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Research Article

Epidermal Growth Factor Receptor (EGFR) Mutation Status in Egyptian Patients with Non-Small Cell Lung Cancer (NSCLC); Can it be Predicted?

Rabab Gaafar[1], Abeer Bahnassy[2], Ahmed Bastawissy[1], Hala Aziz[1], Anan O Abdel-Aleem[3], Emad R Issak[4]* and Nagla Karim[5]

[1]Medical Oncology department, National Cancer Institute, Cairo University, Cairo, Egypt

[2]Tissue culture and Cytogenetics Unit, Pathology Department, National Cancer Institute, Cairo University, Cairo, Egypt

[3]Cancer Biology Department, NCI, Cairo University, Cairo, Egypt

[4]Immunology Department, Ain Shams University, Cairo, Egypt

[5]Medical Oncology Department, Georgia institute, USA

***Corresponding Author:** Emad R Issak, Immunology Department, Ain Shams University, Cairo, Egypt.

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Abstract

Objectives: The study was to assess frequency and patterns of Epidermal Growth Factor Receptor (EGFR) mutation status in a cohort of Egyptian patients with Non-Small Cell Lung Cancer (NSCLC) from the National Cancer Institute (NCI), Cairo University. Also, it aimed to investigate the association of the EGFR mutation status to several clinico-pathological features of patients.

Methods: EGFR mutation status was assessed in tumor tissue samples of 141 NSCLC patients from Egypt presenting to the NCI, Cairo University from December 2014 to January 2018. The association of EGFR mutation status to the relevant clinical and pathological features of the patients was studied using the logistic regression.

Results: EGFR mutations were detected in 33 (23.4%) patients. The most detected were 19 Del (18 patients; 12.77%), exon 21 (13 patients; 9.22%), exon 21+T790M, and G719X (one patient; 0.71%); each. EGFR mutation status was associated significantly to advanced disease stage ($p < 0.001$), brain metastasis 8/19 (42.11%, $p = 0.049$) and contralateral lung metastasis 7/14 (50%, $p = 0.038$). EGFR mutations were also higher in females (non-smokers (35%) and in males smokers (20.9%, $p < 0.001$). Multivariate analysis showed that only the contralateral lung metastasis is an independent predictor for EGFR mutations in the NSCLC patients.

Conclusion: EGFR mutations are relatively common (23.4%) in NSCLC patients from Egypt (NCI experience). Therefore, testing for EGFR mutations in NSCLC should be done routinely for those patients for better treatment outcomes.

Keywords: NSCLC; EGFR Mutations; Exons 19, 21, T790M and Metastasis

Introduction

Despite the advancements in cancer therapy in the previous decades, lung cancer continues to be the leading cause of cancer-related mortality. It accounts for 11.6% of the total malignancies in both sexes, with a mortality of 18.4% [1,2]. In Egypt, according to a National Population-Based Registry Program 2008–2011, it is the third most frequent cancer in males (5.69%) with an age standard rate (ASR) of 10.4. It is also the sixth most common cancer in both sexes accounting for 4.22% of the cases with ASR equaling 7.5 [3]. In addition, the majority of those lung cancer cases (85-90%) are non-small cell lung cancer (NSCLC), with 70% of them detected at a late stage, resulting in a bad prognosis [2,4].

Concurrently and during the last two decades, the molecular profiles of the NSCLC tumors have been well defined to find better treatment. Hence, mutations in some of the driver genes, among which the most commonly involved are the epidermal growth factor receptor (EGFR), the anaplastic lymphoma kinase (ALK), ROS proto-oncogene-1 (ROS1), and other genes, have been discovered [5,6]. Lynch., *et al*. showed in 2004 that EGFR mutations are linked to NSCLC responsiveness to gefitinib [7], bringing NSCLC into the arena of personalized medicine, which led to a paradigm shift in the systemic therapy of those patients. Hence, several approved tyrosine kinase inhibitors (TKIs) targeted therapies have been added to the armamentarium against NSCLC. The first-generation EGFR-TKIs included the first FDA-approved TKI gefitinib (2002), followed by erlotinib (2003). After that, afatinib, dacomitinib (second-generation TKIs), and most recently osimeritinib (third-generation TKI) were developed [5,8,9]. Consequently, in advanced NSCLC patients selected based on the existence of activating EGFR mutations, randomized phase III studies revealed that first-line treatment with EGFRTKIs granted an improved progression-free survival (PFS) compared to standard chemotherapy [10-15]. Thus, the therapeutic response elicited in EGFR-positive patients by those TKIs has been sufficiently documented in the literature [16].

