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Effects of EGFR gene mutation on stage 3A lung adenocarcinomas in the clinical decision-making process for mediastinal invasive staging

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ABSTRACT

Objective: In our study, we aimed to evaluate the effects of EGFR gene mutation on the clinical course of Non-Small Cell Lung carcinoma.

Material and Methods: Our study was conducted retrospectively on patients who were operated on for NSCLC diagnosed as adenocarcinoma. The International Cancer Control Association and the American Cancer Committee eighth TNM classification system were evaluated in our study. Case groups at this stage were divided into two main groups as EGFR gene mutation (+/-) and data between the clinical behaviours of these two main groups were investigated.

Results: There was no statistically significant difference between the two groups in terms of age, gender, smoking, and type of surgery (p = 0.727, p = 0.936, p = 0.463). The relationship between EGFR and surgery type was also not significant (2 = 0.268; p = 0.992). There were no statistically significant difference between the medians of Suv-Max value (z = 1.083; p = 0.279). Among 653 cases in all NSCLC adenocarcinoma subtypes, EGFR gene mutation positivity was 23.89%. When we evaluate the progression of patients with EGFR gene mutation from stage 3A to 3B, it is more aggressive in cases with EGFR gene mutation, but it is not statistically significant (χ^2=2.924; p=0.087).

Conclusions: Knowledge of whether there is an EGFR gene mutation can provide important clinical information. In this respect, EGFR gene mutation positivity in stage 3A cases may constitute an indication for preoperative invasive mediastinal sampling, but we need more data to get statistically definitive results.

Keywords: Egfr, Non-Small Cell Lung Carcinoma, Mediastinal Lymph Node Metastasis

INTRODUCTION

Lung cancer is the most common type of cancer with a high mortality rate. It constitutes 12.4% of all newly diagnosed cancer cases and 17.6% of cancer-related mortality (1). However, the treatment efficacy of first-line chemotherapy agents is very low.

Epidermal Growth Factor Receptor (EGFR) is part of the ErbB family of cell surface receptor tyrosine kinases that control the pathways of signals regulating proliferation, cell growth, cell differentiation, and apoptosis (2). When it binds to EGFR, the receptor dimerizes, autophosphorylates, and activates several pathways (mitogen-activated protein kinase, Janus kinase 2/signal transducer and activator of transcription (STAT 3-5), and phosphatidylinositol 3-kinase (PI3K)/protein kinase B), which lead to cell proliferation, metastasis, and migration while preventing apoptosis (3, 4). In the resting period, EGFR is blocked and does not dimerize. However, when a point mutation occurs in the EGFR gene (on chromosome 7), especially in Exon 19 (deletions) or Exon 21, the EGFR remains dimerized and signals continuous proliferation and avoidance of apoptosis to the cell oncogenic cascade (5).

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The EGFR gene mutation is more expressed in lung cancers than in conjugated normal tissue (6). It is also expressed 60% more in metastatic lung cancers (7). So, tyrosine kinase inhibitors (TKI), gefitinib, and erlotinib have been developed as target therapy (3). EGFR, which has been a very important molecule for targeted therapies in the last decade, may have potential beyond being the target of chemotherapies. If the EGFR gene mutation factor, which is one of the genetic differences in lung adenocarcinoma groups, is evaluated appropriately for metastasis, especially factors that predict metastasis to mediastinal lymph-nodes preoperatively, efficient imaging studies or invasive mediastinal staging may be performed, and clinicians may effectively use limited medical resources (8, 9).

So, we aimed to statistically evaluate the effects of this mutation for the clinical course in the lung cancer adenocarcinoma based on mediastinal lymph node metastases and also the prognosis.

MATERIAL and METHODS

Study population and data acquisition progress

Our study was conducted retrospectively on patients who were operated on for lung cancer and were diagnosed as adenocarcinoma pathologically between February 2016 and February 2021. To reach more specific results, the results of the patients who were stage 3A according to The International Cancer Control Association and the American Cancer Committee eighth TNM classification system were evaluated in our study (10). Data of 653 cases diagnosed with lung adenocarcinoma were scanned and 108 cases were included in the study. Case groups at this stage were divided into two main groups as EGFR gene mutation (+/-), and data between the clinical behaviours of these two main groups were investigated. The patient cohort was derived from medical records of Yıldırım Beyazıt University, Dokuz Eylül Universityand Katip Çelebi University. Besides, the EGFR gene mutation positivity rate of the operated cases at all stages was evaluated in order to determine the EGFR gene mutation (+/-) ratios among all lung adenocarcinoma cases.

