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Epidermal Growth Factor Receptor Expression and Genetic Alterations in Iraqi Lung Adenocarcinoma Patients: A Cross-Sectional Study

Esraa Aldujaily[1*], Ali Al-Fatlawi[2]

[1]Department of Pathology and Forensic Medicine, College of Medicine, University of Kufa, Najaf, Iraq.
[2]Information Technology Research and Development Center, University of Kufa, Najaf, Iraq.
[*]Correspondence to: Esraa Aldujaily (E-mail-esraa.aldujaily@uokufa.edu.iq)
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Abstract

Objective: To characterize the molecular profile of Iraqi patients with lung adenocarcinoma (LUAD), including epidermal growth factor receptor (EGFR) protein expression and mutations.

Methods: This cross-sectional study was conducted at the Middle Euphrates Oncology Center (Najaf, Iraq) between January 2022 and December 2023 and involved 28 patients with histologically confirmed lung adenocarcinoma. EGFR IHC positivity was evaluated using immunohistochemistry (Dako/Agilent system), and the mutation status was assessed via targeted next-generation sequencing (NGS) (Ion Torrent PGM). Written informed consent was obtained from all participants, and the study protocol was approved by the Institutional Review Board of the University of Kufa.

Results: EGFR IHC positivity was observed in 14 (50.0%) cases and was significantly associated with female sex and high-grade tumors ($P < 0.001$). EGFR mutations were detected using NGS in 15 patients (53.6%). Co-occurring Kirsten rat sarcoma viral oncogene homolog (KRAS) and tumor protein p53 (TP53) were found in 12 (42.9%) and 8 (28.6%) cases, respectively. Co-occurrences of EGFR and KRAS mutations have been observed in several tumors. The specific prevalent variants included EGFR exon 19 deletions, L858R, and KRAS G12C/G12V.

Conclusion: This study revealed a distinct molecular profile of Iraqi LUAD, characterized by a high frequency of EGFR and KRAS alterations. These findings support the potential utility of EGFR IHC as a cost-effective screening tool and underscore the need for accessible molecular testing and targeted therapies in Iraq.

Keywords: Receptor, epidermal growth factor, adenocarcinoma of lung, mutation, immunohistochemistry, high-throughput nucleotide sequencing

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide, causing approximately 1.8 million deaths annually.[1] In Iraq, the incidence has increased substantially from 2005 to 2019, with LUAD being among the most common NSCLC subtypes.[2,3] LUAD is commonly driven by somatic alterations in EGFR, KRAS, and TP53, which influence tumorigenesis and response to TKIs.[4,5] This study focuses on the Middle East and North Africa (MENA) region, with a comparative context provided by Western countries (such as the US and Europe) and East Asian populations (e.g. China and Japan). Published data show that EGFR mutations occur in 10–15% of Western LUAD patients and in 30–50% of East Asian patients.[2,6] In contrast, KRAS mutations are reported more frequently in Western populations (25–30%) and are often mutually exclusive of EGFR alterations.[5,7,8] TP53 mutations—markers of genomic instability—exceed 50% in many global LUAD series.[3] Despite the global patterns, genomic data from MENA is still limited. Iraq has a distinct population structure and a high smoking prevalence (approximately 30% of adults), with limited access to molecular diagnostics that may affect mutation detection rates and workflows.[2,3] However, it is important to note that smoking status data were not available for this study. This limitation may hinder the interpretation of the association between smoking and mutations observed in this study. Additionally, the distribution of clinically actionable EGFR mutations (such as exon 19 deletions and L858R) is largely uncharacterized in Iraqi LUAD, while KRAS G12C mutations remain uncharacterized. Owing to logistical barriers, IHC is often prioritized over NGS for testing, despite IHC's lower sensitivity for some EGFR variants.[9,10]

Previous studies have demonstrated substantial variation in LUAD driver mutations across regions: EGFR prevalence is higher in East Asian than in Western cohorts, KRAS is more frequent in Western populations and often inversely related to EGFR, and TP53 alterations are commonly found across global datasets. However, molecular data from MENA, including Iraq, remains sparse, limiting direct regional comparisons. In many Middle Eastern settings, local testing capacity often relies on IHC rather than NGS for EGFR assessment. These factors justify combining EGFR IHC with targeted NGS to generate a molecular profile specific to the Iraqi population and allow for a more accurate comparison with established Western and East Asian datasets.