Because EGFR gene mutations are now considered important prognostic and predictive driver factors in the NSCLC patients worldwide [9]; therefore, testing patients for EGFR mutations becomes mandatory during the planning phase of management. Furthermore, EGFR mutations have been linked to various demographic and clinicopathologic variables in NSCLC, including race, gender, smoking status, and tumour histology [17].

Therefore, the rationale behind carrying out the current study was to provide our share in this area of research through more in-depth investigation of the pattern of EGFR in our patients with NSCLC by determining the frequency and patterns of different EGFR mutations and studying the relationship between the different identified mutations and various clinical and pathological features of the patients.

Patients and Methods

This prospective case-series single-center study included NSCLC patients who attended the outpatient clinics of the National Cancer Institute (NCI), Cairo University, Egypt, during the period from Dec-2014 to Jan-2018. The Ethical Committee of the NCI, Cairo University, Egypt, approved the study protocol, which conformed to the 2011 Declaration of Helsinki principles and the Good Pharmacoepidemiology Practices (GPP) guidelines. All patients enrolled in the study approved to participate and signed informed consent. Inclusion criteria were adult males or females diagnosed histopathologically as NSCLC with age >18 years. Exclusion criteria were non-willingness to participate in the study, refusal to sign the informed consent form or current participation in another clinical trial.

Data collection was carried out using paper methods via a case record form (CRF). The data collected in the CRFs are the eligibility criteria, demographic data, smoking status, diagnosis, and any other relevant clinical and pathological features if available (i.e., the tumor size and site, tumor grade, stage, and other features).

The EGFR mutation status for each patient was determined using tumor specimens from diagnostic or surgical procedures (fresh or paraffin-embedded tissue samples). Patients were prospectively genotyped in the Tissue culture and cytogenetics Unit, Pathology Department, NCI, Cairo University. The following steps were done to determine EGFR mutations in the patients: First, DNA was extracted and purified from paraffin blocks of the tumors (5-10 sections; 4-5 micron thick; each) using the QIAamp DNA FFPE Tissue Kit (QIAGEN Manchester Ltd, Skelton House, Lloyd Street, North, Manchester, M15 6SH, UK) according to manufacturers' protocols. Then, the Therascreen® EGFR RGQ PCR Kit (QIAGEN Manchester Ltd, Skelton House, Lloyd Street, North, Manchester, M15 6SH, UK) was used to detect 29 somatic mutations in the EGFR gene by the polymerase chain reaction (PCR) on the Rotor-Gene Q instrument. The detected mutations are_deletions in exon 19, T790M, L858R, L861Q, G719X (detects the presence of G719S, G719A, or G719C, but does not distinguish between them), S768I, as well as three insertions in exon 20 (detects the presence of any of the three insertions, but does not distinguish between them).

The primary outcome measure in the current study was the percentage of positive EGFR cases, and the secondary outcome measure was the association between EGFR gene status and different relevant clinico-pathological factors including age group, gender, smoking, tumor grade and stage, performance status, and metastasis.

The purposive sampling technique was carried out by inviting all NSCLC cases coming to the NCI at the study period. All statistical tests were carried out using a significance level of 95%. A value for $P < 0.05$ was considered statistically significant. SPSS software (Statistical Package for the Social Sciences, version 25.0, SSPS Inc, Chicago, IL, USA) was used for the statistical analyses. Data was presented as (mean ± SD) for continuous variables, median (IQR) for ordinal and non-parametric data, and frequency & percentage for categorical variables. The frequency of EGFR mutation was compared between demographic and clinical subgroups with the use of $\chi 2$/Fisher's exact test or Phi test with no correction made for multiple testing. To best predict EGFR mutation frequency, factors with p less than 0.05 in the univariate analysis were further analyzed by multivariate logistic regression.

