ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

# Immunohistochemical analysis of epidermal growth factor receptor as a predictive biomarker in lung adenocarcinoma – A cross-sectional study of 33 cases on computed tomography-guided core needle biopsy specimens

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Submission: 07-09-2023 Revision: 03-12-2023 Publication: 01-01-2024

## ABSTRACT

**Background:** Lung cancer is the second most common cause of cancer in all ages and both genders and is the leading cause of cancer death. Two major groups of lung cancers are – small cell lung carcinoma (SCLC) and non-SCLC (NSCLC). Due to discovery of driver mutation such as epidermal growth factor receptor (EGFR) mutation in NSCLC, the paradigm of lung cancer therapy has shifted from cytotoxic platinum based therapy to tyrosine kinase inhibitor (TKI) therapy. In this study, we have analyzed EGFR expression in NSCLC by immunohistochemistry (IHC) and studied the response to TKI therapy in terms of progression free survival (PFS) and overall survival (OS). **Aims and Objectives:** In this study, we have retrospectively analyzed the EGFR overexpression in adenocarcinoma of lung in core needle biopsy (CNB) specimen by IHC analysis and correlated with the therapeutic response and survival rate (OS and PFS) in lung carcinoma patients. **Materials and Methods:** We have analyzed retrospectively EGFR expression in lung adenocarcinoma cases (computed tomography-guided CNB specimen) by IHC. In our study, we have used monoclonal primary antibodies against two most common EGFR mutations, that is, L858R point mutation and E 746–A 750 deletion. **Results:** Out of 33 cases, EGFR expression was seen in 28 cases. For EGFR expression assessment by IHC, both cytoplasmic and/or membranous staining taken into consideration. EGFR positivity was interpreted only when >10% tumor cells having 2+ or more intensely staining pattern. The response to TKI therapy in terms of PFS and OS was also studied. **Conclusion:** IHC analysis of EGFR mutation using specific antibodies has extremely high specificity with good sensitivity. Use of targeted therapy in the form of TKI in EGFR positive lung adenocarcinoma has high response rate and long duration of survival (PFS and OS) with acceptable toxicity profile in contrast to the conventional therapy.

**Key words:** Epidermal growth factor receptor; Immunohistochemistry; Lung adenocarcinoma; Tyrosine kinase inhibitor

**Access this article online**

**Website:** http://nepjol.info/index.php/AJMS

**DOI:** 10.3126/ajms.v15i1.58424

**E-ISSN:** 2091-0576

**P-ISSN:** 2467-9100

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## INTRODUCTION

According to the most recent GLOBOCAN released by International Agency for Research on Cancer on December 14, 2020, lung cancer is the second most common cancer constituting up to 11.4% of all new cases of cancer considering all ages and both genders.[1] Lung cancer represents a group of heterogeneous

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disease both histologically and on the basis of molecular classification.[2,3] Lung cancers are categorized into two major groups-small cell lung carcinoma (SCLC) and non SCLC (NSCLC) constituting up to 15% and 85% of all lung cancers, respectively.[4] Due to poorly understood mechanism, NSCLC has proven difficult to treat in the past. Previously, the protocol for treatment of patients with advanced NSCLC irrespective of the histological subtype was chemotherapy (typically with Platinum doublets).[5] There is poor chemosensitivity and greater cytotoxicity to these traditional platinum therapy. Due to recent advances in understanding of cell signaling pathway and genetic and regulatory mechanism involved in tumorigenesis, the paradigm of NSCLC treatment has started shifting recently. With the discovery of epidermal growth factor receptor (EGFR) mutation in NSCLC specially in adenocarcinoma subtype the way to molecular targeted therapy has opened, as well as the assessment of predictive biomarkers also being done.[6] Predictive biomarkers are defined as markers for which the results are essential for therapeutic decision-making. Over expression of EGFR has been associated in the pathogenesis of many human malignancies including NSCLC.[7] Earlier some studies have shown that expression of EGFR in NSCLC is associated with reduced survival, frequent lymph node metastasis, and poor chemosensitivity.[8,9] Considering the prompt therapeutic response, high EGFR expression may predict higher survival as a response to targeted Geftinib therapy in lung adenocarcinoma.[10] Therefore, a more detailed understanding of EGFR biology for the therapeutic response and survival rate in NSCLC specifically in adenocarcinoma is required.