This study aimed to characterize EGFR protein expression and mutations in Iraqi LUAD patients and assess their associations with clinicopathological features. The primary hypothesis is that EGFR mutations are relatively frequent and may co-occur with KRAS mutations. It was further anticipated that EGFR IHC positivity would be associated with female sex and advanced tumor grade or stage, potentially differing from the patterns observed in Western and East Asian populations.

Materials and Methods

Study Design, Patient Selection, and Clinicopathological Parameters

A single-center, cross-sectional retrospective study was conducted involving patients with histologically confirmed LUAD treated at the Middle Euphrates Oncology Center (Najaf, Iraq) from January 2022 to December 2023. Of the 60 evaluated patients, 28 consecutively eligible patients met the predefined criteria and were included in this study. The inclusion criteria were histologically confirmed LUAD, availability of a formalin-fixed, paraffin-embedded (FFPE) block with at least 10% viable tumor cells, complete clinicopathological data (age, sex, grade, and stage), and written informed consent. The exclusion criteria were insufficient tissue for IHC/NGS and prior targeted therapy. All patients underwent EGFR protein expression assessment using both IHC and targeted NGS. Resource constraints typical of single-center studies, such as limited NGS runs and assay costs, determined the final sample size and framed the study as exploratory.

Sample Size Rationale and Power

Resource limitations restricted the study to 28 cases, resulting in an exploratory design. The study was powered to detect large effects (Cohen's $w \approx 0.6$) at $\alpha = 0.05$, with 80% power, and required a minimum of 24 participants. The final sample size of 28 met this requirement. The study achieved approximately 80% power for large effects but was underpowered for small-to-moderate effects, resulting in wide confidence intervals that necessitate validation in larger cohorts. The single-center design and limited sample size may limit the generalizability of our findings to all Iraqi and MENA patients with LUAD. We extracted the age at diagnosis (continuous and grouped as 35–50, 50–65, and > 65 years), sex, grade (WHO criteria; Grade II = moderately differentiated; Grade III = poorly differentiated), and stage (AJCC 8th edition TNM Stage II–IV).[11–14] The tumor grade was determined based on signed histopathology reports. Data were anonymized and coded prior to analysis.

Immunohistochemistry (IHC)

FFPE lung carcinoma sections (4 μm) were stained for EGFR using the EnVision system (Dako/Agilent, Santa Clara, CA, USA), according to the manufacturer's instructions.[15] The primary antibody used was mouse monoclonal anti-human EGFR (Dako M3563) at a dilution of 1:50 in the EnVision FLEX Antibody Diluent (K8006). Each run included positive (BioSB BSB-5476) and negative (omission of primary antibody) controls. The sections were deparaffinized in xylene, rehydrated, and subjected to heat-induced epitope retrieval in a high-pH Target Retrieval Solution at 90°C for 30 min. Endogenous peroxidase was quenched with Peroxidase-Blocking Reagent, followed by 60-minute primary antibody incubation at room temperature. Detection was performed using the EnVision secondary reagent and DAB chromogen, and the slides were counterstained with hematoxylin. Two pathologists scored the EGFR IHC, and the inter-rater agreement was measured using Cohen's κ.

EGFR IHC Scoring and Interpretation

Tumor cell membrane staining was scored from 0 to 3. Negative: 0 (none) or 1+ (faint, incomplete in ≥10%). Positive: 2+ or 3+ (complete, weak moderate or strong in ≥10% of cases). IHC reflects EGFR abundance and not mutation status. The non-neoplastic epithelium with 0 or 1+ staining was negative. Internal positive and negative controls were also included in the quality assessment. Only membranous staining of the tumor cells was scored, and cytoplasmic staining was ignored. The scores were based on the following patterns and proportions:

- 0: < 10% of tumor cells with membranous staining (any intensity).
- 1+: Incomplete/partial membranous staining in ≥10% of tumor cells.
- 2+: Complete membranous staining of weak to moderate intensity in ≥10% of tumor cells.
- 3+: Complete membranous staining of strong, uniform intensity in ≥10% of tumor cells.

