasive methods for the early screening and diagnosis of oral cancer is essential.[2]

Various devices are used for screening patients, such as those that utilize the principles of autofluorescence and spectrophotometry. Vital rinsing with toluidine blue has also been explored for mass screening for early detection of oral cancer. However, optical imaging technologies have challenges as they often require complex analysis and can have more false-positive results.[3]

Saliva is considered the mirror of oral health. It contains many biomolecules and shows the probability of biomarkers for non-communicable diseases like oral cancer. These biomarkers can be used to monitor and assess systemic health and disease states. The anatomic location of saliva in the oral cavity, coupled with its ease of collection and accessibility, favors using it for oral cancer screening. Unlike blood, saliva does not clot and does not require special equipment for collection and storage. Therefore, several researchers have proposed the term salivaomics, comprising genomics, transcriptomics, proteomics, metabonomics, and RNA analysis.[4, 5]

Amongst the plethora of diagnostic uses of saliva, it can be used for liquid biopsy due to its anatomical proximity with the lesional tissue. Liquid biopsy is a technique that uses various biological fluids and analyzes their components, which can be used for screening, diagnosis, and prognostic evaluation. The majority of the biomolecules included include exosomes, platelets, circulating tumor cells (CTCs), circulating tumor-free DNA (ctDNA), and circulating cell-free RNA (cfRNA).[6]

With this technology, changes in the molecular profile of the entire tumor may be evaluated in real-time, and other features, including disease state monitoring, residual disease observation, tumor dynamics analysis, and burden assessment in metastatic patients, can all be seen. Thus,

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Address for correspondence:
Supriya Kheur,
Department of Oral Pathology and Oral Microbiology, Dr D. Y. Patil Vidyapeeth, Dr. D.Y. Patil Dental College and Hospital, Pimpri, Pune.
E-mail: supriya.kheur@dpu.edu.in

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liquid biopsy can give "real-time" tumor characteristics and heterogeneity analysis, thereby improving the screening. Circulating tumor cells (CTC), exosomes, circulating tumor DNA (ctDNA), and microRNA (miRNA) are factors that contribute to the efficacy of liquid biopsy. This study provides an overview of the current understanding, use, and clinical applications of these biomarkers (ctDNA, CTCs, and exosomal miRNAs) in detecting, managing, and surveilling oral cancer.

Materials and Methods

To conduct a comprehensive literature review on liquid biopsy and its potential applications in the detection and diagnosis of oral squamous cell carcinoma (OSCC) and the identification of biomarkers for cancer detection and treatment, we searched multiple academic databases. These included PubMed, Web of Science, EMBASE, SCOPUS, and Google Scholar, using carefully selected keywords ((liquid biopsy OR oral biopsy)) AND (Oral cancer, head and neck cancer, oral squamous cell carcinoma, OSCC, or mouth cancer) ((Saliva OR Salivomics OR prognostic biomarkers)) AND ((Oral Cancer detection OR Oral cancer biomarkers OR Oral cancer diagnosis and treatment)). After removing duplicates and studies not written in English, we carefully scrutinized the titles and abstracts of the remaining 431 studies to eliminate those irrelevant to our research question. We also excluded studies for which the full text was unavailable. We were left with 35 studies that fulfilled our inclusion criteria after this preliminary screening. These studies explored various aspects of liquid biopsy and its applications in the detection and diagnosis of OSCC, including novel biomarkers and methods for cancer detection. Some studies discussed the potential of saliva-based biomarkers for cancer detection and treatment.

Results and Discussion

The findings of our literature study point to the great potential of liquid biopsy as a noninvasive technique for the identification and diagnosis of oral cancer, as well as the possibility of using new biomarkers to increase the precision and effectiveness of cancer screening and therapy. However, additional research is needed to fully understand the potential of liquid biopsy and biomarkers in the field of oral cancer detection and treatment.

Circulating tumour DNA (ctDNA)

Circulating tumor DNA (ctDNA) is a fragmented DNA derived from tumor cells and found in the blood and saliva. It can be a useful biomarker in oral squamous cell carcinoma (OSCC) patients as it remains viable in the body fluids for less than 2 hours, and its levels increase as the cancer metastasizes. This makes it a reliable indicator of the real-time changes in the tumor burden during cancer therapy.[6-8] The presence of certain somatic mutations like TP53, CDKN2A, NRAS, NOTCH 1, PIK3CA, HRAS, and HPV strain (16&18) can be detected through ctDNA analysis. Also, hypermethylation of genes such as EDNRB, KIF1A, and HOXA9 in salivary DNA can be used for early screening and diagnosis.[9]

