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ROLE OF LIQUID BIOPSY IN DIAGNOSIS OF ORAL CANCER

Hemashree J[1], Sreedevi Dharman[2]

[1]Saveetha Dental College and Hospitals,Saveetha Institute of Medical and technical sciences (SIMATS)Saveetha University,Chennai, India

[2]Reader,Department of Oral Medicine and Radiology ,Saveetha Dental College and Hospitals,

Saveetha Institute of Medical and technical sciences (SIMATS)Saveetha universityChennai, India

[1]151501063.sdc@saveetha.com,[2]sreedevi@saveetha.com

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ABSTRACT

Liquid Biopsies is a new field of study with increased potential for improving early cancer detection, therapeutic modification, surveillance of disease recurrence. Oral cancer is the most common form of cancer.Inadequate screening leads This review provides knowledge of the current biological and clinical significance of circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), and exosomes for diagnosis and prognosis of oral cancer. It also highlights the importance of liquid biopsy using blood and saliva which suggests a potential alternative to solid biopsy for diagnosis and prognosis. Early detection and surveillance of oral cancer is the goal for achieving patients outcomes. Clinical examination and biopsy of the suspected oral lesion followed by histological analysis are usually used for diagnosis. Biopsy is the gold standard ,an invasive procedure in which a portion of the suspected malignant tissue is obtained and further subjected to specialized and sophisticated histopathology or cytology procedures

INTRODUCTION

India has one third of oral cancer cases in the world.Oral cancer accounts for around 30% of all cancers in India.Oral cancers in India estimated new cases were 1,19,992 from 56,000 cases in 2012 – which is a huge 11.4% rise in just 6 years and estimated deaths were 72,616. The number of people suffering

from cancer of the lip and oral cavity increased to 119,992 in 2018[1]. Predominant sites involved in oral cancer are mucosal surfaces of the lips,floor of the mouth, front two-thirds of the tongue, buccal mucosa, lower and upper gingival surfaces, palate and retromolar pad [2]. Cancer has multifactorial etiology and its development is associated with several risk factors such as tobacco smoking, alcohol consumption , human papillomavirus (HPV) , poor immune system, diet with nutritional deficiencies, hereditary predisposition, or radiation[3]. Oral cancer is caused by alterations in specific cancer genes affecting function of certain pathways, most of them present with advanced stage disease leading to poorer outcomes[4].

Early detection and surveillance of oral cancer is the goal for achieving patients outcomes. Clinical examination and biopsy of the suspected oral lesion followed by histological analysis are usually used for diagnosis. Biopsy is the gold standard ,an invasive procedure in which a portion of the suspected malignant tissue is obtained and further subjected to specialized and sophisticated histopathology or cytology procedures [5]. Instead of invasive investigative techniques ,the concept of quick liquid sample can diagnose disease has been appealing [6]. Liquid biopsy first came on the scene in 1984 when Mandel and Metais referred to cfNAs as free floating nucleic acids in blood[7]

It is a noninvasive diagnostic tool, based on detection of circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), circulating tumour RNA (ctRNA), proteins, and exosomes [8]. Each aids in cancer detection and treatment.It is less expensive,other than blood, and bodily fluids such as urine, saliva, seminal plasma, pleural effusions, cerebrospinal fluid, sputum, and stool that can be used for a liquid biopsy [9]. Advantages are it provides a molecular profile of individual,early identification of initial cancers or metastatic tumours, providing importance of the tumour burden gives early evidence of recurrence or resistance to the disease , and helps in therapeutic decision-making [10] .

Our recent research portfolio slides numerous articles in reputed journals [11–15]. Based on this experience we planned to pursue a review on the role of liquid biopsy in oral cancer.