Next-Generation Sequencing (NGS)

DNA Extraction and Quality Control

DNA was extracted from FFPE tissues using the QIAamp DNA FFPE Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. When needed, deparaffinization was extended to 30 min to improve the yield of scant tissue.[16] Extracts meeting A260/A280 > 1.8 and ≥10 ng/μL proceeded with library preparation. Positive and negative extraction controls were included in each batch.

Library Preparation and Sequencing

Libraries were prepared with the Ion AmpliSeq Library Kit 2.0 using the Ion AmpliSeq Colon and Lung Research Panel v2 (22 genes; 504 hotspots). Sequencing was performed on an Ion Torrent PGM to a mean depth of approximately 500× (range 380–720×). Where feasible, runs are targeted to achieve a high on-target rate and uniformity consistent with panel specifications.[17-19] Practical alternative (platform-agnostic) and for amplicon workflows (Ion/Illumina), 10–50 ng FFPE DNA was amplified (e.g., EGFR exons 18–21, TP53 hotspots), adapters/indices were added as required, libraries were purified and quantified (beads, TapeStation, qPCR), and sequenced to ≥1,000–2,000× raw depth when using UMIs to enable a 1–2% VAF limit of detection (LOD), per validation.

Variant Calling and Annotation

The reads were aligned, and the variants were called in Ion reporters (hg19). Filtering thresholds were VAF ≥5% and minimum depth ≥200× to accommodate FFPE DNA and tumor heterogeneity.[5] Variants were annotated using COSMIC v92, dbSNP build 153, and SIFT 4G. Standard pipelines also cross-referenced ClinVar, gnomAD, OncoKB, and VEP/ANNOVAR as appropriate.[17-19] Bioinformatics analysis was performed by a certified analyst and independently reviewed by a second specialist.

Controls and Quality Assurance

Each sequencing run included: (i) positive control DNA with known EGFR mutations (e.g., commercial multiplex reference standards), (ii) no-template controls, and (iii) periodic commercial FFPE reference material to monitor assay drift. Index cross-talk and sample identity were checked when unique dual indices or SNP fingerprinting were available. Library and run

QC followed panel/platform recommendations (e.g., percentage of targeted bases ≥100× and ≥500×, mean on-target coverage, and uniformity thresholds).[17-19]

Assay Performance and Validation

The LOD of the assay was established empirically during validation (typical 2–5% VAF with UMI workflows; 5–10% without UMIs). Analytical sensitivity and specificity were determined using a dilution series of reference standards and orthogonal confirmation (e.g., Sanger or ddPCR) for a subset of variants. It is important to acknowledge that technical challenges, such as the varying quality of FFPE samples and the limited scope of the panel used, may have affected the sensitivity and specificity of the assays. These factors should be considered when interpreting the results.

Variant Interpretation and Reporting

Variants were classified according to the AMP/ASCO/CAP tiers (I–IV). EGFR activating mutations (exon 19 deletions, L858R) and resistance alterations (T790M, C797S, exon 20 insertions) were flagged as actionable, and relevant co-alterations (e.g., TP53, PIK3CA, MET, and KRAS) were reported in the clinical context per the guidelines and database curation.[5,17-19]

Primary Analytic Endpoints and IHC–NGS Concordance

The primary endpoint was the diagnostic concordance between EGFR IHC positivity (2+/3+) and the presence of any pathogenic/likely pathogenic EGFR mutation by NGS. Using NGS as the reference standard, we estimated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), overall accuracy, and Cohen's κ with 95% confidence intervals (Wilson's method for binomial proportions). IHC was considered positive when membrane staining scored 2+ or 3+ per predefined criteria, and negative when 0/1+.

Ethics and Data Abstraction

The protocol was approved by the University of Kufa (MEC-60; 02 Oct 2024).

Statistical Analysis

Statistical analyses were performed using SPSS v28.0 (IBM, USA). Categorical associations were evaluated using Pearson's chi-square or the likelihood-ratio test as appropriate; continuous variables were reported as mean ± standard deviation. All analyses were unadjusted owing to the limited sample size. Confidence intervals (95% CI) were calculated for the prevalence estimates and cross-tabulations. Smoking status data were unavailable; therefore, the analyses were not adjusted for potential confounders. No imputation was required because the complete data were available for the included variables. A post-hoc power calculation (G*Power 3.1) indicated an approximately 80% power ($\alpha = 0.05$) to detect large effect sizes; smaller effects would require larger cohorts. A two-sided $p \leq 0.05$ was considered statistically significant.

