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EGFR MUTATION ANALYSIS ON CIRCULATING PLASMA CELL-FREE DNA FRAGMENTOME AS AN EARLY DIAGNOSTIC TOOL FOR NON-SMALL CELL LUNG CANCER - A SINGLE CENTRE STUDY

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ABSTRACT

Background: Lung cancer is a leading cause of global cancer mortality, with approximately 1.8 million deaths annually and a low 5-year survival rate in developing nations. Early detection is critical for curative intervention, yet traditional tissue biopsies are often invasive and limited by tumor heterogeneity. This study evaluates the diagnostic and predictive utility of plasma cell-free DNA (cfDNA) for identifying Epidermal Growth Factor Receptor (EGFR) mutations as a non-invasive liquid biopsy alternative.

Methods: This experimental study, conducted at Tirunelveli Medical College and Hospital, involved 100 participants divided into two cohorts: Group 1 (N=50, newly diagnosed lung cancer) and Group 2 (N=50, high-risk individuals with a 30-year smoking history but negative conventional imaging and biopsies). cfDNA was extracted from plasma and analyzed via Quantitative PCR (qPCR) to detect mutations in exons 18, 19, 20, and 21 of the EGFR gene.

Results: In the lung cancer group, the mean cfDNA concentration was 2042.9 ng/mL, and EGFR mutations were identified in 78% of cases. The mutation profile showed a regional predominance of "uncommon" variants, specifically Exon 21 L861Q (38%) and Exon 20 insertions (34%). In the high-risk cohort, despite negative conventional investigations, liquid biopsy identified Exon 20 insertion mutations in 12% of patients (6/50). The mean cfDNA concentration in this group was significantly lower at 16.73 ng/mL, with higher levels noted among smokers.

Conclusion: Plasma cfDNA analysis is a highly effective, minimally invasive tool for the molecular profiling of lung cancer, particularly when tumor tissue is inaccessible. The detection of EGFR mutations in clinically suspicious but image-negative individuals underscores the predictive validity of liquid biopsy as an early screening tool. Recognizing regional mutation patterns, such as the high frequency of L861Q and Exon 20 insertions in South Indian populations, is essential for optimizing targeted Tyrosine Kinase Inhibitor (TKI) therapies.

KEYWORDS: EGFR, Lung cancer,cell-free DNA, Mutation

INTRODUCTION

The prevalence of lung cancer remains exceptionally high, with reports indicating approximately 1.8 million newly diagnosed cases and 1.6 million cancer-related deaths annually, accounting for 13% of all cancer diagnoses and 19.4% of cancer deaths respectively. (1 2) The overall 5-year survival rate for lung cancer highlights a significant global disparity, standing at approximately 15 per cent in developed countries compared to just 5 per cent in developing nations. (1 3)

The primary objective of early cancer detection is to identify malignant transformations in asymptomatic individuals through screening tests. (1) Identifying cancers at an early stage, or recognizing high-risk premalignant lesions, allows for curative or earlier interventions that significantly reduce mortality and morbidity. While actualizing early detection is complex, the emergence of blood-based cell-free DNA (cfDNA) testing offers a promising method to bridge current gaps in cancer screening(1 4)

For oncology patients, circulating tumor-derived DNA (ctDNA) is shed into the bloodstream, providing a critical opportunity for detection via liquid biopsy. This approach offers several unique advantages: the collection process is simple and non-invasive, leading to higher patient acceptance than traditional tissue biopsies. Because ctDNA is released from necrotic and apoptotic tumor cells throughout the body rather than from a localized tissue point, it may more accurately reflect a patient's overall tumor burden and metastatic capacity while minimizing the interference of tumor heterogeneity. Furthermore, ctDNA allows for the real-time dynamic monitoring of tumor burden and has demonstrated established clinical utility as a hematological tumor marker (7)