CIRCULATING TUMOR CELLS

Circulating Tumor Cells are free tumor cells in the bloodstream arising from primary tumors in low numbers. Metastasis is the outcome of the circulation of primary tumour cells by passing into the bloodstream and reaching a different location, where a new tumour is formed [16]. Most importantly , it is responsible for 90%of the mortality of cancer patients [17]. CTCs are cells excreted by the primary tumour or by distant metastatic lesions into the bloodstream, so they share most of the mutational profile with tumoral clones present in the primary tumour [18]. They circulate alone or form clusters that have a greater Metastasis is the result of the dissemination of primary tumour cells by reaching the bloodstream and entering a different location, where a new tumour is formed [19]. In order to get into circulation and

form metastases, they must undergo a multistep process called the "metastatic cascade", which includes their epithelial mesenchymal transition (EMT), intravasation, survival in an adverse environment, and their extravasation at distant sites where the mesenchymal–epithelial inverse transformation (MET) takes place [20,21]. EMT occurs in epithelial cells, which develop mesenchymal-like properties, including the down regulation of epithelial markers [22]. Accordingly, the appearance of CTCs with a more epithelial phenotype have been associated with those with a higher ability to form metastases [23]. metastatic potential [20]. In order to get into circulation and form metastases, they must undergo a multistep process called the "metastatic cascade", which includes their epithelial–mesenchymal transition (EMT), intravasation, survival in an adverse environment, and their extravasation at distant sites where the mesenchymal–epithelial inverse transformation (MET) takes place [22–24]. EMT occurs in epithelial cells, which develop mesenchymal-like properties, including the downregulation of epithelial markers [24]. Accordingly, the appearance of CTCs with a more epithelial phenotype have been associated with those with a higher ability to form metastases [27].Pros are highly abundant which is correlated with tumor size and validated isolation methods.Cons are DNA fragmentation.Is applied for prediction of recurrence and prognosis.

There are a large number of technologies for CTCs analyses. These analyses are not being used to manage patients' treatment and monitoring. The main point for further future implementation of CTCs at clinical levels is associated with the sensibility and the versatility of these techniques. Hence ,we need a very sensitive system to detect CTCs in all metastatic patients and also in a high percentage of early stages, which can provide a wide variety of CTCs using both epithelial and mesenchymal markers.

. CIRCULATING CELL FREE DNA

Liquid biopsy test measures fragments of DNA shed by cancer cells in blood.cfDNA originates from apoptotic or necrotic cells that deliver it into the bloodstream and other biofluids by all types of cell consisting both non-malignant host cells and tumour cells [25,26]. ctDNA can be differentiated from cfDNA on the basis of somatic mutations. ctDNA associated with a small variable population within a large population of cfDNA.

Different technologies for ctDNA detection have been organized during the last years, which allows it to be analyzed from the level of a point mutation to that of the entire genome [27]. Classical methods of analyzing ctDNA consist of quantitative real-time PCR, fluorescent assays, and spectrophotometric strategies. Digital PCR-based technologies are greatly sensitive techniques designed for the detection of specific point mutations, copy-number variations, short indels, and gene fusions. These technologies include different systems such as droplet-PCR, microfluidic systems for parallel PCR, and BEAMing (beads, emulsions, amplification, and magnetics). Next-generation sequencing technologies are considered to be an alternative for cfDNA characterisation. These technologies allow high-throughput and relatively low-cost analyses to identify ctDNA alterations across wide genomic regions and they have an

advantage of not requiring prior education of the genetic alterations of the tumour [28].

EXOSOMES

An additional finding for liquid biopsy analysis involves bioactive vesicles described as exosomes by Panand Johnstone in 1983 [29]. Exosomes are small membrane vesicles with diameters ranging between 40–150 nm and a lipid bilayer membrane [30]. Exosomes present on an enriched surface of proteins as in fusion and transport proteins (Rab GTPases, annexins, and flotillin), components of the endosomal sorting complexes which are needed for transport complexes (ESCRT complexes), heat shock proteins (HSP70, HSP90), integrins, and tetraspanins (CD9, CD63, CD81, CD82) [31]. Research has demonstrated the presence of exosomes in the tumour microenvironment, requiring its importance in tumorigenesis, tumour invasion, and metastasis, hence they can act as promoters of tumour progression or possess an antitumor function [32]. Depending on the mode of biogenesis, celltype, and physiological conditions, includes proteins, lipids, mRNAs, microRNAs (miRNAs), long noncoding RNAs (lncRNAs), genomic DNA, cDNA, and mitochondrial DNA(mtDNA) [33] . Exosomes can be abundantly released by different types of cells into numerous biological fluids such as urine, semen, saliva, amniotic fluid, cerebrospinal fluid, lymph, bile, ascites, tears, breast milk, and blood, both in healthy and diseased conditions [34,35] .

