Al-Rafidain J Med Sci. 2025;9(1):209-213.
DOI: https://doi.org/10.54133/ajms.v9i1.2133

AJMS

Online ISSN (2789-3219)

Research Article

Correlation Between Tissue EGFR Mutations and Telomerase Activity in Tissue and Blood of Patients with Lung Cancer: Immunohistochemical and Molecular Study

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Received: 30 May 2025; Revised: 5 August 2025; Accepted: 14 August 2025

Abstract

Background: Lung cancer remains one of the leading causes of cancer-related mortality worldwide. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancer. Epidermal growth factor receptor (EGFR) mutations are known to drive tumor progression, while telomerase activity is a key mechanism in cancer cell immortality. ***Objective***: This study aims to investigate the correlation between EGFR mutations and telomerase activity in the blood of patients with lung cancer. ***Methods***: This study aims to investigate the correlation between EGFR mutations and telomerase activity in blood and tissue of patients with lung cancer. A cohort study was analyzed for EGFR mutations in tissue and telomerase activity levels in blood using molecular techniques such as polymerase chain reaction (PCR) and in tissue biopsy by using telomerase immunohistochemistry (IHC). ***Results***: The findings suggest a significant correlation between specific EGFR mutations in tissue and increased telomerase activity in blood. ***Conclusions***: This study provides insights into tumor prognosis, response to therapy, and the detection of tumor development.

Keywords: Epidermal growth factor receptor, Immunohistochemistry and PCR, Non-small cell lung cancer, Telomerase.

العلاقة بين طفرات EGFR ونشاط التيلوميراز في أنسجة ودم مرضى سرطان الرئة: دراسة كيميائية مناعية وجزيئية

الخلاصة

الخلفية: لا يزال سرطان الرئة أحد الأسباب الرئيسية للوفيات المرتبطة بالسرطان في جميع أنحاء العالم. يمثل سرطان الرئة ذو الخلايا غير الصغيرة (NSCLC) ما يقرب من 85٪ من سرطان الرئة. من المعروف أن طفرات مستقبلات عامل نمو البشرة (EGFR) تدفع تطور الورم، في حين أن نشاط التيلوميراز هو آلية رئيسية في خلود الخلايا السرطانية. **الهدف**: تهدف هذه الدراسة إلى التحقيق في العلاقة بين طفرات EGFR ونشاط التيلوميراز في دم مرضى سرطان الرئة. **الطرائق**: تهدف هذه الدراسة إلى التحقيق في العلاقة بين طفرات EGFR ونشاط التيلوميراز في الدم والأنسجة لدى مرضى سرطان الرئة. تم تحليل دراسة جماعية لطفرات EGFR في مستويات نشاط الأنسجة والتيلوميراز في الدم باستخدام التقنيات الجزيئية مثل تفاعل البوليميراز المتسلسل (PCR) وفي خزعة الأنسجة باستخدام الكيمياء المناعية للتيلوميراز (IHC). **النتائج**: تشير النتائج إلى وجود علاقة ذات دلالة إحصائية بين طفرات EGFR المحددة في الأنسجة وزيادة نشاط التيلوميراز في الدم. **الاستنتاجات**: تقدم هذه الدراسة رؤى حول تشخيص الورم والاستجابة للعلاج والكشف عن تطور الورم.

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Article citation: Ahmed FH, Al-Khalisy MH. Correlation Between Tissue EGFR Mutations and Telomerase Activity in Tissue and Blood of Patients with Lung Cancer: Immunohistochemical and Molecular Study. *Al-Rafidain J Med Sci.* 2025;9(1):209-213. doi: https://doi.org/10.54133/ajms.v9i1.2133