Results

Description of the included cohort

According to the eligibility criteria, only 141 NSCLC patients were included in the analysis. Out of all tested patients, 37 were females (26.24%), and 104 were males (73.76%). The median age of patients was 59 (26-81 years). About half of the patients (51.77%) were less than 60 years old. Most of the patients are smokers, 89 (63.12%). The majority of cases were adenocarcinoma 135 (95.74%), followed by large cell lung cancer 5 (3.55%) and squamous cell carcinoma 1 (0.71%). Metastatic NSCLC was encountered in 107 (74.24%) cases. Other clinicopathological characteristics are shown in table 1.

The majority of cases were adenocarcinomas (135; 95.74%), followed by large cell carcinoma 5 (3.55%) and squamous cell carcinoma 1 case only (0.71%). Only 89 cases out of all the tested cases had data regarding tumor grade (5 were grade I, 57 were grade II, and 27 were grade III). Performance status was assessed according to the Eastern Cooperative Oncology Group (ECOG) scale in which most of the cases were PS1 98 (74.24%), PS2 26 (19.70%), and PS3 8 (6.06%). Metastasis was reported in 107 (75.89%) cases in which each patient had 1.27 sites of metastasis. About 68 (48.23%) of cases had shown one organ metastasis, two organs in 20 (14.18%), three organs in 5 (3.55%), and unknown in 14 (9.93%) of cases.

	n	Proportion of total		EGFR +ve		p-value
All cases		141	100.00%	33	23.40%	
Age	141					0.246
< 60 years		73	51.77%	20	27.40%	
≥ 60 years		68	48.23%	13	19.12%	
Gender	141					0.131
Female		37	26.24%	12	32.43%	
Male		104	73.76%	21	20.19%	
Histological type	141					0.141
Adenocarcinoma		135	95.74%	30	22.22%	
Non-adenocarcinoma		6	4.26%	3	50.00%	
Grade	89					0.372
Grade 1		5	5.62%	0	0.00%	
Grade 2		57	64.04%	16	28.07%	
Grade 3		27	30.34%	8	29.63%	
Stage	141					< 0.001
Early (I and II)		6	4.26%	0	0.00%	
Advanced (III and IV)		135	95.75%	33	44.03%	
Performance status	132					0.887
PS 1		98	74.24%	22	22.45%	

PS 2		26	19.70%	7	26.92%	
PS 3		8	6.06%	2	25.00%	
Metastatic status	141					0.169
Non-metastatic		34	24.11%	5	14.71%	
Metastatic		107	75.89%	28	26.17%	
Site of metastasis	107					
Liver		28	26.17%	7	25.00%	0.969
Pleura		25	23.36%	3	12.00%	0.084
Brain		19	17.76%	8	42.11%	0.049*
Bone		15	14.02%	2	13.33%	0.22
Contralateral		14	13.08%	7	50.00%	0.038*
Adrenal		10	9.35%	2	20.00%	0.53
Lymph nodes		10	9.35%	4	40.00%	0.207
Pericardium		1	0.93%	0	0.00%	0.753
Metastasis number of organs	126					0.389
0		34	26.98%	5	14.71%	
1		67	53.17%	15	22.39%	
2		20	15.87%	7	35.00%	
3 or more		5	3.97%	1	20.00%	

Table 1: Clinical and pathological characteristics and EGFR status.

EGFR mutations analysis

Out of the 141 patients assessed, 33 cases (23.4%) were positive for EGFR mutations. The majority of positive EGFR mutations were exon 19 Del (12.77%), Exon 21 in 13 (9.22%) cases, followed by Exon 21+T790Min one patient (0.71%) and G719X in another patient (0.71%). Only one patient (0.71%) had a resistance mutation (Exon 21 + T790Min) accounted for 3.33% of the overall mutations.

The association between *EGFR* mutation status and the relevant clinico-pathological features of the patients

The EGFR positivity was insignificantly higher in the age group under 60 years (27.40% vs. 19.12%, p = 0.246). Also, it was insignificantly higher in female group (32.43% vs. 20.19%, p = 0.131). In addition, EGFR positivity was insignificantly higher in the non-smoking group (28.85% vs. 20.22%, p = 0.243).

