REVIEW

Liquid Biopsy: A Comprehensive Review

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Abstract

Introduction: Liquid biopsy is a non tissue specimen method uses body fluids for molecular analysis of biomarkers

Objectives: Liquid biopsies has the potential to advance early cancer detection, treatment modification, and disease recurrence surveillance. Biomarkers like exosomes, circulating tumor DNA (ctDNA), and circulating tumor cells (CTCs) and techniques to analyze are discussed. It also emphasizes the significance of liquid biopsy, which proposes a possible substitute for solid biopsy for diagnosis and prognosis of cancer

Conclusion: This review enlightens biological and clinical importance of various biomarkers and their detection for the diagnosis and prognosis of oral cancer.

Keywords: circulating tumor cells; cancer; exosomes; liquid biopsy; miRNA

Introduction

The medical concern of oral cancer is widespread and has a significant impact on many facets of society.[1] One of the most common malignancies in the digestive tract is oral cancer.[2,3] One-third among all oral cancer cases worldwide are found in India. The areas affected by oral cancer are the mucous membranes on the lips, floor of the mouth, anterior two-thirds of the tongue, buccal mucosa, gingiva, palate and retromolar area.[4,5,6] Cancer has a complex aetiology, and there are several risk factors that might contribute to its development, including radiation, familial predisposition, diet with nutritional deficiencies, alcohol intake, smoking, and the human papillomavirus (HPV).[7] In order to reduce the morbidity and mortality caused by complications in oral malignancies, technologies that make it easier to detect recurrence and metastasis early are urgently needed.[5,7]

Over the past ten years, the transformation in the dental and medical studies resulting from human saliva has led to the identification of numerous diseases through compositional alterations in illness situations. Liquid of the human oral cavity comprises of saliva, gingival crevicular fluid (GCF), microorganisms, along with oral epithelial sheddings.[8,9,10] In 1948, Mandel and Metais introduced liquid biopsy, referring to cfDNAs as blood's free floating nucleic acids.[4,5] It's a non-invasive screening technique depends on the identification of proteins, circulating tumour DNA (ctDNA), circulating tumour RNA (ctRNA), circulating tumour cells (CTCs), andexosomes.[1,2,11]

A novel and significant medical method for identifying diseases is liquid biopsy. Each contributes with cancer therapy and diagnosis. Other physiological fluids that can be utilised for a liquid biopsy include urine, sputum, pleural effusions, serum, plasma, urine, saliva, cerebrospinal fluid, and stool.[12]

It aims to provide "bedside" diagnosis by eliminating the time-consuming process of gathering samples and sending them to a central lab for testing.[13,14] In the future, doctors and patients will be able to quickly assess a patient's health status using small, portable devices, catching diseases very early, and allowing ultracustomized treatment based on a patient's unique characteristics.

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LIQUID BIOPSY

Liquid biopsy is defined as "A test done on a sample of blood to look for cancer cells from a tumour that are circulating in the blood or for bits of DNA from tumour cells that are in the blood" by the National Cancer Institute Definition of Oncology (NCI) Terms.[2,13] Perhaps a fluid biopsy done to assist identify earlier stages of malignancy. By utilising the contents of body fluids such as saliva, blood, urine, and surgical drainage.[12] Cancer patients, whose survival rate declines as a result of delayed diagnostic and therapeutic procedures, stand to gain the most from early detection and treatment.[14] When a patient receives a cancer diagnosis, they are typically already in the middle to late stages of the disease and

have missed the best opportunity for treatment. The analysis of saliva normally includes nucleic acid extraction (PCR, qPCR, ddPCR), DNA integrity index calculation, miRNA expression, proteomic analysis, photometric test analysis cytological analysis and other biochemical analysis.[15,16] Saliva has ability to demonstrate potential biomarkers especially in cancer patients hence can be used as an examination technique to enable early detection of oral and oropharyngeal cancers.[7] It can also be utilised to find the human papilloma virus (HPV).[7] Evaluation of circulating tumor DNA (ctDNA) and recently produced or currently existing tumour cells (CTCs) in a cancer patient's blood or body fluids may be a part of liquid biopsy.[2,3] Circulating platelets, exosomes, and cell-free RNA (cfRNA)are additional sample components that might be detected with liquid biopsy.[16,17] Additionally, it could be used to determine the effectiveness of the treatment, how well it is functioning, or whether the cancer has returned.[5,18]

Diagnostic Tools for Liquid Biopsy Sample Analysis

Recent technology developments have opened up new possibilities due to the early identification about human illnesses. Such developments as well enhanced earlier than usual cancer detection, notably for oral cancer.