Pet-Ct Imaging Protocol

The stages of the cases were determined with preoperative Positron Emission Tomography (PET), and standard uptake value-max (SUV-max) values in the primary tumor were recorded. All centers were using the same device and protocol for PET (ECAT model 951/31, Siemens/ CTI, Knoxville, Tenn.). Fluorine-deoxy-glucose (FDG) was synthesized according to the standard method by a high-performance liquid chromatography-controlled synthesis module (10). Patients were instructed to fast for six hours before the imaging, and FDG (370 MBq) was administered intravenously. Data were reconstructed into coronal, sagittal, and transverse sections and a three-dimensional rotating projection (11). A comparison was made between the mediastinal lymph node evaluation performed with PET in the preoperative period and the evaluation considered as the gold standard with the postoperative pathology. Mediastinal lymph node stations of the cases were documented. Lymph node evaluation was done according to Mountain and Dresler's classification (12).

Surgical Approach and Lymph-Node Evaluation Protocol

Lung anatomical resection and mediastinal lymph node dissection with posterolateral thoracotomy were performed in all our cases in our clinics, which is the standard approach to lung cancer surgery. Mediastinoscopy, mediastinoscopy, VATS, and other invasive staging methods cases were not evaluated in this study since mediastinal lymph node stations were not fully exemplified. Pet-Bt evaluation was performed in the preoperative period, and the results of the cases accepted as stage 3A were compared with the results of the pathology evaluation, which was accepted as the gold-standard. The difference between the cases progressing to stage 3B within the stage 3A cases was compared among the EGFR+/- groups.

Histopathological EGFR-gene Mutation Investigations

The immunohistochemistry (IHC) method was studied in tissues from which the EGFR gene mutation was taken from the pathology samples taken from the lung tissue. Formalin-fixed, paraffin-embedded tissue sections were cut into 4-µm-thick sequentially. Deparaffinization and rehydration, sections were boiled in citrate buffer (0.01 M, pH 6.0) for antigen retrieval. Sections were then incubated with 3% H2O2 and 5% serum to block endogenous peroxidase activity and non-specific binding. 2 primary antibodies (delE746-A750 mutation-specific monoclonal antibody (6B6) and L858R mutation-specific monoclonal antibody (43B2); Cell Signalling Technology, Danvers, MA, USA) were used for identification of EGFR mutation. The sections were then incubated with biotinylated secondary antibodies and visualized by DAB. Counterstaining was carried out with hematoxylin. The sections were dehydrated in alcohol and mounted with DPX. The IHC staining score was based on the staining intensity and percentage positivity (0-100%) of cells in the membrane and/or cytoplasm of tumour cells. Four grades were employed: 0, 1+, 2+, and 3+. 0 means no staining; 1+ means faint membrane and/or cytoplasmic staining in less than 10% positive cells; 2+ means moderate membrane and/or cytoplasmic staining in greater than 10% and less than 50% cells; 3+ means strong membrane and/or cytoplasmic staining more than 50% cells positive. 0 and 1+ scores were considered as negative; whereas 2+ and 3+ were considered as positive cases to obtain more specific data (Figure.1) (13,14).

Exclusion Criteria

Diabetes or hyperglycemia defined as plasma glucose> 140 mg / dL before PET was not inc-luded in the study, since standardization problems may occur in the evaluation. Patients with a history of rheumatological, lymphoproliferative disease, or cancer other than lung cancer were not considered. Besides, patients who received chemotherapy and radiotherapy before the operation, who had a central tumor enough to require pneumonectomy, or who had post obst-ructive pneumonia were excluded from the study. Cases with positive N1 lymph nodes were not included in the study. All cases with missing data were not included in the study before and after the operation.

