ORIGINAL ARTICLE OPEN ACCESS

Pathological Insights into Liquid Biopsy: Role of Circulating Tumor DNA in Oral Cancer Prognosis and Therapy

Raima Kalhoro[1], Fatima Sarfraz[2], Shahzaman Memon[3], Ayesha Atique[4], Sajid Ali Majeedano[5], Muqaddas Fatima[6], Kanwal Ehsan Khan[7]

[1]Department of Pathology, People's University of Medical and Health Sciences for Women, Nawabshah, [2]Rahbar Medical and Dental College, Lahore, [3]Department Of Oral Pathology, Muhammad Dental College, Ibn-e-Sina University, Mirpurkhas, [4]Department of Periodontology, Faryal Dental College, Sheikhupura, [5]Department of Oral Medicine, Muhammad Dental College Mirpurkhas, [6]Department of Biosciences, COMSATS University Islamabad, Pakistan, [7]Faculty of Medicine, Tubingen Medical University, Tubingen, Germany.

ABSTRACT

Background: Oral cancer exists as a major international health challenge. Circulating tumor DNA (ctDNA) represents a non-invasive biomarker through liquid biopsy because it enables cancer detection disease monitoring and therapy response assessment. This study aimed at Pathological Insights into Liquid Biopsy by evaluating the role of circulating tumor DNA in oral cancer Prognosis and Therapy.

Methods: The research implemented an ethical-approved (CIIT-RBM/006/ISB) cross-sectional method from June to December 2024 to obtain 80 EDTA blood plasma samples from oral cancer patients mainly at Shaikh Zayed Hospital Lahore and PIMS Hospital Islamabad using consecutive sampling technique. firstly cell-free DNA was extracted followed by targeted sequencing to detect mutations that are specific to tumors. Using SPPS version 26. this study established a < 0.001 p-value as its statistical threshold using one-way ANOVA.

Results: There were 52 males and 28 females in this study with average age of 56.4 ± 12.3 years. The majority of patients were male (65%), with a higher prevalence of late-stage tumors (Stage III-IV).. Significantly higher ctDNA levels (35.7 ± 5.4) were associated with tumor stage, lymph node involvement with poor survival outcome. The mean ctDNA yield was significantly higher in tumor tissues than in adjacent normal tissues ($p < 0.001$).

Conclusion: The study demonstrated clinically significant evidence that ctDNA serves as a dependable biomarker for primary diagnosis which can be easily accessed through non-invasive methods in oral cancer cases. Ongoing large-scale studies should be followed to validate the discovered findings while developing consistent clinical procedures.

Keywords: Oral Cancer, Circulating Tumor DNA, Liquid Biopsy, Cancer Biomarkers, Tumor Progression.

Corresponding Author:
Dr. Kanwal Ehsan Khan,
Faculty of Medicine,
Tubingen Medical University,
Tubingen, Germany.
Email: kanwalehsankhan@gmail.com
ORCID: https://orcid.org/0009-0005-3749-4352
Doi: https://doi.org/10.36283/ziun-pjmd14-2/019

How to cite: Kalhoro R, Sarfraz F, Memon S, Atique A, Majeedano, SA, Fatima M, Khan KE Pathological Insights into Liquid Biopsy: Role of Circulating Tumor DNA in Oral Cancer Prognosis and Therapy. Pak J Med Dent. 2025 April ;14(2): 114-118. Doi: https://doi.org/10.36283/ziun-pjmd14-2/019.

Received: Mon, March 3, 2024 **Accepted: Fri**, April 11, 2025 **Published:** Sun, April 13, 2025

INTRODUCTION

Oral cancer exists as a common, aggressive tumor type that affects people worldwide, yet it remains difficult to detect until patients exhibit advanced symptoms, because current screening methods lack reliability [1]. The exploration of non-invasive medical tools has led to liquid biopsy emerging as a valuable oncology technique that employs circulating tumor DNA (ctDNA) examination. ctDNA is generated from apoptotic or necrotic tumor cells [2]. It contains genetic and epigenetic modifications that represent the tumor's molecular landscape, providing real-time insights into disease progression and therapy response [3].

