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Evaluation of EGFR gene expression and four diagnostic mutations in EGFR in lung cancer patients

Abstract

Lung cancer is the second most common cancer in men and women and is one of the most preventable cancers. a small number of known mutations of the EGFR gene for diagnostic purposes are being studied in laboratories and research centers in our country (Iran), the present study aimed to investigate 4 important EGFR gene mutations in lung cancer patients in the Iranian population in order to provide a method for early diagnosis and improvement of prognosis in lung cancer. Lung paraffin tissue samples were prepared from the pathology department of Imam Hossein Hospital in Tehran. DNA and RNA extraction was performed and mutations studied included 4 diagnostic point mutations in the EGFR gene named G719A (2156G>C) in exon 18, R776C (2326C>T) in exon 20, L861Q (c.2582T> A) in exon 21 and L858R (c.2573T> G) in exon 21. The expression level of the EGFR gene was evaluated using Real-Time PCR. The results of examining the 4 mutations by qRT PCR method in patients showed that G719A, R776C, L861Q, and L858R were positive in 11,7%, 4.2%, 4.2%, and 6% of patients respectively and these frequencies in the case group were significantly higher than the control group. Results of EGFR expression showed overexpression in the case group in comparison with the control group (fold change 8, p-value=0.001). The results of the present study showed that common mutations of EGFR gene including G719A, L861Q, R776C, and L858R mutations in the Iranian population show a significant frequency and this study succeeded in first designing the correct primers and setting up the PCR program in the form of a multiplex reaction to evaluate 4 mutations simultaneously.

Keywords: *EGFR, gene expression, diagnostic mutations, lung cancer*

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Introduction

Lung cancer is the second most common cancer in men and women and is one of the most preventable cancers. There are generally two types of lung cancer Small cell lung cancer (SCLC) and Non-small cell lung cancer (NSCLC). Lung cancers are classified under the microscope based on the appearance of the cells. NSCLC is also divided into three categories including superficial tissue cancer, mucosal and lymph node carcinoma (glandular epithelium), and large cell lung cancer. Among people with lung carcinoma, about 85-90% of cases are NSCLC and about 10-15% are SCLC (1-3).

Like other types of cancer, Lung cancer is often the result of a series of genetic changes, including the activation of proto-oncogenes and their conversion to oncogenes, and the inactivation of tumor-suppressor genes (TSGs) (4, 5). Oncogenes that lead to lung cancer include c-myc, mutant K-Ras over-expression of EGFR, cyclin D1, and BCL2 (6). Epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor that is often overexpressed in NSCLC. These receptors play an important role in the survival of tumor cells, and active phosphorylated EGFR leads to the phosphorylation of downstream proteins, which promotes cell proliferation, invasion, metastasis, and inhibition of apoptosis. The expression seems to depend on histological subtypes, which are often expressed in squamous cell carcinoma but are also often expressed in adenocarcinomas and large cell carcinomas. Not surprisingly, many published reports attempt to link EGFR overexpression to survival. However, the data on the prognostic role of EGFR expression is contradictory and confusing, with some reports suggesting that EGFR is associated with poor survival while no prognostic association has been found in other reports (7-9).

Currently, several mutations of the EGFR gene for diagnostic purposes are being studied in laboratories and research centers in our country (Iran), the present study aimed to investigate four important EGFR gene mutations in lung cancer patients (including G719A in exon 18, R776C in exon 20, L861Q in exon 21 and L858R in exon 21) in Iranian population in order to provide a localized method for early diagnosis and improvement of prognosis in lung cancer.

Methods

Lung tissues were prepared from the pathology department of Imam Hossein Hospital in Tehran. For this purpose, paraffin tissue samples (surgical lung tissue samples, kept in the form of paraffin blocks) from 60 patients with lung cancer and 60 patients as a control group who undergo surgery at Imam Hossein Hospital from 2018 to 2020 were prepared from the pathology department. DNA and RNA extraction from paraffin tissue was performed using the Exgene FFPE Tissue kit (GeneAll Company -South Korea). The mutations studied included four diagnostic point mutations in the EGFR gene named G719A (2156G>C) in exon 18, R776C (2326C>T) in exon 20, L861Q (c.2582T> A) in exon 21 and L858R (c.2573T> G) in exon 21. (Table 1). Primer sequences have shown in table 2.