Technological advancements in ctDNA characterization have enabled the use of Epidermal Growth Factor Receptor (EGFR) gene mutation detection for first-line therapy selection. (3 6) In non-small cell lung cancer (NSCLC), EGFR

overexpression or mutations are observed in 43–89% of patients. (3 9) Over 90% of known tyrosine kinase domain mutations occur as short in-frame deletions in exon 19 or point mutations in exon 21 (3 9). These mutations result in the constitutive activation of signal transduction pathways, driving cell proliferation regardless of extracellular ligands. Currently, the cobas EGFR Mutation Test v2 is the only FDA-approved test for using ctDNA to detect these mutations, where a positive result is sufficient to initiate treatment with EGFR-Tyrosine kinase inhibitors. (3)Recent technological advancements in the characterization of ctDNA, has made first line therapy selection through detection of Epidermal Growth Factor Receptor (EGFR) gene mutations in lung cancer patients.(5)As on date, the only FDAcobas EGFR Mutation Test v2 is an acceptable test for use with ctDNA to detect the presence of EGFR gene mutations and a positive result is sufficient to start a first-line treatment with an EGFR-Tyrosine kinase inhibitors.(8)
This study aims to identify liquid biopsy-based biomarkers specifically through EGFR mutation analysis on plasma cfDNA Fragmentome in patients at risk for lung cancer. The research focuses on the diagnostic and predictive validity of liquid biopsy as a screening or early predictive tool, evaluating its performance against the current gold standard of tissue analysis (3)

MATERIALS AND METHODS

This experimental study, conducted at Tirunelveli Medical College and Hospital with Institutional Ethical Committee approval, utilized a study population divided into newly diagnosed lung cancer cases and individuals categorized as at-risk or clinically suspected cases. Participants were registered following independent clinical examinations by two specialists and met specific criteria: inclusion for Group 1 required positive imaging, biopsy, or FNAC results, whereas Group 2 consisted of individuals with a 30-year smoking history or suspicious clinical features despite negative CT, FDG-PET-CT, and biopsy/FNAC findings. Exclusion criteria across both groups included non-consent, the commencement of chemotherapy or radiotherapy, and significant co morbidities such as Diabetes Mellitus, Hypertension, Acute Coronary Syndrome, renal or liver disease, other cancers, Tuberculosis, or COPD.Institutional ethical committee approval was obtained.

Sample collection and DNA isolation:

For the laboratory analysis, 5 mL of peripheral venous blood was collected from participants in both cohorts using tubes coated with ethylene diamine tetra acetic acid (EDTA) to prevent coagulation and preserve DNA integrity. The plasma fraction was immediately separated from the cellular components through centrifugation at 9000 rpm for 10 minutes at a controlled temperature of 4°C. Following separation, the resulting plasma supernatant was carefully aspirated, labeled, and placed into ultra-low temperature storage at -80°C.
The extraction of cell-free DNA (cfDNA) was executed using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany), following the manufacturer's rigorous spin-column protocol designed for the isolation of highly purified nucleic acids. After the initial extraction, the samples underwent a secondary purification process using a DNA purifying kit to ensure the removal of any residual proteins or PCR inhibitors. The finalized, purified cfDNA was eluted into a volume of 70 µL of AE buffer (a 10 mM Tris-Cl, 0.5 mM EDTA solution) and stored at -20°C for long-term stability prior to molecular testing.
Molecular characterization and quantification were achieved through Quantitative PCR (qPCR) performed on a Real-Time PCR system. This assay was specifically designed to identify the spectrum of clinically relevant alterations—including deletions, insertions, and point mutations—occurring within the tyrosine kinase domain of the EGFR gene, specifically targeting exons 18, 19, 20, and 21. For the final comparative analysis, the patient sequence data were systematically partitioned into two distinct classes based on their clinical status: the confirmed non-small cell lung cancer (NSCLC) group and the high-risk/clinically suspected group

RESULTS

Lung Cancer Group

The lung cancer cohort (N=50) consisted of a study population where 98% of participants were between 40 and 90 years of age, with a mean age of 59.76 ± 9.64 years. Demographic analysis revealed a male predominance, with 36 (72%) males and 14 (28%) females. A history of chronic smoking was present in 56% of these patients, while the remaining 44% were non-smokers. The mean cell-free DNA (cfDNA) concentration was found to be 2042.9 ng/mL (SD: 11.58 ng/mL).

Table:1 EFGR mutations with respect to various exons in lung cancer patients.