In the studied cohort, univariate analysis showed that EGFR mutation status was associated significantly with the advanced disease stage since all patients with EGFR mutations presented with stage III or IV (33/33; p < 0.001). Moreover, EGFR mutations were associated significantly with brain metastasis in 8/19 patients (42.11%, p = 0.049) and contralateral lung metastasis in 7/14 patients (50%, p. = 0.038).

However, no significant association was found between EGFR and any other relevant clinicopathologic features assessed in the study, including the histological type and grade performance status or metastasis (Table 1).

When considering both gender and smoking status in analyzing EGFR status, in the current cohort, most of the males were smokers (86/104, 82.69%) versus only (3/37; 8.11%) of the female group (p < 0.001).

All female-non-smokers had positive EGFR mutations (12/37; 32.4%). However, only 18/104 (17.3% cases) of the male group are smokers and had positive EGFR, versus 3/104 (2.9%) are non-

smokers who had positive EGFR mutations. Thus, positive EGFR were more in the female-non-smoker group and male-smoker group ($p < 0.001$).

Figure 1: EGFR status according to gender and smoking status.

Each patient had 1.31 modalities of treatment. Chemotherapy or EGFR inhibitor was used in 128 (90.78%) cases, surgery 44 (31.21%), radiotherapy 4 (2.84%), and best supportive care (BSC) in 9 (6.38%) of cases. EGFR status did not differ according to these treatment modalities ($p > 0.05$). However, anti-EGFR drugs were used in 8/33 only of the positive EGFR mutation.

Logistic regression was conducted to assess whether the eleven predictor variables, gender, age, smoking, histologic type, brain metastasis, bone metastasis, liver metastasis, adrenal metastasis, pleural metastasis, pericardial effusion, lymph node metastasis, and contralateral lung metastasis, significantly predicted whether or not EGFR is positive. When all eleven variables are considered together, they significantly predict whether or not EGFR is positive, Chi-square = 23.72, df = 12, N = 93, p = 0.022. Table 2 presents the odds ratios, which suggest that the odds of estimating correctly EGFR improve by 36% if one knows the patient's age, by about 34% if one knows the smoking status, et cetera. (Table 2). Overall, 79.6% of the participants were predicted correctly. The independent variables were better at helping us predict who would be EGFR –ve (92.9% correct) than at who would be EGFR +ve (39.1% correct). That means that 40% of those whom the model assigned as positive EGFR are positive, and 60% are false positive.

				95% C.I. for EXP(B)		
Predictor	**B**	**SE**	**Odds ratio EXP(B)**	**Lower**	**Upper**	**p-value**
Age 60 years or more	0.31	0.61	1.36	0.42	4.46	0.610
Gender: male	1.18	0.91	3.24	0.55	19.26	0.196
Smoker	0.29	0.87	1.34	0.25	7.28	0.738
Histologic type: adenocarcinoma	0.78	0.44	2.19	0.93	5.16	0.073
Brain metastasis	1.36	0.87	3.89	0.71	21.21	0.116
Bone metastasis	-0.26	0.96	0.77	0.12	5.02	0.785
Liver metastasis	0.40	0.71	1.49	0.37	5.95	0.573
Adrenal metastasis	-0.92	1.15	0.40	0.04	3.77	0.422
Contralateral lung metastasis	2.38	0.92	10.82	1.77	66.00	0.010
Pleural metastasis	-0.27	0.96	0.76	0.12	5.02	0.776
Pericardial effusion	-18.74	40192.97	0.00	0.00	.	1.000
Lymph node metastasis	0.99	0.98	2.69	0.39	18.38	0.313
Constant	-3.56	1.06	0.03			0.001

Table 2: EGFR status logistic regression.

Discussion and Conclusion

One of the most commonly affected genes in the pathogenesis of the NSCLC is the EGFR gene. The different mutations detected in the EGFR gene represent an important anticancer therapeutic target for those patients apart from other standard treatment modalities (chemo/radiotherapy). Currently, the guidelines-based management plans consider EGFR-TKIs like gefitinib and others as first-line therapy for advanced NSCLC with positive EGFR mutation instead of the conventional chemotherapy [18-20]. Therefore, assessment of the EGFR mutational status in the NSCLC patients is considered a mainstay for managing those patients before the early commencement of treatment [18].

