The Frequency of Epidermal Growth Factor Receptor (EGFR) Mutation in Patients with Lung Cancer Referred to the Lung Diseases Hospital; A Cross Sectional Study

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Abstract

Background

Various studies showed the usage of epidermal growth factor receptors (EGFRs) gene mutations in the therapeutic plan of patients with advanced lung cancer. This study aimed to investigate the frequency and types of EGFR gene mutations among Iranian patients with lung cancer referred to a specialized lung diseases hospital from 2014 to 2019.

Results

The study was included 570 individuals, with an average age of 58.74 years old, of which 51.6% were men and 48.4% were women. Gene mutations were present in 113 of the 570 (19.8%) participants. There was deletion, replacement, and deletion and replacement in 65 (57%), 48 (42.1%), and (0.9%) cases, respectively. The mutation was significantly higher among women (P = 0.001); in detail, deletion was higher among female participants, although the replacement mutation was the same in male and female groups. There were no significant differences in mutation among different age groups (P = 0.05).

Conclusion

EGFR gene mutations, associated with lung cancer and could help patients' therapeutic plan, had the frequency of about 19.8% among Iranian patients with lung cancer.

1. Background

Lung cancer is the most prevalent cause of cancer death, claiming 1.6 million lives in 2012 worldwide, and the number of worldwide deaths is expected to grow up to 3 million by 2035 (1). It has been shown that the total number of breast, prostate, and intestinal cancer victims is lower than the number of patients who died from lung cancer (2). However, limited health care resources in developing countries make a barrier to observe the exact incidence and mortality rate of the disease. Lung cancer has four major histopathological types divided into small-cell lung cancer and non-small-cell lung cancer, accounting for about 80% of lung cancer (3,4).

As the most frequent malignant neoplasm in most countries among both sexes, lung cancer has some advances in surgical, radiotherapeutic, and chemotherapeutic approaches. Still, the long-term survival rate remains low (5), and the median overall survival and the 5-year survival rate are 1 and 3.5%, respectively (6). However, some critical risk factors, including tobacco consumption, lung fibrosis, genetic susceptibility, poor diet, air pollution, and occupational exposure, seem to be correlated with future lung cancer involvement (7–9).

Advances in studying biomarkers as a quantitative factor in diagnosing and treating many diseases result in personalized medicine and appropriate therapeutic plans and drugs for each patient (10–12). One of the genes that affect lung cancer is the Epidermal Growth Factor Receptor (EGFR) (13). It is a protein membrane with tyrosine kinase activity involved in various functions, such as cell growth, proliferation, and differentiation (14). Studies have shown the correlation between EGFR mutation and different cancers, which activate the cell surface receptor (15–17). Besides, this protein would increase cell survival by inhibiting apoptotic pathways (18,19). Consequently, it has been shown that the EGFR mutation is correlated with the non-small-cell lung cancer patients' response to therapy, such as Gefitinib and Erlotinib, as EGFR inhibitors (20–22).

Previous studies have confirmed the impact of this mutation on deciding the appropriate therapeutic plan for the patients. Moreover, it has been stated that the frequency of mutation of EGFR is different among the different population, and it might be related to the race; for instance, a study has shown that the mutation frequency is 2% and 26% among American and Japanese population, respectively (18–21,23). Hence, the appropriate response to EGFR inhibitors is supposed to differ among the different populations.

In Iran, lung cancer is the second and third cause of cancer-related death among men and women, respectively [24, 25], and the country is struggling with the disease. However, there is a lack of investigation about the EGFR mutation among Iranian patients with lung cancer, influencing the selected therapeutic plan. A study in 2018 revealed the frequency of 24.3% mutation among 103 patients with lung cancer (24). In the current study, 570 patients referred to the National Research Institute of Tuberculosis and Lung Diseases (NRITLD) were investigated in terms of EGFR mutation to identify the frequency of this mutation among Iranian lung cancer patients.

2. Results

2.1 Demographic data

570 patients were referred to the molecular and pathology department with the lung cancer diagnosis were included in the study (Table 1). The mean age of the participants was 58.74 ± 11.84. In terms of sex, men and women constituted 294 (51.6%) and 276 (48.4%) of the participants. The mean age of the male patients was 59.8 ± 10.48, and the mean age of the women was 57.81 ± 12.92. Hence, the patients' mean age was not significantly different between male and female candidates ($P > 0.05$).