INTRODUCTION

Lung cancer is a major public health problem, ranking among the most common and deadly malignancies worldwide. NSCLC, the predominant form, is characterized by heterogeneous molecular alterations that drive tumorigenesis and influence treatment responses. Among these, EGFR mutations have been extensively studied, particularly in populations where the incidence of these mutations is high. EGFR mutations, primarily exon 19 deletions and L858R substitutions, lead to aberrant signaling pathways that promote tumor cell survival, proliferation, and resistance to apoptosis [1-5]. Telomerase, a ribonucleoprotein enzyme complex, plays a critical role in cellular immortality by maintaining telomere length, thus enabling continuous replication of malignant cells. The overexpression of telomerase is a hallmark of nearly 90% of human cancers, including lung cancer. Increased activity of telomerase has been linked to tumor progression, therapy resistance, and poor prognosis [6-10]. Recent studies have suggested the presence of a potential mechanistic link between EGFR signaling and telomerase activation. The EGFR pathway can upregulate telomerase reverse transcriptase (hTERT), the catalytic subunit of telomerase, through various oncogenic signaling cascades such as PI3K/AKT and RAS/MAPK pathways. This interplay raises critical questions regarding the role of EGFR mutations in regulating telomerase activity and how this interaction affects cancer progression, particularly in underrepresented populations [11,12]. Several researchers had studied EGFR mutations and telomerase activity independently. Research has shown that EGFR mutations are associated with increased cell

proliferation and resistance to apoptosis [3,13]. Concurrently, high telomerase activity has been linked to enhanced tumor aggressiveness and poor prognosis [12,14]. These studies demonstrated a positive correlation between EGFR mutations and telomerase expression in NSCLC patients, suggesting that telomerase upregulation may be an essential feature of EGFR-mutated tumors. However, regional and ethnic variations in genetic mutations necessitate localized studies to determine specific trends in Iraqi patients. Despite global advances in lung cancer research, there is a gap in understanding the molecular interplay between EGFR mutations and telomerase activity in blood. Identifying such correlations is crucial for improving diagnostic accuracy, personalizing targeted therapies, potentially integrating telomerase inhibitors into existing treatment schedules, and of great benefit in follow up the patient. This study seeks the relationship between tissue EGFR mutations and telomerase activity in blood and explores their clinical significance in the lung cancer cohort.

METHODS

Study design

A cross-sectional study was conducted in Al-Amal Hospital in Iraq, enrolling lung cancer patients diagnosed from October 2023 to November 2024.

Subject selection

Sixty patients have a diagnosis of NSCLC and on treatment with chemotherapy and/or radiation and the presence of other malignancies, primary and secondary tumors such as breast cancer metastasis to the lung. Another twenty samples presented with lung mass; biopsy had been taken and revealed tissue inflammation without any evidence of tumor.

Sample collection and processing

Tissue biopsies were obtained from lung cancer patients at the time of diagnosis for histopathological confirmation. In addition, some patients received radiotherapy or chemotherapy as part of their treatment plan; in these cases, blood samples were collected before each radiotherapy or chemotherapy session. DNA extraction was performed, followed by EGFR mutation analysis via polymerase chain reaction (PCR). Telomerase activity in blood was quantified using qPCR and in tissue using IHC.

IHC for tissue telomerase

Formalin-fixed, paraffin-embedded lung tumor tissue sections (5 µm thick) were mounted on positively charged glass slides. After deparaffinization and rehydration, antigen retrieval was performed using citrate buffer (pH 6.0) in an autoclave. The sections were incubated with a primary antibody against hTERT, followed by a secondary antibody conjugated to horseradish peroxidase (HRP). Visualization was achieved using 3,3′-diaminobenzidine (DAB) as the chromogen, which produced a brown precipitate indicating positive nuclear staining. Counterstaining was performed with hematoxylin. Stained slides were examined under a light microscope, and images were digitally captured for quantitative analysis.

Ethical considerations

Ethical approval was obtained from the Center of Training and Human Development, Baghdad Medical City, Ministry of Health, Republic of Iraq, “prior to data collection." Patient privacy was strictly maintained, and all personal identifiers were removed or coded to protect privacy. The study procedures, including the collection of tissue and blood samples, were explained to participants, and their right to withdraw at any time without affecting their medical care was emphasized. Data were stored securely and accessed only by the research team. No financial or coercive incentives were provided.

