Society of Medical Education & Research

2024, Volume 4, Issue 1, Page No: 1-6
ISSN: 3108-4834

Archive of International Journal of Cancer and Allied Science

Liquid Biopsy in Oral Cancer Diagnosis: A Narrative Review of Emerging Diagnostic Tools

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Abstract

Oral cancer, which originates in the oral cavity tissues, ranks as the eighth most common cancer globally, contributing to high mortality rates due to its often late detection. Although tissue biopsy remains the conventional method for diagnosis, it is invasive. Recently, liquid biopsy has emerged as a non-invasive diagnostic alternative. This method evaluates the tumor and its surrounding microenvironment by analyzing biomarkers found in bodily fluids such as blood, saliva, urine, and breast milk. The key molecular elements identified in liquid biopsy include circulating tumor DNA (ctDNA), exosomes, circulating tumor cells (CTCs), cell-free DNA (cfDNA), and microRNA (miRNA). The potential of liquid biopsy lies in its ability to facilitate early cancer detection, molecular analysis, monitoring treatment responses, and identification of minimal residual disease. This paper discusses the evolving role of liquid biopsy in oral cancer diagnostics, highlighting its advantages, the molecular biomarkers it targets, and its clinical applications.

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

Introduction

Oral squamous cell carcinoma (OSCC) represents the most common form of cancer in the head and neck region, primarily affecting the tongue, lips, and floor of the mouth [1]. The situation is particularly alarming in Southeast Asia, with India contributing to 36% of new cases and 42% of deaths worldwide [2]. Despite advances in treatment methods, the survival rate for OSCC remains discouragingly low, emphasizing the need for more effective early screening and diagnosis [3]. Various tools have been developed for patient screening, including those based on autofluorescence and spectrophotometry, and vital staining with toluidine blue, which has been explored for mass screening purposes. However, challenges such as the requirement for complex data analysis and the higher occurrence of false-positive results limit the effectiveness of optical imaging technologies [4].

Saliva, often called a reflection of oral health, contains a wide range of biomolecules, making it a potential source for biomarkers that could help detect diseases like oral cancer [5]. These biomarkers can be valuable in tracking and evaluating both the state of systemic health and specific diseases. Saliva's easy accessibility, its location in the oral cavity, and its ability to remain unclotted, unlike blood, make it an ideal specimen for oral cancer screening. This has led to the emergence of the term "salivaomics," which includes genomics, transcriptomics, proteomics, metabonomics, and RNA analysis [6, 7].

In the realm of diagnostic innovation, saliva is increasingly used in liquid biopsy—a non-invasive

Access this article online https://smerpub.com/

Received: 19 October 2023; **Accepted:** 14 January 2024

How to cite this article: Pardo-Zamora F, Castellano-Rioja G. Liquid Biopsy in Oral Cancer Diagnosis: A Narrative Review of Emerging Diagnostic Tools. Arch Int J Cancer Allied Sci. 2024;4(1):1-6. https://doi.org/10.51847/CcaLqtzvoN

technique that analyzes biological fluids to aid in screening, diagnosing, and evaluating prognosis [8]. Important biomolecules detected through this method include exosomes, platelets, circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and cell-free RNA (cfRNA) [9].

Liquid biopsy offers the advantage of monitoring real-time changes in the tumor's molecular profile, allowing for comprehensive disease monitoring, detecting residual disease, and analyzing tumor dynamics, particularly in metastatic cases. This technique provides valuable "real-time" data on tumor characteristics and heterogeneity, enhancing early detection capabilities. Biomarkers like CTCs, exosomes, ctDNA, and miRNA significantly improve the utility of liquid biopsy. This review highlights the current understanding and clinical use of these biomarkers—ctDNA, CTCs, and exosomal miRNAs—in the detection, management, and follow-up of oral cancer.

Materials and Methods

This review was carried out to investigate the application of liquid biopsy in diagnosing oral squamous cell carcinoma (OSCC), as well as its potential in identifying cancer biomarkers. A comprehensive search was performed across several academic databases, such as PubMed, Web of Science, EMBASE, Scopus, and Google Scholar, using a variety of keywords: "liquid biopsy," "oral cancer," "head and neck cancer," "OSCC," "salivomics," and "biomarkers." After filtering the results for studies in English and removing duplicates, 431 articles were initially identified. After a thorough screening of the titles and abstracts, irrelevant studies were excluded. Furthermore, articles with unavailable full texts were also excluded, leaving a final set of 35 studies that met the inclusion criteria. These studies contributed valuable information on liquid biopsy's role in OSCC diagnosis, particularly focusing on the use of saliva as a diagnostic fluid.