Table 1
Patients Demographic Characteristics

Subject	Variables
Number of Patients	570
Male	294
Female	276
Age (average)	58.74 ± 11.84
Former or Current Smoker	312
Never Smoker	258

2.2 The Frequency of EGFR Mutation among Participants

Among 570 participants, 113 of them (19.8%) had EGFR mutation; 57% of the mutations were in the form of deletion, 42.1% were in the state of replacement, and 0.9% of mutations, had both replacement and deletion. The most frequent EGFR mutation was detected in exon 19 (54.4%), followed by exon 21 (36.8%). Moreover, 7% of the mutations were detected in exon 18, and 1.8% of the mutations were in two exons. Table 2 depicted the frequency of mutation in different exons.

Table 2
The frequency of each type of mutations in different exons and genders

	Types of Mutation		
	Deletion	Replacement	Deletion and Replacement
18	0	8	-
19	61	1	-
21	4	38	-
18 & 19	0	0	1
21 & 19	0	1	0
Male	18 (27.7%)	26 (54.2%)	0
Female	47 (72.3%)	22 (45.8%)	1 (100%)
Total	65	48	1

2.3 Influence of Gender on Mutations

Among all 113 detected mutations, men and women were constituted 43 and 70 of the mutations, respectively. Although the differences between the number of male and female participants were not significant, the mutations were significantly higher among female candidates than males (P = 0.001). The

frequency of exon 19 mutation was significantly higher in women (P = 0.02) than in men (Table 3). Moreover, among all deleted nucleotides detected (65 cases), 27.7% and 72.3% were found in men and women. Hence, the differences between the men and women in terms of deletion were significant (P = 0.016). However, the difference between men and women in terms of replacement mutation was not significant (P > 0.05), which was detected in 22 men (45.8%) and 26 women (54.2%).

Table 3
The Frequency of Each Exon Mutation among Men and Women

	Sex		
	Male	**Female**	**P-value**
18	5 (62.5%)	3 (37.5%)	> 0.05
19	15 (24.2%)	47 (75.8%)	0.02
21	23 (54.8%)	19 (45.2%)	> 0.05
19 & 18	0	1 (100%)	-
19&21	1 (100%)	0	-

2.4 Mutation among Different Age Groups

Different age groups did not show significant differences in the frequency of deletion and replacement mutations (P > 0.05). Besides, the kind of mutated exons was not significantly different among age groups (P > 0.05).

3. Discussion

The current study aimed to evaluate the frequency and types of epidermal growth factor receptor (EGFR) mutations in lung cancer patients. Following this evaluation, EGFR gene mutations were observed in 19.8% of participants. Most of these mutations (57%) were nucleotide deletions, and the replacement was in the following category (42.1%). In one case (0.9%), nucleotide deletion and replacement were observed simultaneously.

Mutations are not limited to a change in just one nucleotide (replacing one nucleotide with another nucleotide); they also include deletions, insertions, and duplication, which necessitates the study of the genome in different diseases, including cancer. Among the various types of cancer, lung cancer is the most common cause of cancer-related death among American men and women. The number of deaths due to lung cancer is higher than the total number of deaths due to breast, prostate, and colorectal cancer together (2). Hence, it is essential to investigate lung cancer, looking for possible mutations affecting the diagnosis, treatment, and prognosis of this cancer, as the deadliest cancer globally.

One of the biological changes that might be related to lung cancer is the mutation of the EGFR oncogene (25). Epidermal growth factor receptor (EGFR) is a cell membrane protein with tyrosine kinase activity, which has various functions, such as cell growth, proliferation, and differentiation (21). Numerous studies demonstrated an association between the EGFR gene polymorphism and advanced stages of various cancers, such as lung and gastric cancer [26, 27]. The presence of a mutation in the EGFR gene activates the receptor on the cell surface (17). Besides, this receptor induces cell survival by inhibiting apoptotic pathways (18,19). EGFR mutations have been revealed to be associated with responsiveness to receptor antagonist drugs (such as gefitinib) in patients with non-small cell lung carcinoma (NSCLC) (26,27). Reports have also shown that allelic forms of this gene are involved in lung cancer (28,29). In addition, the gene polymorphisms of this receptor might be related to race and geographical conditions and vary in different populations, so that the rate of polymorphism in the American people is 2% and in the Japanese population is 26% (18,20). Therefore, it seems essential for each country and region to investigate the rate and frequency of this mutation among its people.

In the current study, in 113 of the total 570 samples (19.8%), EGFR mutations have been detected. However, in Iranian patients with esophageal cancer, the incidence of EGFR mutation was higher, and Lashkarizadeh et al. reported that 82% of the 60 samples were mutated (30). In our study, 65 cases (57%) of all mutations were in the form of deletion. In 48 patients (42.1%), nucleotide replacement was present, and a combined nucleotide deletion and replacement coexisting simultaneously were observed in only one case (0.9%). In the study of Lashkarizadeh et al., in 52% of cases, the mutation was of the gene deletion type, in 30% of the cases, the mutation was seen as gene duplication, and in other cases, both types of mutation were detected simultaneously (30). In this study, most of the deletion mutations occurred in exon 19 (about 94%), while most of the replacement mutations occurred in exon 21 (about 79%). In the study of EGFR mutations in patients with esophageal cancer, the most deletion mutations were in exon 2 (44%), and the highest rate of replacement mutations (54%) was in exon 27 (30).