EGFR mutation	Number(N=50)	Percentage(%)
Exon 21 L861Q	19	38%
Exon 20 3 Insertion	17	34 %
Exon 20 790 M	2	4%
Exon 21 L858R	1	2%
NO EGFR Expression	11	22%

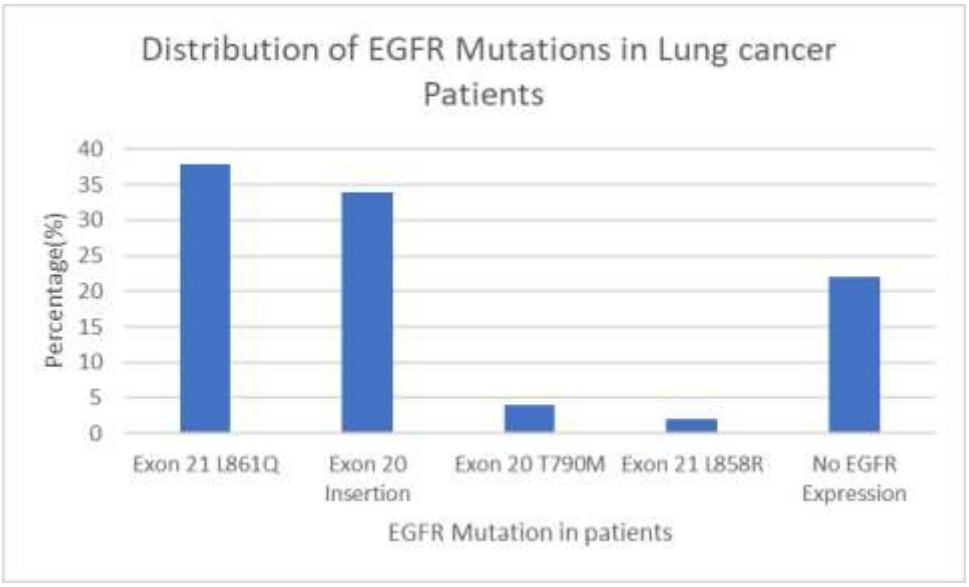

Figure:1 Molecular analysis of the EGFR gene identified expression in 39 patients (78%), while 11 cases (22%) were negative for expression.

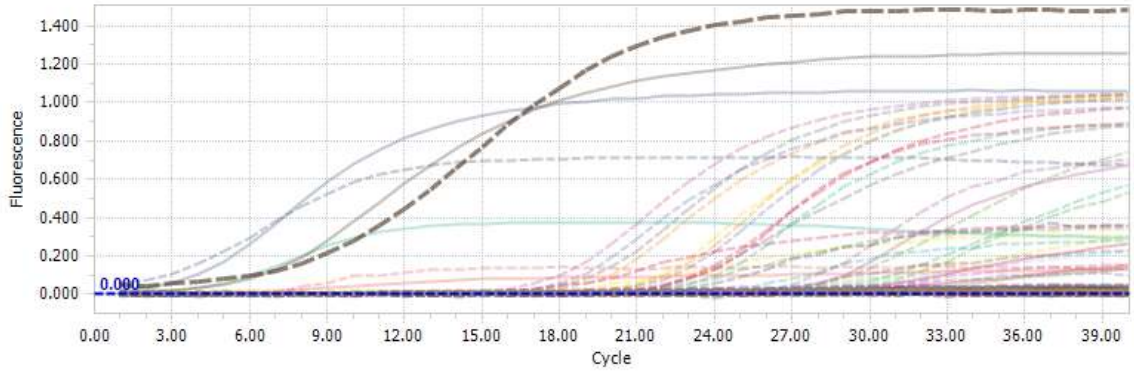

Figure:2 Real-time PCR (RT-PCR) amplification was documented for this group, showing a fluorescent signal proportional to the amount of amplified genetic material (Figure 2).

High-Risk and Clinically Suspicious Group

In the high-risk cohort (N=50), 72% of patients belonged to the 50–69 age group, with a mean age of 62.54 ± 9.99 years. This group was almost entirely male, consisting of 47 (94%) males and 3 (6%) females. A significant 90% of the cohort (45 patients) reported a smoking history exceeding 30 years. The mean cfDNA concentration was notably lower at 16.73 ng/mL (SD: 29.99 ng/mL), with higher levels observed among smokers compared to non-smokers.

Table:2 EFGR mutations with respect to various exons in High risk /clinically suspicious patients.