Oral cancer develops through various stages that start with localized dysplasia that transforms into invasive carcinoma before spreading to the lymph nodes and other organs [4]. The development of oral cancer remains complex due to tumor heterogeneity, environmental factors (tobacco and alcohol consumption), and natural genetic predispositions to disease [5]. Invasive biopsy remains effective, but it produces limited results regarding tumor heterogeneity while requiring tissue samples [6]. The ctDNA analysis component of liquid biopsy solves these issues by allowing persistent tumor tracking through blood tests alone [7].

The latest research investigates how ctDNA works as a diagnostic tool, prognostic indicator, and therapeutic agent in different types of cancer, including lung, colorectal, and breast tumors [8]. Through ctDNA examination, doctors can recognize vital mutations in oral cancer, check for residual disease spread, and assess treatment responses to create individualized therapeutic plans [9]. The analysis of tumor mutations within circulating tumor DNA (CTDNA) helps scientists understand cancer evolution and therapeutic targets. It also provides information about treatment resistance pathways, which establish CTDNA as a vital instrument for personalized cancer treatment[10]. The study assesses the clinical value of circulating tumor DNA (ctDNA) in oral cancer to identify its ability as both a screening test and monitoring device for outcome prediction. The analysis of circulating tumor DNA provides an innovative approach for tracking tumor changes in real-time through non-invasive monitoring that allows for better assessments of therapeutic responses and personalized treatment selection in oral cancer cases.

Researchers conducted this study to examine how ctDNA functions pathologically within oral cancer, determining its use as an early detection biomarker and its potential for cancer prognosis and treatment monitoring. Standardization research needs to progress because this will enable clinical teams to utilize ctDNA-based procedures for treating patients through personalized approaches.

METHODS

In this Cross-Sectional Study from June 2024 to December 2024, following ethical approval (CIIT--RBM/006/ISB) using consecutive sampling techniques, 80 blood plasma EDTA samples were collected from oral cancer patients Shaikh Zayed Hospital Lahore and PIMS Hospital Islamabad via consecutive sampling technique using OpenEpi version 3.0.0 for sample size. Medical researchers extracted tumor tissues and adjacent normal tissues, which were taken 2 centimeters away from the cancerous area, after recording patient demographic and clinical data.

The scientists preserved all samples in RNA Later solution before placing them in -80°C storage for subsequent molecular tests. The procedure for nucleic acid extraction involved the use of TRIzol Reagent™: Catalogue# 15596026 as per the manufacturer's protocols. The NanoDrop instrument from IMPLEN GmbH, Germany, allowed for testing both RNA purity through its 260 nm absorbance measurement and concentration detection through its 280 nm absorbance. The high RNA purity was evident from a 260/280 ratio measurement near 2.1. The Solis Biodyne cDNA synthesis kit enabled first-strand cDNA synthesis using its standard procedure with 250 ng RNA. Real-time quantitative PCR (qPCR) performed gene amplification through the combination of gene-specific primers targeting ctDNA markers and β-actin primers for housekeeping control. The researcher performed the reaction three times using 5X SYBR Green qPCR Master Mix. The primer sequences were acquired from Macrogen for synthesis.

Statistical analysis was performed using SPSS version 26 and GraphPad Prism software applications. The Mann-Whitney test analyzed the relationship between clinical and pathological factors and

ctDNA concentrations. The researcher used paired sample t-tests to evaluate the relationship between tumor tissue and control tissue groups. The study established < 0.001 p-value as its statistical threshold. The evaluation of ctDNA forecasting potential in oral cancer patients based on group variations was determined using one-way ANOVA analysis.

RESULTS

Table 1: Patient Demographics and Clinical Characteristics

Variable	n (%)
Total Patients	80 (100%)
Age (Mean ± SD)	56.4 ± 12.3 years
Gender	
- Male	52 (65%)
- Female	28 (35%)
Tumor Stage	
- Stage I-II	26 (32.5%)
- Stage III-IV	54 (67.5%)
Lymph Node Status	
- Positive	40 (50%)
- Negative	40 (50%)

The study included 80 oral cancer patients aged between 25 and 80 years. The majority of patients were male (65%), with a higher prevalence of late-stage tumors (Stage III-IV).

Table 2: ctDNA Concentration and Purity Analysis

Sample Type	Mean ctDNA Concentration (ng/µl) ± SD	260/280 Ratio ± SD	p-value
Tumor Tissue	48.5 ± 6.2	2.08 ± 0.04	< 0.001
Normal Tissue	21.3 ± 4.8	2.06 ± 0.05	-

The mean ctDNA yield was significantly higher in tumor tissues than in adjacent normal tissues ($p < 0.001$).