Results

Patient Demographics and Clinicopathological Characteristics

Table 1 presents the clinicopathological and molecular characteristics of the patients in this study ($N = 28$). The population

Table 1. **Clinicopathological and molecular characteristics of Iraqi lung adenocarcinoma patients ($N = 28$)**

Characteristic	Study group ($N = 28$) *n* (%)
Age (years):	
35–50	6 (21.4%)
50–65	8 (28.6%)
> 65	14 (50.0%)
Sex:	
Male	13 (46.4%)
Female	15 (53.6%)
EGFR mutation status (NGS):	
Mutated	15 (53.6%)
Wild type	13 (46.4%)
EGFR expression status (IHC):	
Positive (+)	14 (50.0%)
Negative (–)	14 (50.0%)
EGFR expression scoring (IHC):	
Negative (0)	4 (14.3%)
Low (1+)	10 (35.7%)
Moderate (2+)	4 (14.3%)
High (3+)	10 (35.7%)
Genetic driver mutation prevalence:	
AKT1	2 (7.1%)
BRAF	1 (3.6%)
CTNNB1	1 (3.6%)
ERBB4	2 (7.1%)
KRAS	12 (42.9%)
PIK3CA	1 (3.6%)
TP53	8 (28.6%)
Tumor grade:	
II	13 (46.4%)
III	15 (53.6%)
Tumor stage:	
II	14 (50.0%)
III	11 (39.3%)
IV	3 (10.7%)

EGFR; epidermal growth factor receptor; KRAS; Kirsten rat sarcoma viral oncogene homolog; TP53; tumor protein p53; IHC; immunohistochemistry; NGS, next-generation sequencing; AKT1, serine/threonine kinase 1; BRAF, B-Raf proto-oncogene, serine/threonine kinase; CTNNB1, Catenin beta 1 (β-catenin); ERBB4, Erb-B2 receptor tyrosine kinase 4 (HER4); PIK3CA, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PI3Kp110α). Multiple mutations per tumor are possible; percentages reflect the proportion of cases with ≥1 mutation in the specified gene.

had a mean age of 60.8 years, with a majority of patients (50.0%) aged over 65 years. Most patients were female (53.6%). EGFR mutations were detected by NGS in 15 patients (53.6%), while EGFR IHC positivity was observed in 14 patients (50.0%). Among the other driver mutations, KRAS was the most prevalent (42.9%), followed by TP53 (28.6%). Most tumors were poorly differentiated (Grade III, 53.6%) and presented as Stage II (50.0%) or III (39.3%).

EGFR Protein Expression by Immunohistochemistry

EGFR expression, as assessed by IHC, is shown in Figure 1. Positive cases, as represented in Panel A, demonstrated strong, complete membranous staining with cytoplasmic enhancement at 10× magnification. Negative cases, as shown in Panel B, exhibited no specific membranous staining at the same magnification.

EGFR IHC Positivity

Table 2 compares the clinicopathological and molecular features of patients with EGFR IHC-positive (n = 14) and IHC-negative (n = 14) tumors. EGFR IHC positivity was significantly associated with female sex (92.9% vs. 14.3%, $P < 0.001$), Grade III tumors (92.9% vs. 14.3%, $P < 0.001$), and the presence of an EGFR mutation on NGS (100.0% vs. 7.1%, $P < 0.001$). No significant differences were observed in