Exosomes

Exosomes are extracellular vesicles that range in diameter from 40 to 160 nm and are produced by all cells as part of their active function. The process of producing exosomes entails the progressive invagination of the plasma membrane, leading to the development of multivesicular bodies. These entities can communicate with the Golgi network, endoplasmic reticulum, and intracellular vesicles.[10, 11] Multivesicular bodies (MVBs) are known to fuse with lysosomes, autophagosomes, or the plasma membrane, depending on the metabolic status, cellular origin, and microenvironment.[12] Exosomes have emerged as a promising candidate for liquid biopsy analysis due to their ubiquity in biological fluids.[13, 14] In liquid biopsy, they are the most prevalent analyte, with concentrations as high as 1011 particles/ml of blood. Up to 10% of circulating exosomes in cancer patients are tumor-derived, and their composition reflects the DNA, RNA, lipids, metabolites, and cell-surface proteins of their parent cells.[12] Exosome content has the upper hand over other liquid biopsy analytes as they contain different types of RNA and possess DNA and RNA that mirror tumor mutations.[15] Furthermore, their DNA has the original tumor's whole genome and mutational burden, making them a superior option to ctDNA, which contains fragmented DNA.[16] Exosome molecules can be considered prospective biomarkers, either alone or in combination, which enhances the likelihood of success in the search for a viable liquid biopsy marker. Furthermore, the number of exosomes released might be used as a clinical indicator.

Circulating tumor cells (CTC)

The bulk of the mutational profile of CTCs, which are blood-borne tumor cells released into the bloodstream by the primary tumor or distant metastatic lesions, is shared by tumoral clones seen in the underlying tumor. With a greater chance of metastasis, they might circulate alone or in clusters. They must go through a multi-step process known as the 'Metastatic Cascade' to enter circulation and metastasis. One of these cells' hallmarks is their scarcity, with only about 1 CTC for every 107 blood cells in metastatic patients.

CTCs are essential in precision oncology. The discovery of CTCs may constitute a new diagnostic tool for anticipating the occurrence of metastatic illness in Oral Cancer and endowing it with therapy options to treat and prevent cancer spread efficiently. CTCs are one of the various sample groups that dominate liquid biopsy and can be enhanced and detected using multiple technologies, giving a potentially accessible source for cancer characterization and monitoring. CTCs may contain helpful information on tumor composition, invasiveness, therapeutic susceptibility, and resistance to therapy.

Tumor metastasis is caused by CTCs. According to most research, eliminating CTCs might significantly stop tumor spread. Because CTCs serve as a seed for metastasis, they may be a valuable target for therapy. By removing CTCs from the body, we may reduce their recirculation in the blood, decrease the growth of these cells into secondary lesions, and reduce the total tumor burden in cancer patients.[15]

CTCs serve as a biomarker for early diagnosis of cancer.[17, 18]

Researching the molecular and biological characteristics of CTCs can inform clinical judgment and aid in cancer prognosis prediction.[19, 20]

Since metastases and recurrence are characteristic features of cancer, CTCs may serve as an independent marker for assessing tumor invasiveness and directing therapeutic therapy.

These investigations could deepen our knowledge of the therapeutic use of CTCs in dynamic assessment.

The potential of MicroRNAs (miRNAs) as liquid biopsy markers for early cancer detection

MicroRNAs (miRNAs) are short, noncoding RNAs (about 25 nucleotides in length) that play a crucial role in gene expression regulation. In recent years, miRNAs have gained attention as potential biomarkers for cancer detection. Their capacity to form complexes with proteins like Ago2 and high-density lipoproteins or to be packaged into extracellular vesicles (EVs) explains why they can be found in bodily fluids like blood, saliva, and urine through the use of high-throughput platforms like microarray and next-generation sequencing, as well as traditional PCR.[21, 22] MiRNAs are being researched as potential candidates for liquid biopsies to help identify cancer early. A machine learning model for miRNAome-based cancer type prediction, for instance, was developed as part of statewide research in Japan; it had an accuracy of 0.88 for all cancer stages and 0.90 for early stages.[23] In another study, Nakamura *et al*. discovered that serum miRNA expression profiles could accurately differentiate between oral cancer patients and healthy donors.[24] Furthermore, Romani *et al*. revealed 25 miRNAs that varied between OSCC patients and healthy controls. In contrast, Mehterov *et al*. established that a miRNA panel could diagnose OSCC with 98% sensitivity and 60% specificity, respectively.[25, 26]

These were case-control studies; therefore, it's unclear when these putative miRNAs will appear in the patient's body fluids. Further research with prospective cohorts will yield functional solutions for cancer screening utilizing miRNA profiles. As a result, miRNAs show great promise as liquid biopsy markers for early cancer identification, and future research should aim to establish their sensitivity, specificity, and clinical utility in larger, prospective studies. The use of miRNAs as noninvasive biomarkers for cancer detection has the potential to revolutionize cancer diagnosis and treatment. With continued research, miRNA profiles may soon become a routine part of cancer screening, allowing for earlier detection and improved patient outcomes.

