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LIQUID BIOPSY FOR CIRCULATING BIOMARKERS IN ORAL CANCER: A REVIEW

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ABSTRACT
Oral cancer is one of the most common malignancies worldwide, accounting for 2% of all cases annually and 1.8% of all cancer deaths. Over the past decade, invasive techniques for diagnosing and monitoring oral cancer are slowly being replaced by non- invasive methods such as liquid biopsy. Liquid biopsies from cancer patients have opened up newer avenues in detection and continuous monitoring, treatment based on precision medicine and screening of markers for therapeutic resistance. The purpose of this review is to provide an overview of the current methodologies involved in liquid biopsies and their application in isolating tumour markers for detection, prognosis and monitoring oral cancer treatment outcomes.

KEYWORDS: Liquid biopsy, oral cancer, circulating tumour cells, circulating tumour DNA, exosomes.

INTRODUCTION

Molecular profiling of tumours obtained from individual patients has shown to aid in the selection of personalized cancer treatment, therapy, detection of development of any drug resistance, patient responses and monitoring of tumour relapse.[1,2] The gold standard method of profiling tumour involves tissue biopsy. The challenges faced to such invasive procedures include difficulty in acquiring tumour samples for both tumour quality and quantity. Heterogenicity of resected tumour samples as a whole, also limits the use of invasive methods.[3] Moreover, in cases of metastasis, when tumour has spread and constantly evolves both spatially and temporally in response to treatment over time, acquiring multiple biopsies is considered to obtain a holistic image of a tumour.[4] Considering this, recent oncology research has shifted its focus towards biological fluids like- saliva, blood, Cerebral Spinal Fluid and urine for tumour derived components, a technique referred to as Liquid Biopsy.

Difference between Liquid biopsy and tissue biopsy (Fig-1)

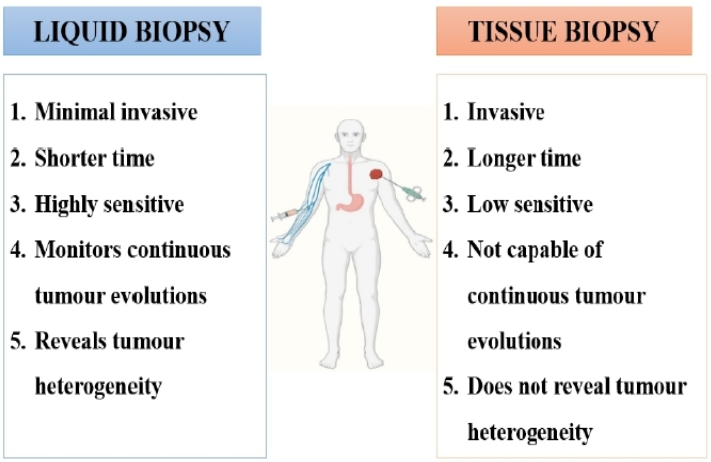

Fig-1 Difference between Liquid biopsy and tissue biopsy

MOLECULAR BIOMARKERS

The detection of circulating biomarkers in liquid biopsy samples reflects the genetic and epigenetic alterations of both the tumour and its microenvironment, providing new information for the identification of novel biomarkers and therapeutics targets in the era of precision medicine. The analysis of ctDNA levels (circulating tumour cells), cfDNA (circulating cell free DNA) and exosomes improves the clinical assessment of cancer and the choice of therapeutic strategies. The detection of altered levels of these parameters in body fluids such as blood and saliva could represent a promising tool for the diagnosis and prognosis of several malignancies including oral cancer.[5]

CIRCULATING TUMOUR CELLS (CTC's)

CTCs are shed from either primary or secondary tumour sites, they migrate into the circulating system and are responsible for the development of distant metastasis.[6,7] The frequency of CTC is very rare with expression of 1 CTC per 10^6- 10^7 leukocytes with even lower numbers in early-stage diseases.[6,8]

However, CTCs provide an ideal approach to molecular cancer diagnosis and treatment options and their investigation is widespread in cancer research. The quantification and the genomic characterization of CTCs have proven to be useful for differential diagnosis, cancer recurrence detection, real time monitoring of tumour evolution and therapeutic efficacy in different tumours.[9,10,11] Grobe et al[12] in his study established that CTCs can act as an independent prognostic marker when OSCC is treated with docetaxel- cisplastin and fluorouracil- induction chemotherapy, surgery and postoperative (chemo)radiation.

To provide more knowledge regarding CTC biology for OSCC, Oliveira- Costa et al[13] in his study concluded that CK9 expression was detected in 80% of the samples from primary tumours and 90% from CTCs. These results confirmed the higher expression of metastasis-associated genes in CTCs facilitating the development of metastasis.[14] Strati et al[15] conducted a prospective study of a cohort of patients with locally advanced cancer of head and neck and reported that the evaluation of PD- L1 overexpressing CTCs in liquid biopsies is feasible and may provide significant prognostic information. CTC analysis is a developing technique in the field of HNSCC in general and in that of OSCC in particular.