Real-time quantitative polymerase chain reaction (qPCR), one of the most commonly utilised methods, is frequently used in clinical practise and research contexts.[19] Although liquid biopsy samples are often analysed using qPCR, other techniques such as Mass spectrometry (MS), digital PCR (dPCR), droplet digital PCR (ddPCR), and next-generation The investigation of circulating cancer biomarkers using sequencing (NGS) has lately become increasingly accurate and focused.[20] Other technologies, including the microarray, the ELISA, the biosensors, and the lab-on-a-chip, have also been proposed as technologies for said high diagnostic precision examination of liquid biopsies (LOC).[21,22]

qPCR

It may be utilised to gauge how much of a certain microRNAs (miRNAs).[21] Nowadays, qPCR is the standard procedure for analysing liquid biopsy samples and finding new oral malignant cells.

ddPCR

For the measurement of relative alleles, somatic ctDNA alterations, DNA methylation, and genetic reconfigurations, this novel method is more sensitive (0.01%) than Qpcr.[19, 21]

The researchers found that Hsa-miR-133a-3p and Hsa-miR-375-3p are the two miRNAs, have been particularly when contrasted with healthy individuals are negatively regulated in cancer sufferers.[22]

NGS

NGS is a potent technique that relies on sequencing by quickly synthesising millions of DNA fragments.[9,23] Additionally, RNA sequencing (RNA-Seq) can be utilised with this method to examine short and non-coding RNAs, post-transcriptional alterations, and changes in gene expression.[20] Consequently two popular bioinformatics, Ion Torrent and Illumina, employ several specialised sequencing strategies and signal detecting techniques.

Microarray

Microarray is a useful biological platform with a variety of uses, including the analysis of DNA methylation and non-cod expression profiles as well as the evaluation of gene expression.[2] It is noteworthy that The bits of DNA are gathered on a flat, accessible surface interact with sensors and release a fluorescence signal.[17] Intriguingly, liquid biopsy samples have also been subjected to microarray applications for the identification of oral cancer-related biomarkers.

ELISA

The identification and measurement of proteins, glycoproteins, hormones, and other peptides such as antibodies and antigens are made possible by the antigen-antibody binding principle.[10] Interestingly, oral cancer biomarkers may also be found using ELISA. Overall, these findings indicate that the ELISA assay is a useful instrument for discovering novel oral cancer-associated biomarkers.[11]

Biosensors

Nucleic acids, enzymes, antibodies, and antigens are just a few of the biological substances that can be detected by biosensors, which are analytical tools. An amplifier, that enables the conversion of the biochemical signal into an electrical signal, and a receptor with the ability to bind the target are specifically included in this device.[24] The early detection of oral malignancies is another use for biosensors.

LOC

With the use of LOC technology, which combines numerous analytical laboratory processes on a single chip, cellular and molecular components can be detected using a miniaturised, automated system.[21,23] Due to its possible use in cancer diagnostics, LOC has recently gained increasing interest. In reality, employing minimal amounts of biological fluid samples, this microfluidic engineering method enables the rapid identification of CTCs, ctDNA, miRNAs, and proteins.[25]

CTCs

Circulating tumour cells (CTCs) are short-lived cancer cells that can invade the nearby vasculature and spread to distant locations.[3] Notably, it is difficult to identify and isolate CTCs from whole blood samples since they are extremely rare, present in just 1-100 CTC/mL amid billions of red blood cells.[12] The process of initial tumour cells spreading via the bloodstream to a new site, where they form a new tumour, is known as metastasis.[13,24] To isolate CTCs, various techniques have been developed. These techniques may essentially be categorised as label-dependent CTCs are selected favourably. using particular indicators are provided through tumour tissues, including such binding proteins on epithelial cells, integrins, label-independent cytokeratins (using no specific markers expressed by tumour cells). Integrins have been a fascinating target for separating CTCs because they are cell surface receptors that are extensively expressed in a variety of tumour types.[20,25]

CLINICAL APPLICATION OF CTC'S

CTCs are now employed clinically as substitute biomarkers for a variety of solid tumours.[14] Many studies have been conducted, mostly in the areas of melanoma, stomach cancer, lung cancer, liver cancer, pancreatic cancer, breast cancer, and prostate cancer.[20] Despite the clinical recommendations don't really specifically address therapeutic application of CTCs, they are

included in the classification of cM0 tumours (i.e., there is no overt metastasis but blood tumour cell detection), and numerous studies have suggested that CTCs have significant potential for use in clinical settings.[25] This section will mostly discuss the use of CTCs as biomarkers for cancer diagnosis, prognosis, and therapeutic monitoring.