Table 1. Mutation Information			
Mutation Name	**Rs code**	**Exon**	**Mutation location**
L858R 2573T>G	)rs1214345568(	21	GATTTTGGGCTGGCCAAACT
L861Q 2582T>A	)rs121913444(	21	GATTTTGGGCTGGCCAAACT
G719A 2156G>C	)rs28929495(	18	CAAAAAGATCAAAGTGCTGG
R776C 2326C>T	(rs1275022697)	20	CAACCCCCACGTGTGCC

Table 2. Primer sequences	
Mutation Name	**Primer Sequence**
G719A 2156G>C	Forward: AGCTCTCTTGAGGATCTTGAAGGAA
	Reverse: CTTACCTTATACACCGTGCCGAA
L861Q 2582T>A	Forward: AAAACACCGCAGCATGTCAAGAT
	Reverse: GCATGGTATTCTTTCTCTTCCGCA
L858R 2573T>G	Forward: AATACACCGCACCATGTCAAGAT
	Reverse: GCATAGTCTTCTGGCTCTTCCGCA
R776C 2326C>T	Forward: CATGCGAAGCCACACTGACGT
	Reverse: TGAGGCAGATGCCCAGCAGTTA

According to the aim of the study, which was to investigate the common mutations of the EGFR gene by multiplex method, an attempt was made to adjust the temperature program of the studied primers in the form of a Cybergreen master mix as shown in table 3. Using this temperature program, it was possible to detect four mutations G719A 2156G> C, L861Q 2582T> A, L858R 2573T> G, and R776C 2326C> T in the form of one reaction in the four microtubes.

Table 3. PCR program			
stage	**cycle**	**Temperature**	**Time**
Denaturation	1	95 °C	15 minutes
Cycling	35	95 °C	35 seconds
		65 °C	25 seconds
		72 °C	35 seconds signal collection

One of the other aims of this study was to determine the expression of the EGFR gene in patients. The probes are labeled using a FAM reporter at the 5′ and the expression level was assessed using the real-time PCR method.

Results

Demographic data. In this study, two groups of patients were evaluated. In the case group, there were 60 Lung paraffin tissue samples from lung cancer patients and in the control group there were 60 Lung paraffin tissue samples from non-cancerous patients. Demographic data showed in table 4.

Mutation detection data

Variables	Result	
	Case group	**Control group**
Gender, male n (%)	44 (73%)	38 (63%)
Age (years)	57.2 ±13.4	53.1± 11.5
BMI (kg/m2)	21.48 ± 4.60	20.7±3.91
Smoking	41 (68.3%)	30 (50%)
Family History of cancer	24 (40%)	18 (30%)
Family history of lung cancer	6 (10 %)	2 (3.3%)

. The results of examining the presence of G719A 2156G> C mutation by qRT PCR method in patients showed that this mutation is present in 14 patients (mutation frequency of 11.7%) and 106 patients (88.3%) have not this mutation. All the patients who were positive for G719A results were in the case group. The results of L861Q 2582T> A mutation by in patients showed that this mutation was present in 5 patients (mutation frequency of 4.2%) and 115 patients (95.8%) have not this mutation. Also, results showed that 4 patients had L861Q mutation in the case group. and 1patient in the control group. The results of L858R 2573T> G mutation in patients showed that this mutation was present in 5 patients (mutation frequency of 4.2%) and 115 patients (95.8%) had not this mutation. All patients who showed L858R mutation were in the case group. The results of examining the presence of R776C 2326C> T mutation by qRT PCR in patients showed that this mutation was present in 8 patients (mutation frequency of 6%) and 112 patients (94%) had no mutation. 6 and 2 patients showed L858R mutation was in the case and group respectively. Results are summarized in table 4.

Table 5. summary of mutation detection in patients

name of Mutation	**Mutation status**	**Number of patients**	**%**
G719A 2156G>C	Positive	14	11.7%
	Negative	106	88.3%
L861Q 2582T>A	Positive	5	4.2%
	Negative	115	95.8%
L858R 2573T>G	Positive	5	4.2%
	Negative	115	95.8%
R776C 2326C>T	Positive	8	6%
	Negative	112	94%

Results of EGFR expression showed overexpression in the case group in comparison with the control group (fold change 8, p-value=0.001).in Figure 1.

