

ALTERATIONS OF CIRCULATING TUMOUR DNA AS A BIOMARKER IN ORAL SQUAMOUS CELL CARCINOMA: A SYSTEMATIC REVIEW

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ABSTRACT

Objective: To investigate the literature on genetic abnormalities of DNA in blood and saliva from OSCC patients, to report diagnostic accuracy, and to address the possibilities for a diagnostic or recurrence monitoring test based on circulating tumor DNA (ctDNA).

Methods and materials: A systematic search was performed in PubMed for articles published between 2013 and 2021. The search terms used were “circulating tumor DNA in oral squamous cell carcinoma”.

Results: We found eleven relevant studies, of which four (36.36%) prospective population studies were included. In all studies ctDNA were used as biomarker in OSCC. Two studies were looked into ctDNA somatic and methylated DNA mutations in OSCC. Another study found 90% of the ctDNA alteration and one study identified PD-L1 gene, which plays an important role in primary cancers based on tumour size, as well as disease-specific survival.

Conclusion: This study concluded that the use of ctDNA as biomarkers in OSCC clinical routine will help to establish consistent strategies for the early detection of cancer lesions enable early prevention and support the development of targeted therapies.

Key words: OSCC, ctDNA, Biomarker, Mutation, Alterations

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most prevalent oral malignancy, representing up to 90 % of all malignant neoplasms of the oral cavity. It is the sixth most frequent cancer in the world and it is usually the result of a multi-step carcinogenic process.¹ Furthermore, several lesions may appear simultaneously and spread over significant sections of the mucosa, and become cancerous. This could explain the increased rate of OSCC recurrence after treatment.² As a result, more biomarkers that are sensitive and specific for

OSCC are widely sought for in order to improve the diagnosis of those at risk as well as the treatment of patients.³ OSCC is diagnosed through biopsy, which is considered the gold standard. However, there are many shortcomings. For example, invasive sampling carries risks, especially when used in fragile organs like the lungs, and the sensitivity is suboptimal, often resulting in the inability to detect early-stage cancers. Furthermore, tumors are heterogeneous and constantly developing,⁴ tissue biopsy-based examinations often cannot accurately determine tumor progression.⁵

Similarly, they have difficulty detecting minor, persistent lesions after treatment. It has been hypothesized in recent years that a plasma biomarker-based approach can be used to assess tumor onset, progression, and recurrence. This method is

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minimally good compliance.⁶ Circulating tumor DNA (ctDNA) has now been widely evaluated as a new biomarker for liquid biopsy in cancer diagnosis and prognosis. The Food and Drug Administration as approved ctDNA for disease monitoring and personalized treatment of non-small cell lung cancer for EGFR mutations,⁷ as well as hypermethylation of the SEPT5 gene as a colorectal cancer screening marker.⁸ During physiological cell turnover or, in particular, pathological conditions, apoptotic and necrotic cells release RNA/DNA molecules and residues into body fluids. Under physiological conditions, cell-derived molecules and debris are removed from phagocytes; instead, in cancer patients.⁹ As a result, cancer patients have increased levels of ctDNA in body fluids.¹⁰

CtDNA generally contains genetic changes that could be useful in detecting cancer. Several laboratories have reported outstanding clinical data with cancer detection sensitivities of 50% to 70% calculated with specificities of 90% to 95%.¹¹ It has other important uses, such as estimating tumour volume, prognosis and monitoring therapy.¹² In particular, patients with advanced pancreatic, gastro-oesophageal colon and breast cancers showed a higher level of ctDNA than those with early-stage.¹³ In addition, the researchers also looked ctDNA in relapsed and non-recurrent patients and found that it could be used to monitor relapse status, resulting in a 10-month lead time to detect relapse compared to traditional follow-up.⁹ As a result, ctDNA has the potential to be used to assess tumour progression and prognosis.¹⁴ The use of ctDNA as a biomarker has shown significant results in the evaluation of OSCC. In addition, it is a minimally invasive procedure that can provide detailed information on tumor differentiation, biological behavior, and response to different therapeutic modalities even before the manifestation of the disease.¹⁵ CtDNA may be used as an alternative when tissue biopsies from a metastatic tumor are possible. Haematological analysis is simple, and sample collection can be done many times.^{16,17} Various molecular techniques have showed promise in detecting ctDNA in solid metastatic cancers with hematogenous dissemination.¹⁸ In current clinical practice, genotyping is accomplished using DNA obtained from a tissue biopsy. However, tissue biopsy can only obtain local and static tumour information and,

due to the heterogeneity and constant evolution of tumours, cannot reflect tumour genotyping in real time¹⁹ CtDNA analysis overcomes these problems by reflecting genetic mutations throughout tumour tissue. Furthermore, ctDNA from the same patients at different stages can be used to dynamically monitor genetic mutations during cancer progression.^{20,21}