Based on the result of the current work, EGFR mutations were detected in 23.4% of all tested cases. As for the types of mutations detected in our series, mutations in exon 21 and Del 19 were the most prevalent, being detected in 9.22% and 12.7%of the patients assessed, respectively. Those were followed by Exon 21+T790M mutations detected in 0.71% of the patients and G719X in 0.71% of the patients. The results are within the ranges reported in other studies in this context. Comparable frequencies were also reported in other countries, including Italy (36.9%), Turkey (32.0%), and the Gulf region (Saudi Arabia) in 28.7% of the tested cases [21-24]. However, previous research studies have shown that EGFR mutations in NSCLC vary significantly among various populations. Vietnam and Taiwan had the highest rates of EGFR mutations, accounting for 64 percent and 62.1 percent of the NSCLC patients studied, respectively. China (37%) and Japan (29%), while the United States and Australia had the lowest 14% and 7% rates, respectively. The rate of EGFR mutation in India ranged from 23.2 to 51.8 percent [25-28]. In general, Asians have a greater rate of EGFR mutation (47%) than Caucasians (13%) [29].

In Egypt, one study in Alexandria (coastal city) by Zaki & colleagues (2015) showed that exon-19 mutations were detected in 22% and 18% using two methods. However, they tested only for exon-19 and exon-21 mutations in a sample of 50 cases [30]. Eid., *et al*. (2020) reported that the rate of EGFR mutation in Upper Egypt in 34 NSCLC cases was (44.1%) [18]. In a separate study from Cairo, Egypt, 68 (34.8%) of 195 adenocarcinoma patients had tumor-associated EGFR mutations. Forty cases (58.8%) had codon 19 deletion, 16 (23.6%) codon 21 deletion, six (8.8%) codon 20 insertion, three (4.4%) patients had codon 18 insertion, and three (4.4%) patients had multiple mutations [31].

This discrepancy in the results from different areas of the world could be attributed to different factors, including the population under study (homogenous or heterogeneous), genetic and ethnic variations, the adequacy and the nature of the samples tested (tissue or liquid biopsy), the technique(s) used for assessment of mutations (NGS, Sanger sequencing, conventional PCR, line strip assay, or immune-histochemistry), the kits used for the detection and the prevalence of smoking in the tested cohort.

However, according to our data, positive EGFR mutations were significantly higher in female non-smokers (35%) and 0% of total 3 cases of female smokers. As for males' smokers (86 cases), EGFR positive was 20.9% (18 cases), while for non-smokers (18 cases), EGFR positive was 16.6% (3 cases). So, the number of female smokers was minimal, and the number of male smokers was considerable. This could be attributed to the fact that in Egypt, as in most Arab countries, smoking is not common in females due to cultural and religious beliefs.

The data also demonstrate significant associations between EGFR mutations and advanced disease stage, the incidence of brain metastasis, and the presence of contralateral lung metastasis. In contrast, we did not find any significant correlation between EGFR gene status and the histological subtypes, tumor grade, performance status, or the incidence of metastasis. Our data in this context agree with Gaur., *et al*. [32], who found no significant association between EGFR mutations and gender, smoking, or the histological subtypes. In contrast, Demiray., *et al*. [33] and Jazieh [34] were able to find a highly significant association between the presence of EGFR mutations and gender, non-smokers, and histological types (adenocarcinoma). Similar to these mentioned studies, females had a higher rate of EGFR mutations (60.3%) than males (39.7%). Non-smokers were more likely than smokers to have activating EGFR mutations in an Egyptian study [31].

Almost all research studies that tackled the EGFR mutations in NSCLC tried to find how to predict the EGFR status from the clinic-pathologic parameters. Also, we tried to carry out the same goal in the current work. According to the logistic regression analysis model, 79.6% of the participants were predicted correctly. However, the variables were better at helping us predict who would be EGFR negative than at who would be EGFR positive with 60% false-positive results. Therefore, we cannot rely on the model to predict the EGFR mutation status in practice. Thus, testing EGFR is a must for our patients' better management.

In conclusion, like other countries, EGFR mutation is relatively common in the NSCLC patients from Egypt as they represent about one-fourth of the studied cohort. Therefore, testing for the EGFR gene status in the NSCLC cases should be routinely performed according to the standard guidelines.

Declaration of Interest

All authors declare that there is no conflict of interest related to the manuscript.

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