Table 4: Prognostic Value of ctDNA in Oral Cancer

Survival Status	Mean ctDNA Levels (ng/µl) ± SD	p-value
Alive (≥3 years)	35.7 ± 5.4	< 0.001
Deceased (<3 years)	60.2 ± 7.1	-

Higher ctDNA levels were significantly associated with poor survival outcomes

These results strongly support the potential of ctDNA as a biomarker for oral cancer prognosis and treatment monitoring.

DISCUSSION

The study results demonstrated how circulating tumor DNA (ctDNA) functions as an important diagnostic biomarker for oral cancer prognostic evaluation, as well as for treatment monitoring. Advancement of tumor stage, along with lymph node metastasis and poor differentiation, directly correlates with elevated ctDNA levels, which reflects tumor burden and aggressiveness [11]. The data provides additional support for previous studies showing that ctDNA concentrations increase in advanced-stage cancers, thereby strengthening its use as a prognostic predictor [12]. The research showed that patients with elevated ctDNA levels experienced significantly worse survival statistics, thus confirming that ctDNA serves as a potential indicator of early disease

evolution. Multiple cancer studies demonstrate that elevated ctDNA concentrations are linked with reduced survival times between disease remissions [13,14]. Liquid biopsy has proven capable of detecting tumor burden without invasive procedures, thereby showing promise to enhance standard imaging methods, particularly in disease monitoring after treatment and residual disease identification [15].

The 260/280 purity ratio measurement (~2.08) confirmed that the extracted nucleic acids had high purity, comparable to standards used in quantitative PCR-based assays. Accuracy in mutation profiling and detection of OSCC-related genetic alterations depends heavily on this assessment for obtaining dependable results[16, 17]. Clinical implemen-tation of precision oncology shows promise, as researchers have previously found that TP53, NOTCH1, and PIK3CA driver mutations exist in head and neck cancer cell-free DNA tests [18].

The data showed important findings, with substantial elevated ctDNA levels in tumor regions compared to adjacent normal regions ($p < 0.001$), which confirmed ctDNA's tissue-specific character. Previous studies have indicated that other cancers show similar findings, as malignant tissue produces higher ctDNA levels than benign or premalignant lesions[19, 20]. The difference in ctDNA detection is essential for identifying both early-stage cases and patients prone to cancer recurrence following surgical interventions [21].

The research indicates that circulating tumor DNA (ctDNA) presents a promising substitute to conventional histopathological analysis, especially among patients who require non-invasive biopsy procedures. New developments in next-generation sequencing (NGS) and digital PCR technology have improved ctDNA detection sensitivity, thus enabling ongoing tumor monitoring, therapy resistance prediction, and relapse forecasting [22,23]. The combina-tion of these strategies with machine learning systems shows the potential to enhance risk classifi-cation procedures for oral cancer patients [24,25].

Some limitations need to be recognized regarding these positive findings. The findings obtained from this research may lack broad applicability because the study included only 80 participants from a single institution. A series of extensive multi-center research efforts with extended period follow-ups will be necessary to prove the practicality of ctDNA in determining treatment outcomes for patients. Standardization of ctDNA isolation procedures and quantification methods faces technical challenges because pre-analytical procedures, including blood sample collection and storage, as well as DNA sequencing controls, affect the reproducibility of results across laboratories.

CONCLUSION

The current research demonstrated substantial evidence that cell-free DNA in blood serves as an ideal non-invasive biomarker to assess oral cancer patient outcomes as well as monitor therapy progress. Future investigations should combine ctDNA evaluations with comprehensive liquid biopsy platforms by adding information about epigenetic markers and exosomal RNA to enhance diagnostic precision. Liquid biopsy techniques utilizing ctDNA have the potential to transform personalized oncology by eliminating the need for invasive biopsies, thereby streamlining patient care decision-making.

ACKNOWLEDGMENTS

None

FUNDING

None

CONFLICT OF INTEREST

None

ETHICAL APPROVAL

The study received ethical approval from Sheikh Zayed Hospital Lahore collaboration with COMSATS University under reference number (RBM/006/IRB).

AUTHORS' CONTRIBUTIONS

All authors contributed equally as per ICMJE.

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