In this systematic review we present specific DNA abnormalities in OSCC patients based on ctDNA in plasma. The purpose of this study was to investigate the literature on genetic abnormalities of DNA in blood and saliva from OSCC patients. Therefore, a liquid biopsy based on ctDNA analysis could improve tumor genotyping and targeted cancer therapy, which would be of considerable benefit in the field of personalized medicine.

MATERIALS AND METHODS

Strategy for Searching of Data

The methodology of the subject systematic review is schematized in a flow chart diagram (in figure 1). Articles on oral squamous cell carcinoma were searched using online resources such as Google Scholar, PubMed, Research Gate, and requested articles from foreign friends who have access to SciFinder. Data such as Conference preceding and web of science and other sources were also searched to collect data for this review. Only those articles were included which were in English language. The data was searched using titles of relevant articles, MeSH and keywords. The limit for data collection was set from the last seven years. More than 120 research articles were downloaded highlighting the role of ctDNA in OSCC. Our study just focused on oral squamous cell carcinoma. The study's main focus was to check the relationship between the types of a cell and their division cycle causing cancer. All articles published in last 8 years were used for review study.

Inclusion and Exclusion Criteria

All articles related to squamous cells were used for gathering data. Articles with recent dates were included in review. Only research studies on humans as subject were included for further analysis. Those articles which were focusing on ctDNA as a biomarker for OSCC were considered for further review. The exclusion criteria were the studies, which did not check the specific gene alterations either mu-

tation or methylation, review articles, power point presentations, case reports and studies that did not validate their results.

Extraction Strategy for Data

The titles and abstracts of all downloaded articles were screened by two independent reviewers (PhD scholars) for further data analysis. After this, full texts of the pieces were also reviewed by these two independent reviewers. For data extraction from all papers selected for this review, a preliminary analysis was conducted on the most relevant data specified on the topic. For data extraction, extraction forms with the headings, author's name, year of publication, and type of neurodegenerative disorder being studied, disease treated, and possible outcomes were used. Focus of the study was all those article targeting genetic alteration, gene examined, study design, sensitivity, and specificity to the subject topic. The outcome was to determine the association of ctDNA in OSCC.

RESULTS

The literature search generated 100 articles using the predefined search terms, of which 11 studies were eligible for inclusion. Four (36.36%) prospective population studies were included. All studies were published between 2013 and 2021. These studies varied in the technologies used to detect genetic alterations, which included polymerase chain reaction (PCR), immune-histochemistry, immunofluorescence, nano string, the cancer genome atlas (TCGA) and catalogue of somatic mutation in cancer (COSMIC) data bases. In all studies ctDNA were used as biomarker in OSCC.

Somatic mutation was examined in a study reported by Cui et al, 2021 in OSCC using ctDNA. They showed that ctDNA might be used to diagnose

somatic mutations in OSCC. It could be owing to the use of ctDNA genetic analysis to detect early tumor recurrence.²² One study (Uzawa K, et al 2015) looked into mitochondrial DNA mutations in OSCC patients. The findings reveal a novel method for detecting circulating mut-mtDNAs, which are promising molecular markers for assessing tumoral micro metastasis in OSCC.²³

Another study (Kakimoto, et al, 2008) found that 90 % of the ctDNA had been altered. Accord-