EGFRmutation	**Number(N=50)**	**Percentage(%)**
EXON 20 3 INSERTION	6	12%
NO EGFR Expression	44	88%

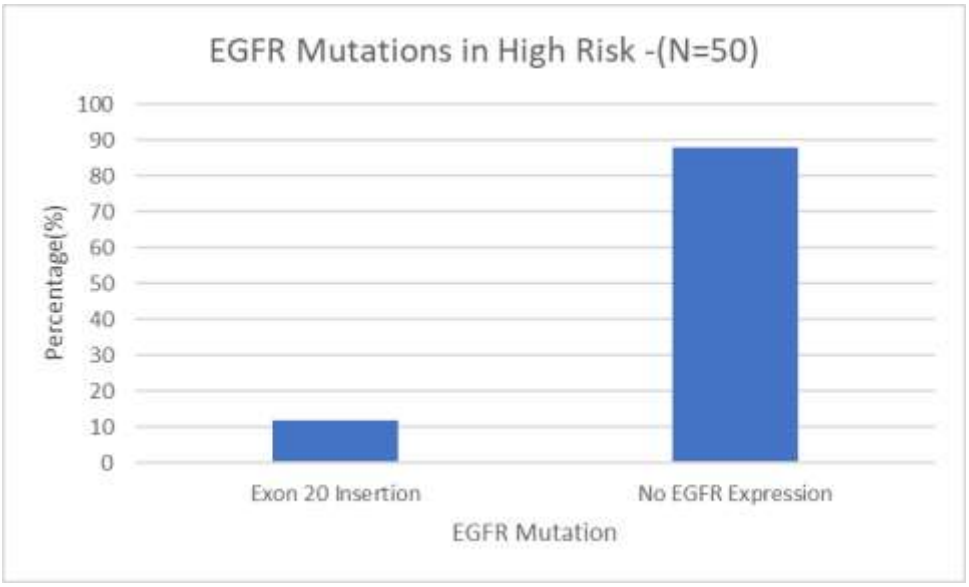

Figure:3 Within this group, EGFR Exon 20 insertions were identified in 6 patients (12%), while the remaining 44 patients (88%) showed no EGFR expression.

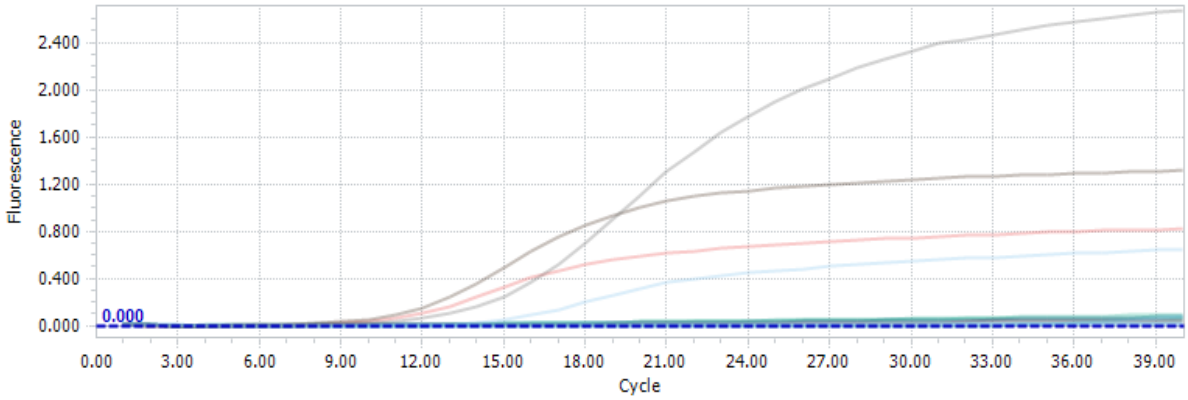

Figure:4 RT-PCR amplification graphs for this population demonstrated genetic material proportional to the fluorescent signal (Figure 4))

DISCUSSION

Demographic and cfDNA Profile

The present study observed a peak incidence of lung tumors in the 61–70 age group, with a significant male predominance (72%).This demographic trend aligns with global observations and regional data from Southern India, which report a higher incidence of lung carcinoma in males, largely attributed to higher smoking rates. In our lung cancer cohort, 56% were smokers, and their mean cell-free DNA (cfDNA) concentration (2126.9 ng/mL) was notably higher than that of non-smokers (1463.3 ng/mL). This disparity supports the established link between smoking-induced cellular damage and increased DNA shedding into the bloodstream.(1)