### Aims and objectives

1. Retrospective analysis of EGFR overexpression in adenocarcinoma of lung in CNB specimen by Immunohistochemistry.
2. Correlation of EGFR overexpression with the therapeutic response and survival rate (overall survival and progression free survival) in patients of lung adenocarcinoma.

## MATERIALS AND METHODS

### Study design and duration

This was a retrospective study conducted over a period of 2 years (from January 2020 to January 2022) with a follow-up period of 1.5 years (from January 2022 to June 2023).

### Clinical data

All the relevant clinical data of the patients such as – age, sex, clinical presentation including stage, radiological findings, therapy received, and follow-up details were obtained from clinical records.

### Inclusion and exclusion criteria

All patients having primary NSCLC favoring adenocarcinoma were included in the study. Patients with SCLC, Squamous cell carcinoma metastatic NSCLC, and other malignancies were excluded from the study.

### Informed consent

Written informed consent was taken from each patient or patient's relative (as applicable) for invasive procedure of core needle biopsy (CNB) and for chemotherapy.

### Tumor specimen

Formalin fixed Paraffin embedded blocks were prepared from the computed tomography guided CNB specimens of lung tumors. Thirty-three cases of adenocarcinoma were diagnosed from hematoxylin and eosin stained slides based on the 2021 World Health Organization classification.[2]

### Immunohistochemistry (IHC)

IHC was performed using the standard protocol. Four micrometer sections from respective paraffin blocks of each tumor specimen were cut and then deparaffinized in xylene and hydrated with graded series of ethanol. Antigen retrieval was done by heat induced epitope retrieval method (boiling citrate buffer, pH - 6.0 for 5 min). Then the microsections were incubated with avidin & biotin blocking reagent successively. Then, the sections were incubated with primary antibody which include L858 R specific monoclonal antibody (prediluted clone SP125, Ventana Medical System Inc.) and DEL-specific monoclonal antibody (pre-diluted, clone SP111, Ventana Medical Systems Inc., Tucson, AZ) followed by secondary antibody. DAB/AEC (3,3'Diaminobenzidine/ 3- Amino-9 Ethylcarbazole) chromogen was added followed by DAB enhancer if required. Till this every step was followed by rinse with wash buffer. Counterstained with hematoxylin followed by mounting done.

### IHC interpretation

EGFR immunore activity was scored based on membrane and/or cytopylasmic staining as follows.

- 0: No staining or faint staining in <10% of tumor cells
- 1+: Weak staining in >10% or more of tumor cells
- 2+: Moderate staining in >10% or more of tumor cells
- 3+: Strong staining in >10% or more of tumor cells.

The staining scores obtained in three cores (at least) were averaged and the final result was taken as a representative score for each case. To diagnose EGFR positive lung cancers by IHC, recent optimized protocol was taken into account intensity of 2+ or more in membrane and/or cytoplasm of >10% tumor was considered positive.

### Statistical analysis

Statistical analysis was done using software IBM Statistical Package for the Social Sciences 20.0. All the data were represented as number and percentage. P<0.05 considered statistically significant.

### Ethical clearance

The study was approved by the Institutional Ethics committee.

## RESULTS

In our study, out of 33 cases, 21 were female and 12 cases were male with a female-to-male ratio of 1.57:1 (Table 1). Smoking history was present in 7 cases (21.21%) with 26 cases were nonsmoker (78.79%). Brain metastasis was present in 13 cases (39.3%) at the time of diagnosis. Out of 33 cases of lung adenocarcinoma cases (Figure 1A, 2A, 3A), EGFR expression was seen in 28 cases (84.8%). For EGFR expression assessment, both cytoplasmic and membranous staining taken into account. Pneumocytes served as internal control (Figure 1C). By taking the 4-scale grading system, intensity scoring was done as follows-0 (3 cases 9.09%), 1+ (2 cases 6.06%) (Figure 1b), 2+ (19 cases 57.5%) (Figure 2b), and 3+ (9 cases 27.3%) (Figure 3b) (Table 2). Response to tyrosine kinase inhibitor (TKI) therapy in terms of progression free survival (PFS) and overall survival (OS) are described in Table 3. Out of 28 cases showing EGFR positivity, all showed PFS and 25 cases had increase in OS. Patients with EGFR positivity but without brain metastasis seen in 20 cases. PFS observed in all 20 cases and in 17 cases, there was increase in OS (Table 3).