Results and Discussion

The results from the literature suggest that liquid biopsy represents a promising, non-invasive method for detecting and diagnosing oral cancer. Additionally, the exploration of novel biomarkers within body fluids may enhance the sensitivity and accuracy of cancer screening and therapeutic monitoring. Nevertheless, more research is necessary to fully unlock the potential of liquid biopsy techniques and biomarkers for OSCC management.

Circulating tumor DNA (ctDNA)

Circulating tumor DNA (ctDNA) is DNA released by tumor cells into the bloodstream or saliva. It serves as a valuable biomarker for OSCC due to its short half-life in body fluids (less than two hours) and its increase in concentration as the cancer progresses or metastasizes. This makes ctDNA an important marker for tracking tumor changes in real time during therapy. ctDNA analysis can identify somatic mutations, including TP53, CDKN2A, NRAS, NOTCH1, PIK3CA, HRAS, and certain strains of HPV (types 16 and 18). Additionally, salivary DNA analysis can reveal hypermethylation of genes like EDNRB, KIF1A, and HOXA9, offering the potential for early detection and diagnosis of oral cancer [10-14].

Exosomes are extracellular vesicles, ranging from 40 to 160 nm in size, that are released by all cell types during normal cellular activity. Their formation begins with the inward folding of the plasma membrane, resulting in the creation of multivesicular bodies (MVBs). These MVBs can interact with intracellular components such as the Golgi apparatus, endoplasmic reticulum, and other vesicular structures. Depending on the cell's metabolic state and its microenvironment, MVBs may fuse with lysosomes, autophagosomes, or the plasma membrane. Exosomes have garnered attention in liquid biopsy research due to their abundance in biological fluids, including blood, where their concentrations can reach as high as 1011 particles per milliliter. In cancer patients, approximately 10% of these exosomes are derived from tumors, and their contents—such as DNA, RNA, lipids, and proteins—reflect the molecular profile of the parent tumor cells. Exosomes offer several advantages over other liquid biopsy analytes, including the ability to carry both RNA and DNA, which mirror the mutations present in the tumor. Additionally, exosomal DNA retains the full genomic and mutational characteristics of the original tumor, making it a more reliable source for genetic analysis compared to ctDNA, which consists of fragmented DNA. This makes exosomes a promising option for cancer diagnosis and monitoring. Furthermore, exosome concentration can serve as a clinical indicator of disease progression [15-19].

Circulating tumor cells (CTCs) are another critical component of liquid biopsy. These are tumor cells that have detached from the primary tumor or metastatic sites

and entered the bloodstream. CTCs are considered a hallmark of metastasis, as they can travel throughout the body and establish secondary tumors. Typically, only one CTC is found per 107 blood cells in metastatic patients, highlighting their rarity. Despite their low numbers, CTCs provide valuable insights into the mutational profile of the tumor and its metastatic potential. They play a central role in precision oncology, offering opportunities for early detection of cancer, assessment of metastasis, and the identification of therapeutic targets. CTCs may also reveal critical information regarding tumor composition, its invasiveness, and how it might respond to treatment. Therefore, studying CTCs offers the potential for improved cancer management, especially in the context of metastasis and recurrence. Removing CTCs from the bloodstream could not only limit their ability to spread but also reduce overall tumor burden. Consequently, CTCs represent an essential biomarker for monitoring cancer progression and tailoring treatment strategies [18-22].

MicroRNAs (miRNAs) are small, non-coding RNAs approximately 25 nucleotides in length that play a pivotal role in regulating gene expression. In recent years, miRNAs have attracted significant interest as potential biomarkers for early cancer detection due to their stability and presence in various biological fluids, including blood, saliva, and urine. Their ability to bind with proteins such as Ago2 or be incorporated into extracellular vesicles (EVs) explains their wide distribution in the body. Detection of miRNAs in these fluids is facilitated by advanced techniques such as microarray, next-generation sequencing, and traditional PCR methods. As a result, miRNAs have emerged as promising candidates for liquid biopsy, allowing for early cancer detection. For example, a machine learning model developed in Japan demonstrated an 88% accuracy rate in cancer detection across all stages, with a 90% accuracy for early stages. Similarly, studies have shown that miRNA expression profiles in serum can accurately distinguish between oral cancer patients and healthy controls. In a separate study, a panel of 25 miRNAs was identified that differentiated between patients with oral squamous cell carcinoma (OSCC) and healthy individuals. Additionally, a miRNA panel with 98% sensitivity and 60% specificity has shown promise in diagnosing OSCC. However, as these findings are based on case-control studies, the timing of miRNA appearance in bodily fluids remains unclear. Future prospective cohort studies are needed to validate the potential of miRNAs as reliable biomarkers for cancer detection. With further research, miRNAs could become a routine part of cancer screening, offering a noninvasive method for early diagnosis and improving patient outcomes [23-28].