Statistical analysis

A *t*-test analysis was used to assess the relationship between tissue EGFR mutations and telomerase activity in blood. A p-value < 0.05 was considered statistically significant. The PCR results revealed a statistically significant difference in mean telomerase levels in blood and EGFR expression in tissue. Specifically, patients with EGFR levels > 20 IU/µL exhibited a significantly higher mean telomerase level compared to those with EGFR ≤ 20 IU/µL. With a p-value of 0.036, the null hypothesis was rejected at the 5% significance level.

RESULTS

IHC staining targeting telomerase expression in the tissue highlights regions with brownish coloration, signifying positive telomerase protein expression. These regions suggest active telomerase involvement, potentially correlating with areas of high proliferative activity or malignancy. Individual nuclei are clearly visible, with some cells demonstrating stronger telomerase staining (brown) compared to other nearby cells (Figure 1).

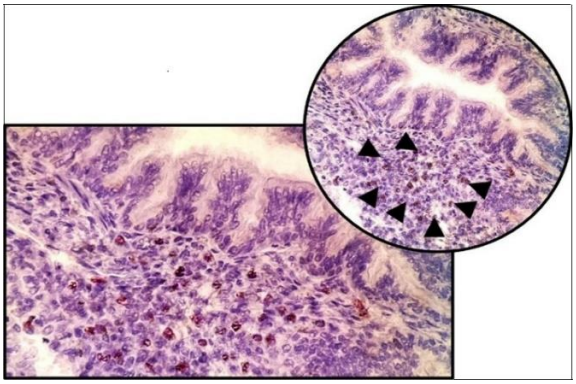

Figure 1: The lung tissue shows a dense population of cells, with varying levels of staining intensity. Areas with brownish coloration represent positive telomerase expression. Individual nuclei are visible, with some cells showing stronger telomerase staining (brown) compared to others. 400X. Immunohistochemistry (IHC) targeting telomerase expression.

Dense clusters of proliferating cells disrupt the normal alveolar architecture, with noticeable tissue thickening (cellular hyperplasia). These findings indicate pathological alterations in the lung tissue. Immunohistochemical (IHC) staining targeting telomerase expression in tissue highlights brown-stained areas (Figures 2 and 3A), indicating regions of positive telomerase activity.

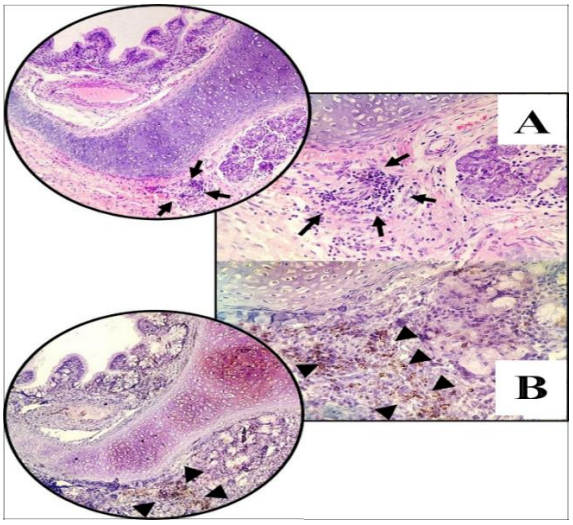

Figure 2: The slide showing bronchial area (Insite A: H&E, 40X and insite B: IHC, 40X) as follows: **A**) significant structural disorganization, with evidence of proliferative and abnormal cellular arrangements (arrows). 100X, H&E staining. **B**) Dense clusters of proliferating cells are visible, disrupting the normal bronchial architecture (head arrows). 100X, (IHC) targeting telomerase expression.

There is an apparent thickening of the lung tissue, likely due to cellular hyperplasia. Brown-stained areas indicate positive expression of telomerase. These regions correspond to cells actively expressing the telomerase enzyme (Figures 2 and 3). At higher magnification, the IHC staining reveals clearer details of telomerase expression at the nuclear level. The presence of inflammatory infiltrates, stained purple due to hematoxylin counterstaining, further supports the ongoing active pathological process (Figure 3).