In a similar study by Dr. Basi and colleagues on lung adenocarcinoma, EGFR mutations were observed in 25 of 103 patients (24.3%), which is inconsistent with the value obtained in our study (24). In Dr. Basi's study, the most common sites of mutations were exon 21 (15 patients; 60%) followed by exon 19 (10 patients; 40%); although in our study, it was the other way around, and the mutations in exon 19 (62 patients; 54.9%) occurred more frequently than Exon 21 (42 patients; 37.2%), following by exon 18 mutation (8 patients; 7.1%). In this study, the overall incidence of mutation in women (70 patients; 61.9%) was significantly higher than in men (43 patients; 38.1%). It was in contrast with the study of Dr. Basi et al., in which the incidence of mutations was equally distributed between men and women (24). In our study, the incidence of deletion mutations in women was significantly higher than in men. Still, there was no significant difference between the two sexes in terms of replacement mutations. However, in the study of Dr. Basi, no similar investigation has been done.

Investigating biomarkers in blood, urine, and tissue is becoming appropriate markers for early cancer detection (31,32). Accordingly, various studies have been performed on the possibility of using different tumor biomarkers for lung cancer screening. Plasma microRNAs, circulating tumor cells, and

autoantibodies are presented as possible biomarkers for lung cancer diagnosis (33–35). In other studies, the serum level of Carcinoembryonic antigen (CEA) in patients with non-small-cell lung cancer was higher than the other types of cancer (36,37). Moreover, another study on 184 patients revealed a relation between lung cancer involvement and CK19 and CEA serum levels (38). Hence, advances in biomarkers and genomic fields are considered the future of cancer investigation, and EGFR as an important proven marker could be used in this regard. Consequently, its frequency in different populations could play an important role in planning treatment guidelines for lung cancer in each country.

In conclusion, the association of the EGFR gene mutation with lung cancer has been indicated, so investigation of EGFR mutation could help decide about patients' therapeutic plan, such as anti-EGFR drug usage. The frequency of EGFR mutation in lung cancer patients referred to a specialized lung disease hospital has been investigated. A higher frequency among Iranian patients than the western population has been obtained. However, the frequency seems to be almost the same as the eastern population.

Further multi-centric studies with a higher number of participants, other genomic evaluations, and investigating the possible application of this mutation for screening and early detection of cancer among the different population are recommended.

4. Conclusion

The frequency of EGFR mutation among the Iranian population with lung cancer referred to a specialized lung diseases hospital is 19.8%, and it is higher among female patients compared to males. Most of the mutations were in the form of deletion and were present in exon 19.

5. Material And Methods

This was a retrospective descriptive study. The data of patients with lung cancer referred to the Masih Daneshvari hospital, Tehran, Iran (National Research Institute of Tuberculosis and Lung Diseases (NRITLD)), from 2014 to 2019, were gathered. Patients' demographics (including age, sex, and the city they have lived in), history of the disease, and therapeutic plan were obtained from Hospital Information System (HIS). The patients were referred to the Pathology and Molecular Department of the hospital for genomic investigation.

These patients had undergone a biopsy, or the specimens were resected surgically. The samples were formalin-fixed paraffin-embedded (FFPE) tissues from primary or metastatic sites. The mutation analysis was conducted, the genomic DNA was extracted, and exons 18, 19, and 21 of the EGFR gene were amplified by polymerase chain reaction (PCR). Analysis of the genomes was performed in line with company protocols. All these molecular analysis data were available at the hospital information system (HIS).

The investigation was performed following the Declaration of Helsinki's ethical standards and national and international guidelines. It has been approved by the Institutional Review Board of Shahid Beheshti University of Medical Sciences, with written informed consent obtained from all patients.

The data are shown as mean ± standard deviation. The statistical analysis was performed using the Chi-square test and Student's t-test to compare the categorical variables and independent groups, respectively. P-value < 0.05 was considered statistically significant. All analysis was conducted with SPSS v25.0 (IBM, Armonk, NY, USA).

6. Declarations

6.1 Ethics and Consents

Investigation has been approved by Institutional Review Board of Shahid Beheshti University of Medical Sciences, with written informed consent obtained from patients.

6.2 Consent for Publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

6.3 Availability of data and materials

All data generated during this study are included in this published article.

6.4 Competing Interest

The authors declare that they have no conflicts of interest.