mi-RNA- Micro-RNAs (miRNAs) are non-invasive biomarkers and important components of the cell-free nucleic acids available in different body fluids. Mir-371, mir-150, mir-21 and mir-7d were potential prognostic markers, mir-134, mir-146a, mir-338 and mir-371 were associated with metastases. Prognostic markers, mir-21 and mir-7d were found to be significantly correlated with resistance to chemotherapy, while mir-375, mir-196 and mir-125b were significantly correlated with sensitivity to radiotherapy. Despite the promising roles of circulating miRNAs, challenges still remain in unravelling the exact regulation of these miRNAs before using them for targeted therapy.MiRNAs are usually secreted into the body fluids in membrane bound vesicles known as exosomes. Release of miRNAs from exosomes is a significant mechanism of genetic exchange between cells. Circulating miRNAs are extremely stable and can be conveniently used as informative bio- markers for complex diseases such as cancers [36]. Recent studies of circulating miRNAs in plasma, serum, and other body fluids show that miRNAs secreted from a particular cell type not only has a local action, but may also act at distant sites.

Salivary Biomarkers

The use of salivary biomarkers has gained great attention as a novel, noninvasive method for oral cancer diagnosis. Currently, multiple salivary molecules can be utilised as biomarkers in cancer for diagnosis, prognosis, treatment monitoring, and pharmacogenetic studies [37,38]. Keeping in mind that saliva can be considered as “the mirror of the body” [39], it has become an attractive clinical tool because it's easy to collect and simple storage [40]. Five diagnostic alphabets have already been characterized in salivary samples: proteome, transcriptome, micro-RNAs, metabolome, and microbiome [39]. Only

a few studies analyze saliva to find biomarkers for oral cancer, although the results are promising. In a study performed by Spafford et al. [41], a total of 44 head and neck squamous cell carcinoma patients (13 of which were located in the oral cavity) and 43 healthy subjects were analyzed to find tumour-specific microsatellite alterations in the DNA from exfoliated salivary oral cells. Salivary miRNA biomarkers have recently become an emerging field for monitoring both oral and systemic diseases . In carcinogenesis, overexpression of certain miRNAs could result in downregulation of tumor suppressor genes, while reduced expression of certain miRNAs could cause oncogene upregulation salivary miRNA screening emerges as a valuable diagnostic method for detection of human cancers, especially for those of the salivary glands and of oral mucosa. The expression patterns of certain miRNAs have shown positive correlation with clinical stage, lymph node metastasis and patient survival, indicating that these miRNAs can act as prognostic predictors in OSCC[42].

HPV ASSOCIATED ORAL CANCER

A novel liquid biopsy test showed specificity and sensitivity in detecting minute traces of cancer-specific HPV DNA among patients who underwent chemotherapy for HPV –associated oropharyngeal Squamous cell carcinoma [43,44]. This blood test had exceptional performance in monitoring patients for cancer recurrence after radiotherapy. Circulating tumor HPV DNA to detect cancer recurrence after treatment.If a patient develops HPV DNA detectability in blood during follow up subsequent imaging were done as they were promising.They have high accuracy ,with a negative predictive value of 100%.

CONCLUSION

A better knowledge of the biology and origin of circulating biomarkers would be the key for the development of effective therapies for the management of oral cancer. It can monitor and surveil post treatment for recurrence and detect new cancers. Liquid biopsy for oral cancer is in its infancy, so research efforts should be addressed to perform large, prospective multicenter studies that investigate the role of CTCs, ctDNA, and exosomes in oral cancer.It is a personalised treatment and there is improved quality of life and higher survival rates. More evidence is required for further optimization of liquid biopsies and incorporating them into routine clinical use.

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