cfDNA

When compared to healthy individuals and in later-stage tumours as opposed to early-stage tumours, there have been more cfDNA detected in patients with malignancy.[26] Notably, the level of cfDNA can rise under physiological (hard exercise) and paraphysiological (during pregnancy) circumstances, and daily oscillations are seen as a result of the circadian cycle.[27,28] In addition, certain non-tumor circumstances like vasculitis, last-stage kidney failing, trauma, surgery, myocardial infarction, and stroke are connected to elevated cfDNA amount. In both translational and clinical research, cfDNA has become a prominent potential biomarker, particularly for cancer.[29] The development and use of cfDNA-based tests, for example, was hampered by conflicting information regarding total nuclear cfDNA concentration: plasma amounts of cfDNA in people with several ng/mL to several thousand ng/mL are the range for cancer, which interlinks using the variety of concentration for normal individuals.[18]

EXOSOMES

Exosomes are tiny membrane vesicles with a lipid bilayer membrane with sizes between 40 and 150 nm in diameter cell-derived vesicles that are released into the extracellular space by a variety of cytotypes.[23,24] Due to their significance in carcinogenesis, invasion, and metastasis, exosomes are present in the tumour microenvironment and can either promote tumour progression or act as an anticancer agent. Different cell types have the capacity to release exosomes in large quantities into a variety of bodily fluids, including blood, lymph, bile, tears, amniotic fluid, cerebrospinal fluid, urine, semen, saliva, and amniotic fluid.[30]

They are believed to play a role in cell communication and metastasis and contain mRNA, microRNA, DNA, lipids, proteins, and other non-coding molecules.[31] Exosomal molecules were good biomarkers for many therapeutic applications since they are shielded against proteinase- and RNase-dependent deterioration, and may be discovered persistently on the circulating shelf, which is why there is increased interest in these nanosized vesicles.[32]

ctDNA

The fraction of cfDNA known as ctDNA is only found in apoptotic, necrotic, or live tumour cells that are actively releasing DNA into the bloodstream.[14] When phagocytosis becomes tired or inhibited within the tumour, ctDNA reaches the bloodstream in greater levels in malignant situations.[26] So, in cancer patients, cfDNA is a mixture of ctDNA from cancer cells and noncancer cell DNA from healthy cells.[31] Typically, only a small portion of the overall cfDNA is made up of ctDNA.

Several researches looked into the relationship between lung cancer patients' ctDNA levels and treatment outcomes. The focus has shifted to tracking of malignant cells changes in real-time for evaluating overall reaction such as particular treatments or even the possibly earlier establishment since medication resistance, however, the absolute ctDNA level is insufficiently relevant as a diagnostic tool. New approaches like Next-generation sequencing (NGS) with digital PCR (dPCR) surpassed the sensitivity threshold of conventional techniques like Sanger coding, enabling even if ctDNA was found when only accounts for the small portion on the whole cfDNA.[19,21,23]

SALIVA

Use of Saliva in the Diagnosis of Systemic and Oral Diseases

Saliva can sometimes referred to as "the mirror of the body" its ease of collection and straightforward storage have made it a desirable clinical tool.[2,13] It contains a wide range of proteins, nucleic acids, enzymes, and electrolytes, antibacterial agents, antibodies, cytokines, and hormones.[18,31] Extracellular vesicles (EVs), circulating and tissue-derived cells, RNA and DNA molecules, cytokines, and extracellular fluid (biofluid) are all new biomarkers or indicators found in saliva.[16,21,33] The patient should refrain from eating, drinking, smoking, or brushing their teeth for at least an hour prior to collection because some variables can affect the analysis of the many biomarkers taken into account. The lipid profile of cystic fibrosis patients is significantly different from that of healthy persons, reflecting the consequences of the altered salivary composition.[17] As opposed to a normal participant, the saliva from the submandibular gland in cystic fibrosis patients has 66% higher per 100 mL of lipids. In addition to having twice as much cholesterol as the average healthy individual, Sjogren's syndrome patients also have greater levels of antibodies, IgA, IgG, interleukin-6, prostaglandin-E2, and cytokines (IgG).18,20 Numerousproteins, such that profilin, MxA, CXCL13, IL-4, IL-5, anhydrase-I, have been described as biomarkers for the diagnosis of SS.[20,21]

Conclusion

Liquid biopsy is an additional or substitute technique for tissue biopsy. According to the current state and future prospects salivary study and liquid biopsies, saliva holds potential significance in oral cancer governance. Exosomes, microvesicles, platelets, circulating nucleic acids free of cells, and CTC are the primary components of liquid biopsy. Future investigations on the use of ctDNAs, EVs, miRNAs, and CTCs as salivary biomarkers in oral cancer detection, may undoubtedly assist to develop reliable techniques to identify cancer at an early stage promotes rapid detection and support the creation of focused treatments; which will enhance the current state of early detection and treatment of oral cancer.

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