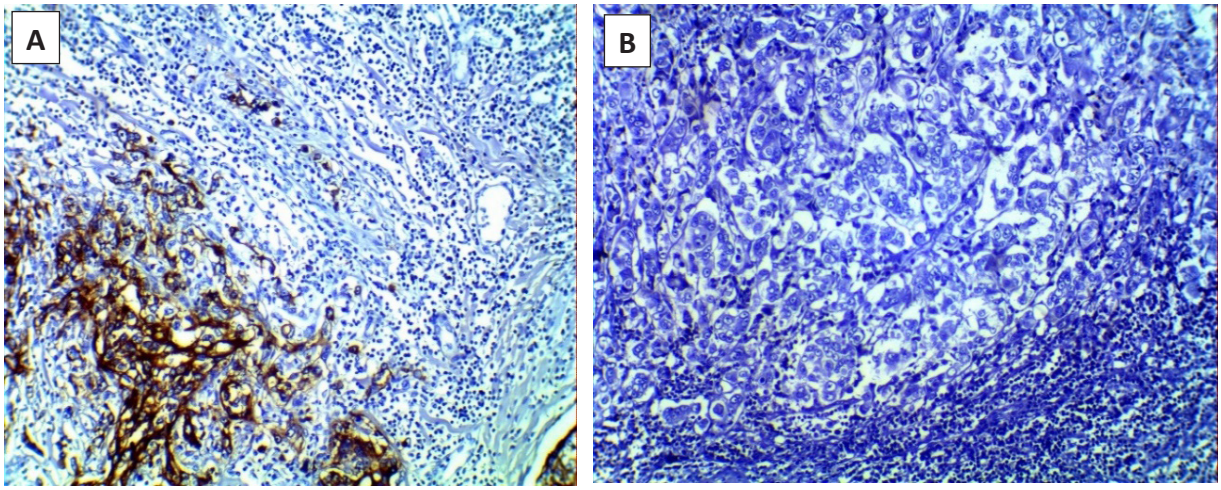

Fig. 1 **Representative images of EGFR immunohistochemistry expression. (A) A case with positive EGFR expression, demonstrating strong membranous staining with cytoplasmic enhancement (10× magnification). (B) A negative case, showing no specific staining, at the same magnification (10×).**

Table 2. **Clinicopathological and molecular characteristics by EGFR expression status (positive and negative expressions)**

Variable	Total ($N = 28$) n (%)	EGFR positive expression ($n = 14$) n (%)	EGFR negative expression ($n = 14$) n (%)	P-value
Age > 65 years	14 (50.0%)	6 (42.9%)	8 (57.1%)	0.70
Female	15 (53.6%)	13 (92.9%)	2 (14.3%)	< 0.001*
Grade III	15 (53.6%)	13 (92.9%)	2 (14.3%)	< 0.001*
Stage II	14 (50.0%)	5 (35.7%)	9 (64.3%)	0.13
Stage III	11 (39.3%)	7 (50.0%)	4 (28.6%)	0.45
Stage IV	3 (10.7%)	2 (14.3%)	1 (7.1%)	1.00
EGFR mutated	15 (53.6%)	14 (100.0%)	1 (7.1%)	< 0.001*
KRAS	12 (42.9%)	6 (42.9%)	6 (42.9%)	1.00
TP53	8 (28.6%)	3 (21.4%)	5 (35.7%)	0.68
AKT1	2 (7.1%)	1 (7.1%)	1 (7.1%)	1.00
BRAF	1 (3.6%)	0 (0.0%)	1 (7.1%)	1.00
CTNNB1	1 (3.6%)	1 (7.1%)	0 (0.0%)	1.00
ERBB4	2 (7.1%)	1 (7.1%)	1 (7.1%)	1.00
PIK3CA	1 (3.6%)	0 (0.0%)	1 (7.1%)	

EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma viral oncogene homolog; TP53, tumor protein p53; IHC, immunohistochemistry; AKT1, serine/threonine kinase 1; BRAF, B-Raf proto-oncogene, serine/threonine kinase; CTNNB1, Catenin beta 1 (β-catenin); ERBB4, Erb-B2 receptor tyrosine kinase 4 (HER4); PIK3CA, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PI3K p110α). Percentages of genetic mutations reflect the proportion of patients within each column with ≥1 mutation in the specified gene.
*A P-value less than 0.05 was considered statistically significant.

the distribution of KRAS, TP53, or other driver mutations between the two groups.

Concordance Between EGFR IHC and NGS Mutation Status

We evaluated the diagnostic concordance between EGFR IHC positivity and EGFR mutation status (NGS) as specified in the analytical plan. Among the 15 patients with EGFR mutations identified by NGS, 14 were IHC-positive (93.3%). Of the 13 wild-type EGFR cases, all 13 were IHC-negative (100%). This resulted in a sensitivity of 93.3% (95% CI 68.1–99.8), specificity of 100% (95% CI 75.3–100), positive predictive value (PPV) of 100% (95% CI 76.8–100), and negative predictive value (NPV) of 92.9% (95% CI 66.1–99.8). The 2×2 counts were as follows: True Positive (TP) = 14, False Negative (FN) = 1, True Negative (TN) = 13, and False Positive (FP) = 0. However, these estimates were derived from a small study and associated with wide confidence intervals.