Statistical analysis

All of the statistical analyses were carried out with IBM SPSS (IBM Corp. Armonk, USA, Ver.20.0). Descriptive statistics were presented as mean ± s.dev for normal distributed data or median IQR, P25(25 th percentile) and P75 (75 th percentile) for nonnormal data. The relations between the categorical data were investigated with the Chi-squared test. SUV-max values of egfr positive and negative groups were tested with Mann Whitney U test. The p-value was set at $p \leqslant 0.05$.

Normality of data was tested by P-P and Q-Q graphs, value of Std/Mean value <0.20, Shapiro Wilks test and Skewness/SEM<1.96 and Kurtosis/SEM<1.96 tests. Variables provided all these criteria were assumed as normally distributed. The time variable was not considered in the progression from stage 3A to stage 3B, as there was no significant time between the preoperative period and the postoperative period.

The study was approved by the Ethical Committees of the Ankara Bilkent City Hospital (25/062020-E1-20-817). The study was conducted according to the principles of the Helsinki Declaration. All patients gave written informed consent.

RESULTS

When we evaluated the baseline characteristics of the two groups compared in terms of age, gender, and smoking, there was no statistically significant difference between the two groups (p=0.727,p=0.936,p=0.463) (**Table.1**).

Differences in surgical approach between groups may be important in terms of the value of the results. In terms of homogeneity between EGFR +/- groups, the association between EGFR mutation and surgery type was also not significant (χ^2=0.268; p= 0.992) (**Table.2**).

There were no statistically significant difference between the medians of SUV-Max value, EGFR negative 9.80 (IQR = 5.22; P25=7.20 ; P75 =12.42) and EGFR positive groups 10.00 (IQR = 6.32; ; P25= 8.00; P75=14.32) (z = 1.083; p=0.279).When we evaluate the progression of cases with EGFR gene mutation from stage 3A to Stage 3B, it was seen that cases with EGFR gene mutation tend to progress to more stage 3B by more aggressive behaviour, but this is not at a statistically significant level (χ^2=2.924; p=0.087) (**Table.3**).

Table 1. A) Gender distribution of the groups, **B)** Distribution of surgery type between groups, **C)** Difference in mediastinal Lymph Node progression in the groups

		EGFR (+)	EGFR (-)	X^2	P
Gender	Female	15(36,59)	24(35,82)	0,006	0,936
	Male	26(63,41)	43(64,18)		
Surgery type	LLL	10(24.39)	15(22,39)	0,268	0,992
	LUL	13(31,71)	22(32,84)		
	RLL	6(14,63)	11(16,42)		
	RUL	9(21,95)	13(19,40)		
	RBL	3(7,32)	6(8,96)		
Mediastenal Lymph-Node Positivity Changed	No	24(58,54)	51(76,12)	2,924	0,087
	Yes	17(41,46)	16(23,88)		

Table 2. Distribution of surgery type between groups

	Surgery Type					
	LLL n (%)	**LUL n (%)**	**RLL n (%)**	**RUL n (%)**	**RBL n (%)**	**Total n (%)**
EGFR Negative	15 (22.39)	22 (32.84)	11 (16.42)	13 (19.40)	6 (8.96)	67 (100.00)
EGFR Positive	10 (24.39)	13 (31.71)	6 (14.63)	9 (21.95)	3 (7.32)	41 (100.00)
Total	25	35	17	22	9	108

Table 3. Difference in Mediastinal Lymph Node progression in the groups

	Mediastenal Lymphadenopathy Changed		
	No n(%)	**Yes n(%)**	**Total n(%)**
EGFR Negative	51 (76.12)	16 (23.88)	67 (100.00)
EGFR Positive	24 (58.54)	17 (41.46)	41 (100.00)
Total	75	33	108
$\chi^2 = 2.924$; p = 0.087			