**Table 1: Characteristics of patients with lung adenocarcinoma (n=33)**

| S. No. | Characteristics | Range | Mean |
|---|---|---|---|
| 1. | Age | 33–87 years | 58.3 year |
| | | **Number** | **Percentage** |
| 2. | Gender | | |
| | Female | 21 | 63.63 |
| | Male | 12 | 36.36 |
| 3. | Smoker | | |
| | Yes | 7 | 21.21 |
| | No | 26 | 78.78 |
| 4. | Brain metastasis | | |
| | Yes | 13 | 39.39 |
| | No | 20 | 60.60 |
| 5. | Pathologic stage | | |
| | Tumor stage | | |
| | T1/T2 | 10 | 30.30 |
| | T3/T4 | 23 | 69.69 |
| | Nodal stage | | |
| | NO/N1 | 17 | 51.51 |
| | N2/N3 | 16 | 48.48 |
| | Metastasis | | |
| | Present | 19 | 57.57 |
| | Absent | 14 | 42.42 |
| 6. | EGFR expression | | |
| | Yes | 28 | 84.8 |
| | No | 5 | 15.1 |

EGFR: Epidermal growth factor receptor

## DISCUSSION

According to the recent GLOBOCAN 2020, lung cancer is the most common cause of cancer deaths constituting up to 18.0% of all cancer deaths.[1] The overall prognosis of lung cancer is poor due to several factors such as detection at late stage, poor response to chemotherapy, and high toxicity of chemotherapy. In addition to this, approximately 50–60% of lung cancer patients present in advanced stage that have no chance of receiving radical surgery or chemoradiotherapy.[11] On the contrary to this, due to new advances in the field of oncology with the discovery of driver mutation, there is a paradigm shift of therapeutic approach in patients with NSCLC.

Target specific therapy for these driver mutation in NSCLC and application of orally administered TKI (targeting these driver mutation) produced a relatively high response rate and long duration of PFS with acceptable toxicity.

The various oncogenic drivers detected in advanced NSCLC for stratification of therapeutic approach are

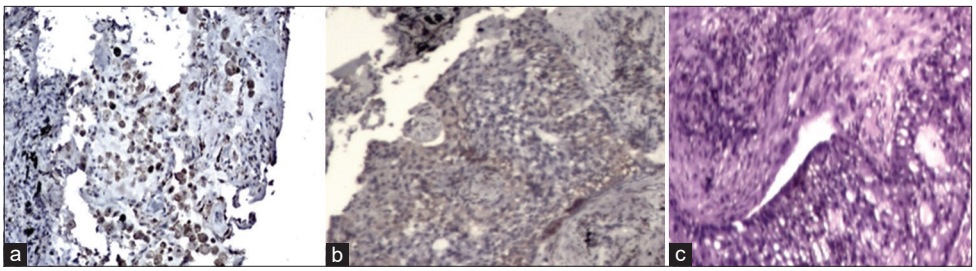


**Figure 1:** (a) Features of lug adenocarcinoma (hematoxylin and eosin, ×400), (b) weak nuclear and cytoplasmic positivity of >10% of tumor cells (score 1+) (epidermal growth factor receptor (EGFR), ×400) and (c) EGFR positive pneumocytes serving as internal control for EGFR immune histochemistry