Associations Between EGFR IHC Positivity and Co-occurring Mutations

Table 3 shows the association between EGFR IHC positivity and the presence of concurrent off-target KRAS or TP53 mutations. There was no significant association between EGFR IHC positivity and KRAS mutations (OR = 1.00, 95% CI 0.22–4.56, $P = 1.00$). A non-significant inverse relationship was observed between EGFR IHC positivity and TP53 mutations (OR = 0.49, 95% CI 0.09–2.59, $P = 0.68$).

EGFR and Co-occurring Genetic Alterations Identified by NGS

Table 4 shows the spectrum of genetic alterations detected by NGS. Concurrent mutations in *TP53* included both missense and truncating events such as Y234C, L299fs, and R273L. *KRAS* mutations were present in 12 patients (42.9%), with G12C and G12V being the predominant variants. *EGFR* mutations across exons 18–21 were identified in 15 patients (53.6%), with exon 19 deletions and exon 20 insertion/substitution variants being the most frequent. The observed co-occurrence of *EGFR* and *KRAS* mutations in this cohort contrasts with the pattern of mutual exclusivity often reported in Western populations.[4,7,20]

Discussion

This exploratory study of patients with LUAD revealed a molecular profile with a high prevalence of both *EGFR* (53.6%) and *KRAS* (42.9%) mutations. The observed *EGFR* mutation frequency approaches the upper range reported in East Asian populations, while the *KRAS* rate notably exceeds common regional averages and is higher than that reported in Latin American and Western consortia.[20,21] Several tumors harbor co-occurring *EGFR* and *KRAS* mutations, a pattern that contrasts with the typical mutual exclusivity reported in many Western cohorts and may suggest distinct underlying biology.[4,7,20] In this study, we observed a high degree of concordance between EGFR protein expression by IHC and the presence of an *EGFR* mutation by NGS, with a sensitivity of 93.3% and specificity of 100%. This preliminary finding suggests that IHC could serve as a useful and cost-effective initial screening tool in resource-constrained settings to triage patients for confirmatory molecular testing. However, it is critical to emphasize that these performance estimates are derived from a small sample size and are associated with wide confidence intervals. The single false-negative case underscores that IHC cannot replace gold-standard genomic assays for definitive treatment decisions. Therefore, while promising, the diagnostic performance of EGFR IHC requires validation in larger, prospective Iraqi and regional cohorts. The variant spectrum includes canonical *EGFR* alterations (exon 19 deletions, L858R) and less common variants, highlighting the importance of comprehensive genotyping to inform treatment selection for both common and uncommon mutations.[22] *EGFR* IHC positivity was significantly associated with female sex and higher tumor grade ($P < 0.001$), aligning with established clinicopathological correlations.[4,5,7] The enrichment of *EGFR* IHC positivity in higher-grade tumors may reflect its role in more aggressive disease phenotypes, consistent with studies linking specific molecular profiles to tumor progression and heterogeneity.[23,24] Prevalent *KRAS* codon 12 mutations (e.g., G12C, G12V) and various *TP53* mutations were identified, supporting the clinical relevance of emerging targeted therapies.[3,8,25] The prevalence of *TP53* mutations (28.6%) was lower than in some large series, a finding potentially influenced by panel design or cohort size;

Table 3. **Association between EGFR expression status and concurrent off-target mutations**

Mutation	EGFR negative expression ($n = 14$) n (%)	EGFR positive expression ($n = 14$) n (%)	OR (95% CI)	*P*-value
KRAS	6 (42.9%)	6 (42.9%)	1.00 (0.22–4.56)	1.00
TP53	5 (35.7%)	3 (21.4%)	0.49 (0.09–2.59)	

KRAS, Kirsten rat sarcoma viral oncogene homolog; TP53, tumor protein p53; EGFR, epidermal growth factor receptor; OR, odds ratio; CI, confidence interval.