However, a factor of crucial importance in any future clinical implementation is the need to improve the sensitivity of the techniques used to quantify and characterise the presence of CTCs.[16]

CIRCULATING TUMUOR DNA

CT DNA can be differentiated from cfDNA on the basis of somatic mutations, it represents less than 1% of cfDNA and has a significant potential as a biomarker in oncology.[9,16] cfDNA (circulating cell free DNA) originates from apoptotic or necrotic cells that release it into the bloodstream and other biofluids by all type of cells including both non-malignant host cells and tumour cells.[9] Lawrence et al[17] describes the molecular profile of HNSCC, reporting TP53 (72%), PIK3CA (21%), FAT1 (23%) and CDK2NA (22%) as key mutations in these tumours.

On the other hand, Wang et al[18] explored the usefulness of ctDNA as a biomarker in plasma and saliva in 93 patients with HNSCC at different stages and in different anatomical locations. The results showed that when a joint evaluation of plasma and saliva was performed, ctDNA was detected in 79% of the patients. Moreover, they found that sensitivity for the detection of ctDNA in saliva was dependent on the primary tumour (the test was more effective in tumours of the oral cavity) and on the disease stage. In early stages, saliva appears to be more sensitive predictor than plasma (100% vs 70% respectively), while the latter is more sensitive in advanced stages (92% vs 70% in saliva). Therefore, further determinations are required, whether conducted singly or in conjunction with the determination and or sequencing of ctDNA, in order to offset the field effect of the OSCC.

EXOSOMES

Exosomes are small membrane vesicles with diameters ranging from 40- 150 nm and a lipid bilayer membrane.[19] Exosomes present an enriched surface of proteins such as fusion and transport proteins. In OSCC, exosomes have proved to be key components in the tumour microenvironment, increasing the transforming growth factor- β signalling pathway, which contributes to progression and drug resistance of OSCC.[9]

Recent data also suggested that tumour exosomes play an important role in immune suppression, enhancing tumour development and progression.[20]

Furthermore, exosomes could be used to discriminate between active- disease cancer patients and those with no evident disease after oncologic therapies.

Zlotogorski- Hurvitz et al[21] have analysed the expression of oral fluid derived exosomes- CD9, CD81 and CD63 between cancer patients and healthy individuals, although statistically significant differences were found only for CD81.

The discovery of microRNA in both the tumours and plasma of patients with tongue SCC highlights the importance of microRNAs – both free and within exosomes as potential biomarkers for the diagnosis of tongue cancer.[22]

PROTEIN BASED SALIVARY BIOMARKERS FOR ORAL CANCER

Recently, proteomic analysis has been applied to discover novel reliable biomarkers for the early diagnosis of tumours. Such analysis performed on liquid biopsy samples are effective in identifying new potential protein biomarkers for oral malignancies.

1. CYTOKINES

Panneer Selvam et al[23] in their study observed that salivary IL-6 (interleukin-6) levels were significantly higher in OSCC, and oral leukoplakia patients compared to controls. [132.88 ± 59.09 pg/ml, 52.14 ± 43.00 pg/ml and 12.84 ± 9.68 pg/ml respectively]

2. CRP [C REACTIVE PROTEIN]

CRP is a plasma protein encoded by the CRP gene, which is secreted by hepatocytes. Metgud R and Bajaj S[24] reported that salivary and serum concentrations of CRP were higher in OSCC and oral premalignant lesion patients when compared to controls.

3. MATRIX METTALOPROTEINASES (MMP's)

Saleem Zet et al[25] in their study highlighted that MMP12 was differentially expressed among the OSCC patients mean value (14.92 ng/ml), oral mucous fibrosis patients (mean value 12.53 ng/ml) and controls (mean value of 0.82 ng/ml) with a specificity and sensitivity of 100%.

4. CA-125 (CANCER ANTIGEN- 125)

CA-125 is mainly considered as a serum biomarker for the diagnosis of ovarian cancer, studies have also suggested the detection of salivary CA-125 levels for oral cancer lesions.

Balan JJ et al[26] found that the mean salivary concentration of CA-125 in OSCC patients and the controls was 320.25 U/ml and 33.14 U/ml respectively.[5]

We would like to condense the potential role of different liquid biopsy technical approaches for the diagnosis and prognosis of OSCC. CtDNA and microRNA have a greater diagnostic value than a prognostic value. Exosomes have high diagnostic value whereas CTC has a greater prognostic value.

CONCLUSION

Liquid biopsy holds great potential as a rapid, non-invasive and repeatable approach for the diagnosis of oral cancer. According to the various studies described in the present review article, the detection and analysis of biomarkers from serum plasma and saliva (ctDNA, microRNA and exosomes) it could significantly improve the current screening programs and diagnostic strategies, improving early diagnosis and real- time monitoring of disease in the era of precision and personalized medicine. Today the practical applications are in the stage of infancy but stands to change treatment permanently in future and achieve astonishing goals for mankind tomorrow.

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Conflicts of interest

There are no conflicts of interest.

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