EGFR Mutation Prevalence and Clinical Discrepancies

A study by Feng et al. suggested that serum EGFR mutation rates are typically higher among females and non-smokers. In contrast, our findings revealed a higher frequency of mutations among male patients and non-smokers.(6)This variance underscores the potential influence of regional genetic or environmental factors specific to the South Indian population (9)

Our cohort displayed a distinct mutation profile compared to global and regional benchmarks. While global meta-analyses indicate that exon 19 deletions (~49%) and exon 21 L858R substitutions (~28–49%) are the most common sensitizing mutations, our study found exon 21 L861Q (38%) and exon 20 insertions (34%) to be the most prevalent.(12) This differs significantly from an Egyptian cohort where exon 19 deletions predominated (66.67%), suggesting that South Indian patients may harbor a higher frequency of "uncommon" variants(10)EGFR mutations in lung cancer occur primarily in exons 18-21, with significant variations in frequency and treatment response across different mutation types, such as the typically common L858R (exon 21) and exon 19 deletions that together comprise 85-90% of cases globally, versus uncommon variants like L861Q and exon 20 insertions that show distinct geographical distribution patterns and therapeutic sensitivities.(11,12)

Diagnostic Utility of Plasma cfDNA

The EGFR mutation detection rate of 78% in our study's plasma samples exceeds the 60.5% rate reported by Zhang et al.(13)This high detection rate further validates plasma cfDNA as a valuable tool for molecular profiling, especially when

tumor tissue is inaccessible.(13) Literature confirms that a positive plasma EGFR mutation result has a greater than 90% chance of being a true positive, indicating that the patient will likely benefit from EGFR Tyrosine Kinase Inhibitors (TKIs).(14) However, due to a reported false-negative rate of approximately 30% in liquid biopsies, a negative result should still be followed by a traditional tissue biopsy when possible(14)

Early Detection in High-Risk Populations

In the high-risk and clinically suspicious group, liquid biopsy identified EGFR exon 20 insertion mutations in 6 patients (12%).Remarkably, for four of these cases, conventional investigations including biopsy, bronchial wash, and PET scans were negative or inconclusive. (15)The remaining two patients expired before biopsy procedures could be performed, illustrating the aggressive nature of suspected malignancies in this cohort. These outcomes emphasize the utility of liquid biopsy as a predictive screening tool that can identify genetic markers of malignancy even when imaging and tissue-based methods fail.(15)

CONCLUSION

This study demonstrates that plasma cell-free DNA (cfDNA) analysis is a highly effective and minimally invasive tool for the molecular profiling and early detection of lung cancer. By identifying EGFR mutations in 78% of confirmed cases, our findings underscore the diagnostic validity of liquid biopsy as a robust alternative to traditional tissue analysis, particularly when malignancy is suspected but tissue is inaccessible.

The identification of EGFR Exon 20 insertion mutations in 12% of the high-risk cohort—including cases where conventional imaging and biopsies were negative—highlights the significant predictive value of cfDNA Fragmentome analysis. This suggests that liquid biopsy can bridge critical diagnostic gaps, enabling earlier intervention in asymptomatic or clinically suspicious individuals before advanced disease stages are reached.Furthermore, the mean cfDNA concentrations observed in this study suggest that elevated cfDNA levels serve as a clear indicator of malignancy, with notably higher levels found in smokers compared to non-smokers(1)Our results also reveal a distinct regional mutation profile in the South Indian population, characterized by a higher prevalence of "uncommon" variants such as Exon 21 L861Q (38%) and Exon 20 insertions (34%). Recognizing these regional variations is vital for selecting appropriate first-line Tyrosine Kinase Inhibitor (TKI) therapies.

In conclusion, while tissue biopsy remains the gold standard, plasma cfDNA testing offers a powerful complementary tool that enhances diagnostic accuracy, overcomes challenges associated with tumor heterogeneity, and provides a real-time monitoring capability that is more acceptable to patients. Future integration of this technology into routine clinical workflows is essential for reducing the high mortality and morbidity associated with delayed lung cancer diagnosis in India(2,7,10)

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Conflict of Interest

The authors have no conflicts of interest to disclose.

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