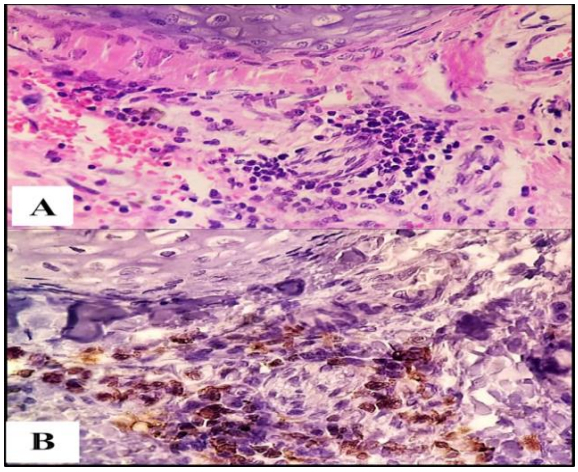

Figure 3: The slide showing bronchial area. **A**) high magnification of proliferative and abnormal cellular arrangements. 400X, H&E staining. **B**) Many nuclei are surrounded by telomerase-positive staining, which could inflammatory infiltrate seen in the background (stained purple due to hematoxylin counterstaining) further supports an active pathological process. 400X, (IHC) targeting telomerase expression.

The PCR outcomes, with a *p*-value of 0.036, reject the null hypothesis at the 5% significance level. This suggests that the mean telomerase level in the blood is significantly higher when tissue EGFR > 20 compared to when EGFR ≤ 20 (Table 1). 20 patients showed inflammatory changes without evidence of malignancy. These samples served as an important control group, helping to confirm the specificity of hTERT expression.

Table 1: Patients with EGFR mutations showed significantly higher telomerase activity in blood compared to the tissue levels of EGFR ($p< 0.01$).

Biomarkers	(< 20 IU/µL)	Correlation coefficient	(> 20 IU/µL)	Correlation coefficient
Telomerase (blood)	13.63±1.1	0.974	263.38±4.9	0.954
EFGR (tissue)	15.45±0.6		259.58±5.3	

Values were expressed as mean±SEM.

Immunohistochemistry and qPCR results showed no significant telomerase activity in these non-tumor tissues, supporting the conclusion that hTERT overexpression is associated with malignant transformation rather than inflammation. This distinction reinforces the potential diagnostic value of telomerase as a cancer-specific biomarker.

DISCUSSION

Telomerase activity exhibits significant variability in its distribution levels (wide variation across samples) [6]. The analysis of the correlation between EGFR in tissue and telomerase levels in blood reveals distinct relationships that may have biological implications. Our findings align with previous studies suggesting that EGFR-mutated lung cancer cells exhibit enhanced telomerase activity in the blood. This correlation may be due to EGFR-driven activation of telomerase reverse transcriptase (hTERT), the catalytic subunit of telomerase [15]. The strong association between these biomarkers suggests that targeting telomerase alongside EGFR inhibitors may enhance therapeutic efficacy. The differential staining of lung tissue indicates variability in telomerase activity among the cells, consistent with its known upregulation in cancerous tissues. These findings provide valuable insights into the role of telomerase in lung cancer pathology (Figures 1, 2, and 3). The intensity of staining varies, with some areas showing stronger signals, suggesting higher telomerase activity. Many nuclei are surrounded by telomerase-positive staining, correlating with cells actively expressing the enzyme (Figures 1, 2B, and 3B). This finding supports the hypothesis that elevated EGFR expression is associated with increased telomerase