6.5 Funding

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6.7 Authors' contributions

All authors met the authorship contribution criteria based on the international committee of medical journal editors' recommendation. All authors met the authorship contribution criteria based on the international committee of medical journal editors' recommendations. The contributions are as follows:

M.R: conceived of the presented idea; S.M and F.Sh.: wrote the manuscript in consultation with all authors; M.R, S,M, M.P , and L.MZ: conceived the study and were in charge of overall direction and planning; M.R, M.P, B.S, S.S, A.D, and A.Kh: were involved in the plan of the patients; M.R and L.MZ have conducted the pathological analysis; S.M and M.R gathered the data, S.M and F.Sh conduct data analysis. All authors discussed the results and commented on the manuscript.

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Figures

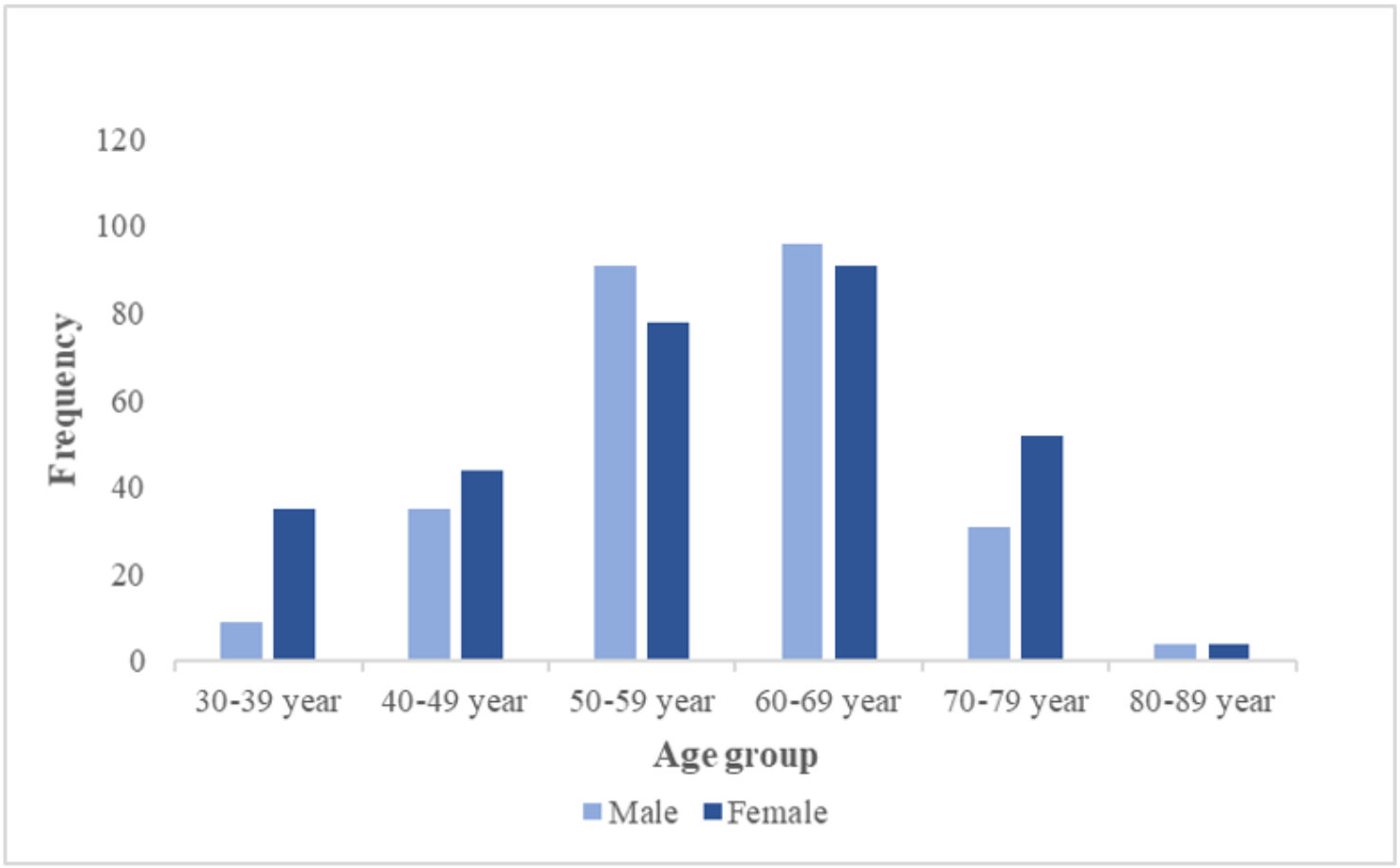

Figure 1

The Frequency of age groups (among male and female)