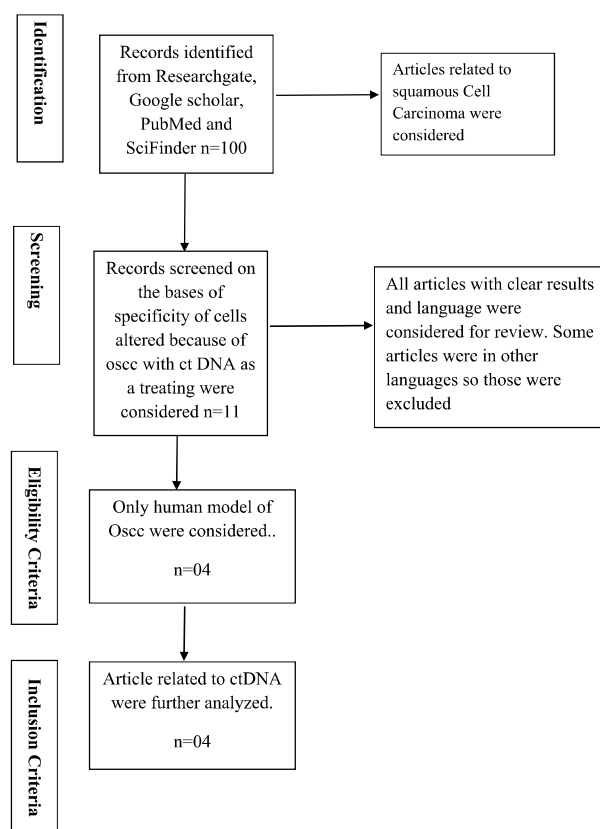


Figure1. Schematic illustration of selection process of studies

TABLE: 1 Studies showed the ctDNA as a biomarker in OSCC

S. no	Study reference	Genetic markers	Patients (n)	Protocol	Type of Mutation
1	Kakimoto, et al, 2008	D2S1327 (on 2q32-35)	20	PCR	Allelic imbalance
2	Cui et al, 2021	TP53, CASP8, AJUBA, CDKN2A, and NOTCH1	11	NGS	Somatic mutation
3	Uzawa et al 2015	ND2, ND3 regions of the genome	240	RT- qPCR	Mitochondrial DNA mutation
4	Costa et al, 2015	PD-L1	36	RT-qPCR	Expression of gene during tumor progression

ing to this study, blood testing for circulating tumor genetic markers could provide significant prognostic information as well as a roadmap for future treatment.²⁴Costa et al, 2015 identified biomarkers by searching over and under expressed genes and they found that PD-L1 plays an essential role in primary cancers based on tumour size, as well as disease-specific survival.²⁵table 1.

DISCUSSION

To the best of our knowledge, this is the first systematic review to explore the role of ctDNA in OSCC patients. This study investigated the use of ctDNA in blood and saliva of OSCC patients. The goal was to highlight existing knowledge of OSCC ctDNA alterations in blood and saliva as a diagnostic test, as well as to improve OSCC patient outcomes. The included studies revealed that both genetic and epigenetic alterations could be detected in ctDNA. Of the 4 studies included in the present analysis reported different mutations in OSCC using to ctDNA.

The first comprehensive analysis was performed by Fan G, et al in 2017 with CRC using the currently available literature. The findings of the study were, the patients with ctDNA positive CRC have an unfavorable prognosis.²⁶Same study was performed by AH Pall in 2020, reported specific DNA alterations based on ctDNA in plasma of HNSCC patients. The literature search generated 1535 articles. Five studies evaluated mutations found in ctDNA of HNSCC patients. Eleven studies investigated methylation as a diagnostic marker for HNSCC. Six studies investigated nasopharyngeal squamous cell carcinoma (NPSCC), and five studies investigated all HNSCC sites.²⁷cullinane et al analyzed 69 studies to determine the prognostic value of liquid biopsy in predicting lymph node metastases, recurrence, and survival in breast cancer.²⁸Since ctDNA has a short half-life, it can indicate the patient's status including distant metastasis and tumor burden, in real time. Therefore, in the future, it may be possible to recommend a less invasive ctDNA test rather than an expensive CT test that increases risk of exposure as has also been suggested by others.²⁹ In the detection of metastatic breast cancer, ctDNA shows superior sensitivity to that of other circulating biomarkers and has a greater dynamic range that correlates with changes in tumor burden and often provides the earliest measure of treatment response, as has been

supported by recent analyses of ctDNA in other solid cancers. It represents a “liquid biopsy” alternative, allowing for sensitive and specific serial sampling to be performed during the course of treatment (Jane et al., 2013). CtDNA analysis may serve as a tool to monitor treatment and can identify local and systemic relapse with greater precision than tissue biopsy in OSCC. It will be an innovation in the field of oral cancer research as it has the ability to detect genetic aberrations, that are periodically noticed in oral tumors and they have a significant role in its malignant transformation.

CONCLUSION

According to the current status and future prospects of ctDNA, it is possible that future studies on the use of ctDNA as biomarkers in OSCC clinical routine will help to establish consistent strategies for the early detection of cancer lesions, enable early prevention, improving the prognosis/ survival rate and support the development of targeted therapies; this will improve OSCC patient treatment outcomes and also will have less side effects of the adjuvant therapy.

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