The role of cell-free DNA analysis in oral squamous cell carcinomas

Cell-free DNA (cfDNA) is a kind of DNA derived from apoptotic or necrotic cells, and it may be distinguished from cfDNA by somatic mutations.[27] It is highly fragmented and can be found in various bodily fluids, including blood, saliva, plasma, urine, cerebrospinal fluid, and other bodily fluids.[28] cfDNA analysis has recently been applied to oral squamous cell carcinomas (OSCCs), with no significant changes detected across groups. Perdomo *et al*. used two techniques to describe the identification of circulating tumor DNA (ctDNA) mutations in head and neck cancers: evaluating TP53 mutations in plasma and sequencing TP53 mutations in tumor tissue, plasma, and oral rinses.[29] Additionally, HPV detection with cfDNA could be helpful in OSCCs as well. Mazurek *et al*. discovered that HPV cfDNA testing might be utilized to detect and monitor HPV-positive head and neck squamous cell carcinomas early.[30] They found that 14% of individuals tested positive for HPV, with the majority (96.4%) testing positive for HPV16. SPEPT9 and SPEPT3 are methylation markers with the highest validity in biofluids from malignancies not in the oral cavity. The significance of these indicators for tumor diagnosis and treatment efficacy has been confirmed in a sizable prospective cohort of 649 patients with head and neck cancer. However, there are still challenges to be addressed before detecting ctDNA in low ratios in early stages, the requirement for multiplexed assays due to tumor heterogeneity and evolution, and a lack of standardized methodologies for detecting ctDNA **(Figure 1)**.

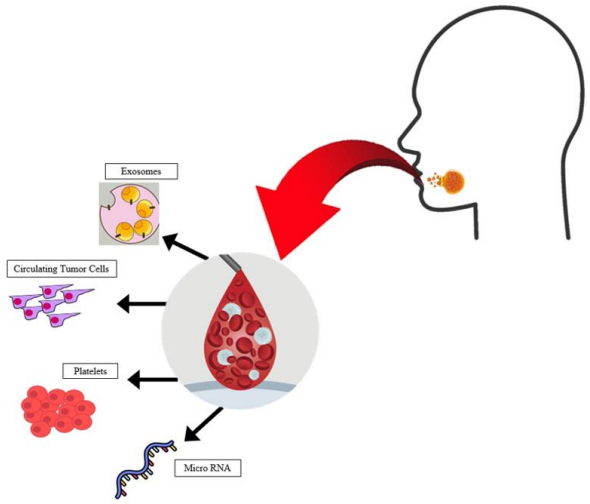

Figure 1. The essence of using Saliva as a Liquid Biopsy in oral cancer diagnostics.

Future perspectives

As a noninvasive method for early cancer diagnosis, molecular profiling, treatment response tracking, and minimum residual sickness discovery, liquid biopsy has great promise. However, its impact on oral cancer is modest, necessitating additional investigation. Personalized medicine has begun by developing a solid panel of unique and sensitive circulating biomarkers to help improve the diagnosis and prognosis of patients with oral cancer.[31]

Recent studies have shown that liquid biopsy can detect oral cancer-specific alterations in circulating tumor DNA, RNA, and proteins.[1] These alterations can serve as potential biomarkers for early detection, treatment monitoring, and predicting patient outcomes.[2]

Additionally, liquid biopsy can provide insights into the clonal

evolution of oral cancer, helping to identify new therapeutic targets.[3] The biology and origin of these biomarkers are crucial for developing effective therapies and managing oral cancer. For example, understanding the mechanisms of tumor-derived exosomes (TDEs) that carry biomolecules such as RNA and proteins could provide valuable information to improve the accuracy of liquid biopsy.[4] Furthermore, developing a sensitive and specific panel of biomarkers may provide a noninvasive alternative to tissue biopsy, allowing for more convenient and less costly monitoring of disease progression and therapy efficacy. Liquid biopsy is still in its early stages for oral cancer; hence, large prospective multicenter trials are required to determine the clinical utility of these biomarkers. Such research could pave the path for new therapeutic options and personalized medicine approaches for patients with oral cancer.

Conclusion

A reproducible, noninvasive diagnostic method that gives real-time information on specific cancers is liquid biopsy. It has shown encouraging results in detecting oral cancer and offers several benefits over conventional histological investigation. Even though they haven't been accepted as biomarkers for oral cancer, molecular possibilities such as the proteome, metabolome, microRNAome, extracellular vesicles, cell-free DNAs, and circulating tumor cells offer great potential for liquid biopsy in diagnosing oral cancer. More study is needed to confirm these indicators and show their therapeutic value in treating oral cancer.

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Conflict of interest

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Ethics statement

None.

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