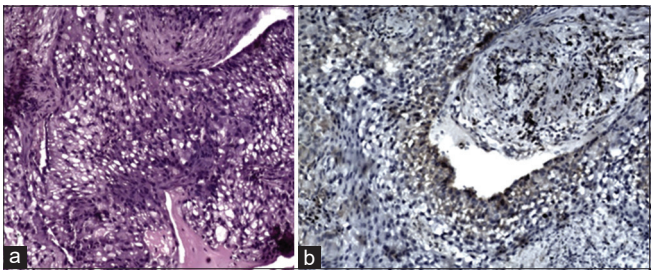


**Figure 2:** (a) Features of lug adenocarcinoma (hematoxylin and eosin, ×400) and (b) moderate nuclear and cytoplasmic positivity of >10% of tumor cells (score 2+) (epidermal growth factor receptor, ×400)

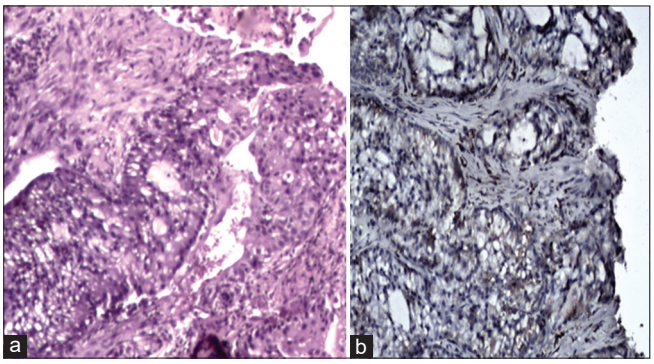


**Figure 3:** (a) Features of lug adenocarcinoma (hematoxylin and eosin, ×400) and (b) strong nuclear and cytoplasmic positivity of >10% of tumor cells (score 3+) (epidermal growth factor receptor, ×400)

EGFR mutation, anaplastic lymphoma kinase, ROS1 rearrangement, etc.[12] Out of these, somatic mutation in EGFR is the most common mutation in NSCLC.[11]

EGFR is a 170 kdalton protein of Erb B family present on the cell surface. Mutation in EGFR gene leading to EGFR overexpression is frequently noted in NSCLC[13] specially in adenocarcinoma subtype.

EGFR is a transmembrane receptor having three portion – an extracellular ligand binding domain, a transmembrane domain and an intracellular tyrosine kinase domain.[14] Out of various types of EGFR mutation detected in NSCLC, two most common mutations are L858R point mutation in exon 21 and E746-A 750 deletion (DEL) in exon 19 comprising up to 90% of all EGFR mutation.[15] These mutations are responsible for high sensitivity to TKI therapy.[16,17] According to American Lung Association, 10–15% of lung cancers in United States are EGFR positive.

According to the study by Kohno et al., EGFR mutations are found approximately 15% of lung adenocarcinoma cases in the Europe and United States and 55% of cases in East Africa.[18] The population frequently showing EGFR positive lung cancers are classically as Asian nonsmoking female with adenocarcinoma.[19] Apart from

**Table 2: IHC analysis of EGFR expression in lung adenocarcinoma (n=33)**

| A. | Primary monoclonal antibody used | Clone | Antigen retrieval |
|---|---|---|---|
| | 1. L858R specific | SP 125 | Heat induced |
| | 2. DEL specific (E 746-A 750) | SP 111 | Heat induced |
| B. | Assessment of EGFR expression<br>Membrane and/or cytoplasmic staining | | |
| **C.** | **Four grading scale scoring for intensity of EGFR expression** | **Number of cases** | **Percentage** |
| | 0 | 3 | 9.09 |
| | 1+ | 2 | 6.06 |
| | 2+ | 19 | 57.5 |
| | 3+ | 9 | 27.3 |
| D. | Criteria for positivity (optimized protocol)<br>1. Membrane and/or cytoplasmic staining<br>2. Intensity 2+ or more<br>3. >10% of tumor cell staining | | |
| **E.** | **EGFR expression** | **Number of cases** | **Percentage** |
| | Yes | 28 | 84.8 |
| | No | 5 | 15.1 |

EGFR: Epidermal growth factor receptor, IHC: Immune histochemistry

**Table 3: Response to TKI in lung adenocarcinoma with/without EGFR expression and brain metastasis in terms of PFS and OS (n=33)**

| A | EGFR expression | PFS | OS | P-value |
|---|---|---|---|---|
| | Yes - 28 cases | 28 | 25 | <0.001 |
| | No - 5 cases | 2 | 1 | <0.001 |
| B. | Brain metastasis | | | |
| | Yes - 13 cases | 7 | 5 | <0.001 |
| | No - 20 cases | 20 | 7 | <0.001 |