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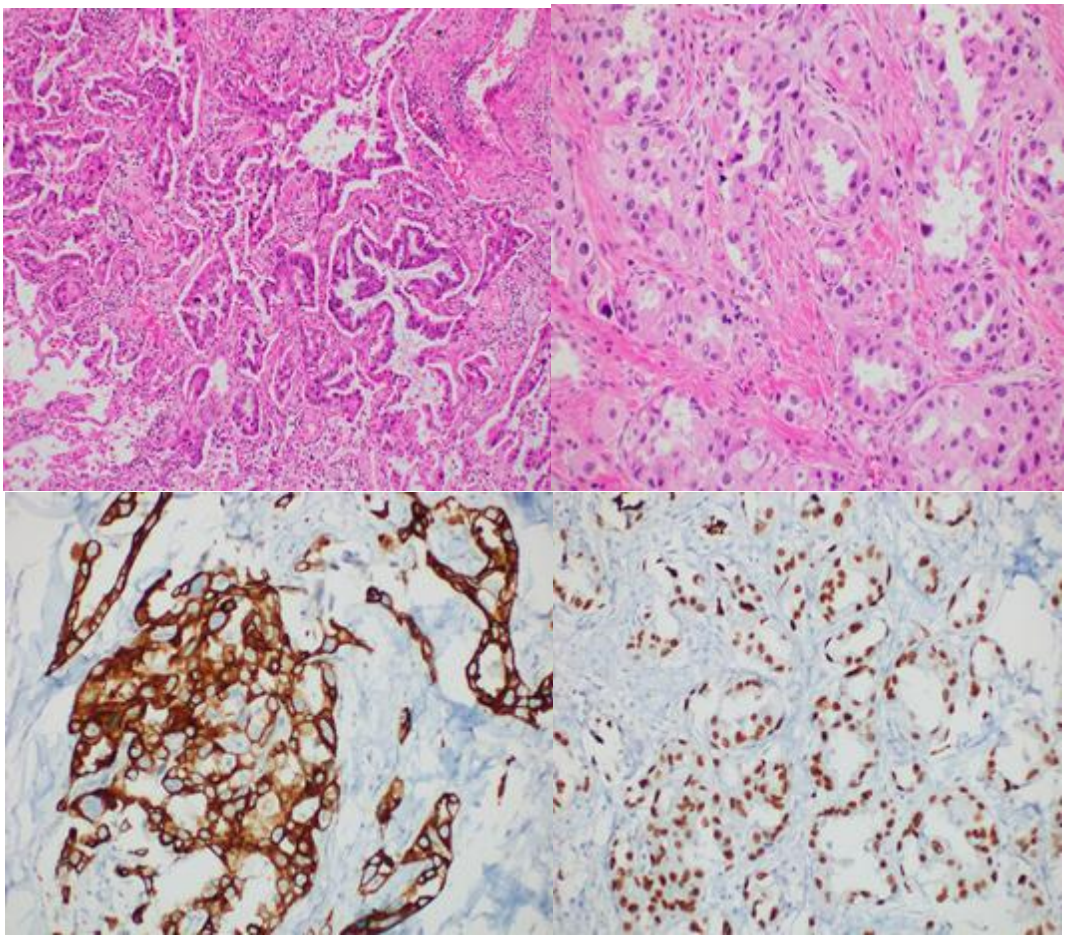

Figure 1: 1. Right Upper Lobe Lesion, Invasive Lung Adenocarcinoma Biopsy (H&E). **2.** Primary Antibodies for EGFR (delE746-A750 mutation-specific monoclonal antibody and L858R mutation-specific monoclonal antibody).

DISCUSSION

Overexpressed EGFR plays a critical role in the oncogenic process. It can prevent effects on cell growth, proliferation, and apoptosis by blocking the EGFR. Erlotinib and gefitinib, which are first-generation reversible TKIs, target the EGFR domain by competing with adenosine triphosphate. Compared with placebo, erlotinib has been shown to provide a significant survival benefit in unselected individuals in a randomized study with advanced-stage NSCLC (15). Even in recent studies, Osimertinib seemed to be the most preferable first-line treatment in advanced EGFR-mutated NSCLC (16). So, recent clinical guidelines recommend all patients with advanced or metastatic non-squamous NSCLC to receive EGFR mutation testing (17).