Cell-free DNA (cfDNA) refers to DNA fragments that are released from apoptotic or necrotic cells into bodily fluids such as blood, saliva, plasma, urine, and cerebrospinal fluid. These fragments can be analyzed for somatic mutations and provide valuable insights into the genetic changes associated with various cancers, including oral squamous cell carcinoma (OSCC). Recent studies have explored the use of cfDNA in detecting mutations in OSCC patients, although no significant differences were found across groups. Perdomo *et al.* employed two methods to identify circulating tumor DNA (ctDNA) mutations in head and neck cancers, including TP53 mutations in plasma and tumor tissue. HPV detection in cfDNA has also shown potential in OSCC, with a study by Mazurek *et al.* highlighting that HPV cfDNA testing could be used for early detection and monitoring of HPV-positive head and neck squamous cell carcinomas. In their study, 14% of participants tested positive for HPV, with a majority (96.4%) testing positive for HPV16. Additionally, methylation markers such as SPEPT9 and SPEPT3 have shown promise for identifying malignancies in head and neck cancers outside of the oral cavity. However, challenges remain in detecting ctDNA at low levels in early-stage cancers. The need for multiplexed assays to address tumor heterogeneity and evolution, along with the lack of standardized detection methods, are significant barriers that must be overcome before ctDNA can be routinely used in clinical practice **(Figure 1)** [29-34].

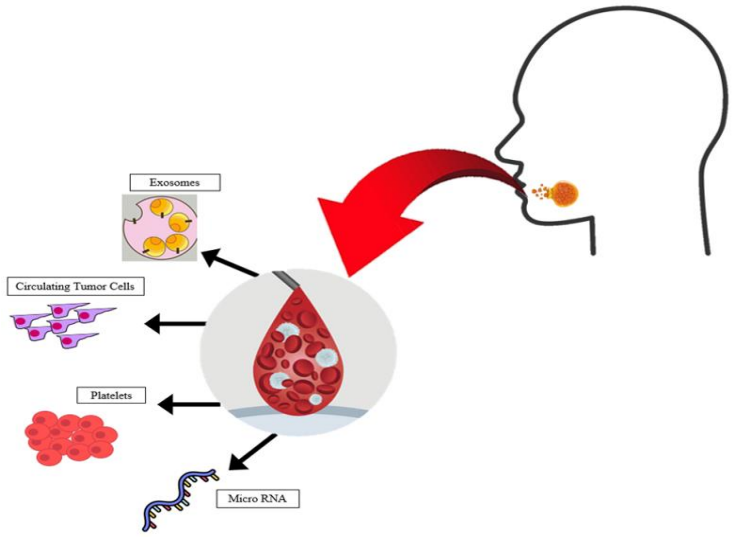

Figure 1. The essence of using saliva as a liquid biopsy in oral cancer diagnostics.

Future perspectives

Liquid biopsy presents a promising noninvasive approach for early cancer detection, molecular profiling, monitoring treatment responses, and identifying minimal residual disease. While its application in oral cancer diagnosis is still under exploration, its potential benefits suggest a need for further investigation. Advancements in personalized medicine are paving the way for the development of a sensitive and specific panel of biomarkers, which could enhance the diagnosis and prognosis of patients with oral cancer.

Recent findings indicate that liquid biopsy can detect oral cancer-specific changes in circulating tumor DNA, RNA, and proteins. These biomarkers could be valuable for early diagnosis, monitoring treatment efficacy, and predicting patient outcomes. Liquid biopsy also provides insights into the clonal evolution of oral cancer, which can aid in the identification of new therapeutic targets. Understanding the underlying biology of these biomarkers, such as tumor-derived exosomes that carry RNA and proteins, is crucial for improving the accuracy and efficiency of liquid biopsy.

Furthermore, the creation of a reliable and precise panel of biomarkers could offer a noninvasive alternative to tissue biopsy, making it easier and more cost-effective to monitor disease progression and treatment outcomes. Despite the promising results, liquid biopsy for oral cancer is still in the early phases, and large-scale prospective studies are necessary to fully assess the clinical value of these biomarkers. These studies could ultimately lead to personalized treatment strategies and provide novel therapeutic options for patients with oral cancer.

Conclusion

Liquid biopsy stands out as a noninvasive diagnostic method, offering real-time insights into cancer characteristics. It shows promising results for detecting oral cancer and provides several advantages over traditional histology. Although biomarkers such as extracellular vesicles, cell-free DNA, and circulating tumor cells have not yet been established for oral cancer, they hold significant promise for improving early diagnosis and treatment. Ongoing research is essential to validate these markers and establish their therapeutic potential in oral cancer management.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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