EGFR: Epidermal growth factor receptor, TKI: Tyrosine kinase inhibitor, PFS: Progression free survival, OS: Overall survival

ethnicity Western/Asian), various other factors (histological subtypes and smoking status) also affect incidence rates of EGFR mutations.[20]

In our study, out of 33 cases, 21 (63.63%) are female and the age ranges from 33–87 years similar to the study of Kim.[21] In another study, nearly 60% of the patients having EGFR mutations were never smoker.[22] Another important phenomenon regarding NSCLC is that many patients (30–35%) have brain metastasis which is a life threatening condition. The risk of brain metastasis is high in patients with EGFR mutation at the time of diagnosis. Shin et al., reported a dramatic connection of brain metastasis with EGFR mutation.[23] Out of 33 cases in our study, 13 cases had brain metastasis at the time of diagnosis.

### Detection of EGFR mutation by IHC and its interpretation

Although DNA based molecular methods are gold standard technique for EGFR detection, these methods are tedious, expensive and not routinely done in clinical laboratories. Recently generated mutation specific rabbit monoclonal antibodies against two most common NSCLC associated EGFR mutations for detection by IHC is a reliable screening method used routinely.[24] In our study, EGFR expression was detected by IHC only.

To diagnose EGFR positive lung cancers by IHC, we have used a recent optimized protocol, that is, intensity of 2+ or more in membrane and/or cytoplasm of >10% tumor cells were considered positive.[25]

### Treatment response

In our study, all the NSCLC subtypes (i.e., adenocarcinoma) cases received TKI therapy (Geftinib). Various previous studies showed that EGFR overexpression has been associated with poor survival. However, in our study, there was a survival benefit in terms of PFS and OS in patients with EGFR positive lung cancers in response to TKI therapy. This was similar to a recent study by Wang et al.[10]

### Limitation of the study

1. In our study, IHC analysis was done using antibodies against the most common EGFR mutation, that is, L858R point mutation in exon21 and E746-A 750 DEL in exon 19. Other mutations such as EGFR exon 20 insertions were not detected which might be contributing to the EGFR negative results and also these mutations though rare have different treatment strategy contributing to one of the important limitation of our study.
2. Only IHC analysis for EGFR expression was studied. Data on molecular (DNA) based methods were very limited and not available in majority of cases.

### Future perspectives

NSCLC harboring EGFR driven mutations showed TKIs are the primary therapeutic choice. However, development of drug resistance is the most common challenge associated with these therapy. Some recent advances to combat these are as follows.

a. Treatment of EGFR T790M acquired resistance by third-generation EGFR-TKI monotherapy (Osimentinib).
b. EGFR-TKI and cytotoxic agents or angiogenesis inhibitors as combination therapy.
c. Immunotherapy targeting immune checkpoint inhibitors (including anti-PD1/L1) and anti CTLA-4 antibodies.

## CONCLUSION

EGFR mutations in NSCLC are significant driver mutations commonly seen among Asian females who were never smokers with adenocarcinoma histology. NSCLC patients with active EGFR mutations have different biological behavior and treatment algorithm. IHC analysis with EGFR mutation specific antibodies have extremely high specificity (99.5%) with good sensitivity (75.6%). Use of targeted therapy in the form of orally administrable small molecule TKI produce relatively high response rate and long duration of PFS and OS with acceptable toxicity profile.

## ACKNOWLEDGMENTS

We acknowledge the faculties of Chest Medicine and Radiotherapy Department of our institution for contribution of the cases of our study.

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**Author's Contributions:**
**SM**- Concept, design, data collection, result analysis, and first draft of manuscript; **SC**- Design, result analysis and interpretation, and review of literature; **MS**- Concept, coordination, and review of literature; **RP**- Concept, design, coordination, review of literature, preparation, and final revision of manuscript.

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**Source of Funding:** Nil, **Conflicts of Interest:** None declared.