activity, suggesting a potential molecular linkage that may be relevant to tumor progression or responsiveness to targeted therapies in lung cancer. However, the Chenet *et al*. 2024 study confirmed that inhibiting human telomerase reverse transcriptase (hTERT) enhances the therapeutic efficacy of Osimertinib, a third-generation EGFR tyrosine kinase inhibitor (TKI), in EGFR-mutant lung cancer [12]. This finding suggests that telomerase activity contributes to the survival and proliferation of EGFR-mutant cancer cells, and its inhibition can improve treatment outcomes [12]. These suggest a potential link between EGFR activity in tissue and telomerase expression in blood. This relationship could indicate that higher levels of EGFR are associated with increased telomerase activity, which might be relevant in contexts such as tumor progression or the response to cancer treatment. EGFR is a well-known oncogene, and its positive association with telomerase could reflect mechanisms that promote cellular proliferation and resistance to apoptosis. Another study by Wei *et al*. (2015) indicated that telomerase and telomere function might be essential for the carcinogenesis of EGFR-mutant NSCLC. The research found an association between the TERT polymorphism rs2736100-C and EGFR mutations in NSCLC patients, implying a potential link between telomerase activity and EGFR mutation status [16]. Furthermore, Goh *et al*. (2018) demonstrated that NSCLC caused by EGFR mutation is related to the telomerase reverse transcriptase gene, confirming the link between telomerase activity and EGFR mutations [17]. These findings suggest a potential correlation between telomerase activity and EGFR mutation status in NSCLC, with implications for understanding tumor biology and developing targeted therapies. Furthermore, those patients with high telomerase activity and EGFR mutations tend to have more aggressive tumors and may respond differently to EGFR-targeted therapy.

Study limitations

There are various restrictions on this study. The sample size was relatively small, which may affect the reliability and generalizability of the findings. It focused exclusively on non-small cell lung cancer (NSCLC), limiting applicability to other lung cancer types such as small cell lung cancer (SCLC). Only a selected group of molecular markers (EGFR and telomerase) were assessed, while other relevant biomarkers were not included, such as ALK, ROS1, KRAS, TP53, PD-L1, ctDNA, and CEA. Additionally, the study required long-term follow-up, which is important for evaluating treatment response.

Conclusion

This study provides evidence that tissue EGFR mutations are significantly correlated with increased telomerase activity in blood of Iraqi lung cancer patients. These findings highlight the potential for integrating telomerase inhibitors into EGFR-targeted therapy treatments. Future studies with larger sample sizes and diverse populations are necessary to validate these results. The study provided that EGFR mutation is significantly correlated with an increase in telomerase activity in tissue of lung cancer. Besides, the level of tissue telomerase reflected its level in the blood. Since telomerase activity reflected tumor aggressiveness and response to chemotherapy and radiotherapy, the level of telomerase in blood will also reflect this point. To summarize all these results, the researchers provide that the level of telomerase enzyme in the blood could be used as a preliminary, easy method to insure or exclude lung cancer from other benign lung masses. Also, it could be easier to follow up with the patients with lung cancer regarding their response to treatment and also to follow the patients instead of doing a CT scan of the chest to follow up with the patient or a PET scan to follow up on secondary metastases since examining telomerase level in blood serum is easier, noninvasive, and does not expose the patient to radiation, besides its cheaper cost. The inclusion of 20 inflammatory, non-tumor lung samples strengthened the study's conclusions by serving as a negative control. The absence of hTERT expression in these samples confirmed that telomerase activation is specific to malignant tissue and not a feature of inflammation. This supports the reliability of hTERT as a cancer-specific biomarker for lung cancer diagnosis and monitoring. This work adds to earlier findings by directly comparing the expression of telomerase (hTERT) in peripheral blood and lung tumor tissue from the same patient group. Few studies have systematically connected circulating hTERT with matched tissue expression, despite earlier research suggesting that it may be a useful diagnostic marker. Our findings show a high degree of agreement with blood-based detection, in addition to confirming the specificity of telomerase activation in malignant tissue. The therapeutic usefulness of liquid biopsy for noninvasive monitoring is supported by this dual analysis, which provides a more reliable and useful substitute for tissue-based diagnostics documented in previous research.

Conflict of interests

The authors declared no conflict of interest.

Funding source

The authors did not receive any source of funds.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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