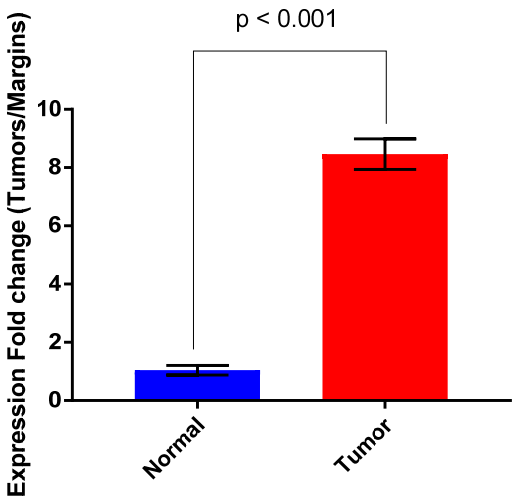

Figure 1: Chart of changes in EGFR gene expression in the study groups

Discussion

Mutations that lead to overexpression of EGFR (known as regulation or amplification) have been linked to several cancers, including lung adenocarcinoma, rectal cancer, glioblastoma, and head and neck epithelial tumors (10). These somatic mutations in EGFR lead to its permanent activation, which causes uncontrolled cell division. Mutations, amplification, or inappropriate regulation of EGFR or family members are involved in about 30% of all epithelial cancers.

Among the EGFR mutations tested in lung cancer, several rare types are treated differently from the more common EGFR mutations. The main example of this in lung cancer is the insertion of exon 20 EGFR. This is a type of EGFR mutation that does not respond to the usual treatment for lung cancer EGFR positive, called tyrosine kinase inhibitors, or TKI. There are generally two ways to diagnose an EGFR mutation. The best way is through Next Generation Comprehensive Sequencing (NGS). This type of test puts the patient's tumor tissue (collected from a biopsy) into a device that looks for a large number of possible biomarkers at a time. There may be situations where the patient is unable to perform the biopsy required to perform NGS, so a liquid biopsy is recommended. A fluid biopsy can look for specific biomarkers in a patient's blood. Knowing whether EGFR is positive for lung cancer has the most therapeutic implications for stage IV patients. For most patients with stage IV EGFR-positive lung cancer, a pill called EGFR-targeted tyrosine kinase inhibitor (TKI) or EGFR inhibitor may be prescribed. In addition to patients with stage IV lung cancer, patients with stage IB-III lung cancer who have had their lung cancer surgically removed are also eligible for postoperative EGFR inhibitors.

In a study by Wu et al., The effectiveness of two chemotherapy drugs, Afatinib, and cisplatin + gemcitabine, was evaluated in Asian patients with NSCLC lung cancer who had mutations in the EGFR gene. The results of this study, which was performed as a phase 3 clinical trial on 900 people with lung cancer, showed that Afatinib is more effective in patients with mutations in the EGFR gene (11).

In a study by Satoshi et al., They compared survival in patients with rare mutations in gefitinib-treated lung cancer (including L858R, G719X, and L861Q) with common mutations in the EGFR gene. Overall survival was significantly shorter among patients with abnormal EGFR mutations compared to patients with common EGFR mutations in the general population as well as in the gefitinib group (12 vs. 28.4 months, p = 0.002 and 11.9, respectively. Versus 29.3 months; P <0.001)). A significantly shorter survival time was observed in patients with abnormal EGFR mutations compared to survival time in patients with common EGFR mutations (12).

In a study by Thress et al., They examined the effect of C797S and T790M mutations on the EGFR gene in response to treatment with tyrosine kinase inhibitor chemotherapy (AZD9291). The results of this study showed that people with these mutations are resistant to the chemotherapy drug under study and it is suggested that these mutations be examined at the beginning of the chemotherapy course with this method (13).

Conclusion

The results of the present study showed that common mutations of the EGFR gene including G719A, L861Q, R776C, and L858R mutations in the Iranian population show a significant frequency. Considering the main purpose of this study, which is to prepare a lung cancer diagnostic kit based on EGFR mutations in the Iranian population, this study succeeded in first designing the correct primer probes and setting up the PCR program in the form of a multiplex reaction to evaluate 4 mutations, the frequency of mutations in the

Iranian population studied was obtained and showed its potential to become an experimental method of replacing expensive laboratory methods. It is suggested that in order to study more accurately the effects of mutation on the treatment process, prognosis, and survival of patients, the course of treatment of patients with mutation and no mutation should be compared and better conclusions should be obtained.

Acknowledgments

None.

Conflict of interest

None.

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None.

Ethics Statement

All Permissions to conducting this research has been approved.

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