Detection of the EGFR gene mutation is crucial for target therapy, but it has been limited to identifying cases suitable for target therapies. However, EGFR gene mutation may be important for evaluating the prognosis of the cases and lymphatic metastasis potential. Molecular tests are also used with the most sensitive methods since lung cancer cases can be diagnosed with small tissue or cytological material in the preoperative period. For this reason, nowadays real-time PCR and ICH are used most frequently to investigate EGFR mutation (15, 18). Even when samples are limited, successful results can be obtained with these methods. Therefore, the presence of the gene mutation can be detected even in the preoperative period.

This situation creates the potential of the mutation to be used in clinical decision-making in the preoperative period. However, when the literature is evaluated for the EGFR gene mutation, there is no data on the prognosis and lymphatic metastasis potential of the mutation. Therefore, we focused our study on stage 3 cases, which is the most important threshold in NSCLC cases and completely changed the treatment approach.

Anatomical resection is the most curative treatment of lung cancer, it continues to gain wide acceptance despite the development of targeted therapies such as TKI (19). However, it is much more important to operate the cases that will benefit from surgery at the right stage. Therefore, the correct evaluation of stage 3A cases in the preoperative period constitutes a critical threshold. Although evaluation made by PET in stage 3A cases is sufficient in NSCLC adenocarcinomas by some clinics, some clinics take anatomical criteria into account, while others perform routine mediastinal sampling (mediastinoscopy, eus, ebus, vats, etc.) (20, 21). Considering that mediastinal sampling is indicated, both ACCP and ESTS guidelines recommend Ebus/ Eus biopsy, which is a minimally invasive method, as the first-line method for staging NSCLC (22). Thorax computed tomography has been used for mediastinal staging before, but nowadays it is used for anatomic information. PET shows metabolic activity in both primary lesions and lymph nodes. PET has inherently lower spatial resolution than Computed

Thomography (CT). Some disease groups such as infectious diseases, inflammatory and granulomatous diseases may cause false-positive results or we may encounter false-negative results with PET in some types of NSCLC.To completely eliminate such problems, the EGFR gene mutation information to be obtained in the preoperative period may be a guide in terms of mediastinal sampling. 100% accuracy can be achieved with combinations of data that can be reduced to many clinical and anatomical numerical standards and algorithms that can be developed based on software. Thus, complications caused by unnecessary mediastinal sampling and unnecessary surgery will be avoided.

The EGFR mutation varies considerably between populations. EGFR mutation was found in 10% to 20% of the European population and in 30% to 60% of Asian patients with non–small cell lung cancer (23, 24). In our study, when the data of all our cases were scanned, a value of 23.89 % was encountered. For all NSCLC patients, the ratio of work done in Turkey before was determined to be 16.4% (25). In our study, NSCLC adenocarcinoma subgroup cases were evaluated and it contains results representing a much larger population in terms of being multicentric. Since very different results were obtained in different populations in previous studies, it is necessary to evaluate each racial region separately and in detail in terms of this mutation. Besides, it would be a more appropriate approach to consider each NCSLC pathological type as a separate disease and to evaluate the data only for subgroups. Apart from additional information in terms of prognosis and lymphatic metastasis, EGFR gene mutation positivity and regulation of TKI treatment are recommended by some experts only in adenocarcinomas. EGFR rates are found at a much higher rate in lung adenocarcinomas. The fact that we found this mutation higher in the NSLCS adenocarcinoma group compared to other subgroups is consistent with the literature (26, 27, 28). Available Literature shows considerable variation between populations. Therefore, the data to be obtained on certain populations are critical in predicting the potential for TKI therapy and determining the treatment strategy (29).

Although our study is a multi-Center study, it reflects a certain population with a limited number of cases. Because it is a retrospective study, there is a risk of bias. Besides, although many clinically important gene mutations are popular, our study focused only on EGFR mutation. More specific results can be obtained by combining EGFR Gene mutation with many other clinical features in larger series.

CONCLUSION

In conclusion, apart from diagnosis and subtyping in pathology samples to be obtained preoperatively, it may be important to detect EGFR among clinically important mutations. Tumors with this mutation may have a more aggressive nature. EGFR gene mutation positivity in stage 3A cases may constitute an indication for preoperative aggressive, invasive mediastinal sampling but we need more data to get statistically definitive results

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Ethical approval: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by Local Ethical Committee.

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