Table 4. **Spectrum of detected gene mutations and alteration types**

Gene	Mutations	Alteration types
TP53	Y234C, L299fs, R337L, M160I, R280I, R273L	Missense, Truncating
AKT1	W22C	Missense
ERBB4	F279L, G336S	Missense
KRAS	G12C, G13R, G12V, G12D, G19F, G12R, G12A	Missense
BRAF	D594G	Missense
EGFR	Exon18 (G719X), Exon 19 (Ex19Del), Exon 20 (S768I, Ex20Ins), Exon21 (L858R, L861Q)	Missense, In-frame insertion/duplication
PIK3CA	E545K	Missense
CTNNB1	S33F	Missense

EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma viral oncogene homolog; TP53, tumor protein p53; AKT1, serine/threonine kinase 1; BRAF, B-Raf proto-oncogene, serine/threonine kinase; CTNNB1, Catenin beta 1 (β-catenin); ERBB4, Erb-B2 receptor tyrosine kinase 4 (HER4); PIK3CA, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PI3K p110α).

TP53 disruption is a common event in cancer pathogenesis and is linked to diverse clinical outcomes.[26-28] No significant association was found between *EGFR* IHC positivity and concurrent *KRAS* or *TP53* mutations, reflecting the complex, heterogeneous biology of LUAD.[5,7,25] When considering regional context, the high *KRAS* prevalence in this Iraqi cohort also appears distinct from rates reported in neighboring countries, such as Iran.[29] These differences may arise from genetic, environmental, or methodological factors and highlight the importance of local epidemiological data to guide resource allocation in precision oncology, particularly in regions with a high and growing burden of lung cancer.[30] Future studies should incorporate multicenter Iraqi and MENA cohorts to assess sex-stratified EGFR effects and KRAS G12C prevalence. To address the limitations of this preliminary study and build upon its findings, we recommend several key directions for future research. These include establishing multicenter collaborations within Iraq and the MENA region to validate our observations in larger, more diverse cohorts. Such efforts should prioritize the standardized collection of clinical data, including smoking history, and the implementation of broader molecular profiling. Expanding testing panels to include other actionable targets (e.g., *ALK*, *ROS1*, *MET* exon 14, *BRAF*, *RET*, and *NTRK*) with orthogonal validation would provide a more comprehensive genomic landscape. Furthermore, linking molecular data to clinical outcomes, such as response to tyrosine kinase inhibitors (TKIs) or anti-KRAS therapies and survival metrics, is essential to translate genetic findings into improved patient care. Finally, the development of external quality assessment programs for molecular testing across regional laboratories will be crucial for ensuring the reliability and consistency of results in future studies

Limitations

The primary limitations are the small single-center sample size (N = 28) and the enrichment for locally advanced (Stage II-III) disease" or "underrepresentation of metastatic (Stage IV) disease, which constrain statistical power and generalizability. Therefore, the reported mutation frequencies and co-occurrence patterns should be considered preliminary and hypothesis-generating.

Conclusion and Implications

This study suggests a distinct molecular landscape in Iraqi LUAD, characterized by frequent *EGFR* and *KRAS* alterations. The preliminary concordance between *EGFR* IHC and NGS supports the further evaluation of cost-efficient testing algorithms. These findings underscore the need to expand local molecular capacity and access targeted therapies. Validating this study in larger multicenter regional cohorts is essential to inform precision oncology strategies in Iraq and the wider MENA region.

Abbreviations

LUAD, lung adenocarcinoma; NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; NGS, next-generation sequencing; KRAS, Kirsten rat sarcoma virus oncogene; TP53, tumor protein p53; TKIs, tyrosine kinase inhibitors.

Financial Support

Self-Fundings.

Ethical Approval

The study protocol was approved by the Institutional Review Board of the University of Kufa, Faculty of Medicine (approval number MEC-60, dated 02 October 2024), and was conducted in accordance with the ethical principles of the Declaration of Helsinki (1995; as revised in Edinburgh in 2000). Written informed consent was obtained from all the participants prior to their inclusion in the study.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgement

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Author Contributions

EA contributed to the design and implementation of the study. AA performed the bioinformatics analysis, interpreted the data, and drafted the manuscript. All authors have read and approved the final manuscript.

Use of Artificial Intelligence Tools

An artificial intelligence tool (ChatGPT) was used for language editing and rephrasing. The authors verified all contents and took full responsibility for the scientific accuracy of the manuscript. ■

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