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Potential Effects of EGFR Exon 21 L858R Mutations in Lung Cancer

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Abstract

Objective: The mainly significant reason in the etiology of lung cancer is smoking, which is more important in other environmental pollutants and genetic susceptibility. Lung cancer is separated two major groups as mainly non-small cell and small cell according to the growth rate, spread, timing of metastasis, response to chemotherapy and radiotherapy. Epidermal growth factor receptor (EGFR) constitutes the highest rate with 50-80% in gene mutations which are prognostic value in lung cancer. Many studies have shown that EGFR is overexpressed in lung cancer. In our study, we aimed to investigate the relationship between EGFR gene exon 21 L858R mutations in lung cancer.

Material and Methods: Our sample consisted of a healthy group of 190 healthy volunteers with the same age and gender characteristics as the patient group of 178 patients who were diagnosed as lung cancer in the Mersin University Medical Faculty Oncology clinic. The DNAs were obtained according to the standard salt precipitation method. Mutation detection and genotyping analyzes were identified by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyzes.

Results: Smoking was one of the other risk factors for lung cancer, smoking rates are 130 (68.33%) in the control group and 156 (87.5%) in the lung cancer group. The 27 person of the lung cancer (15%) were female and 151 (85%) were male. In the control group, 92 people (48.33%) were in the wild genotype and 98 persons were in the mutant genotype (51.66%). In the lung cancer group 80 (45%) were wild-type and 98 (55%) were mutant genotypes. According to the histopathological types of lung cancer, EGFR-21 mutation heterozygous or homozygous carriers are proportionally compatible ($p = 0.90$).

Conclusion: According to our findings, the EGFR-21 mutation is not associated with histopathological types of lung cancer.

Keywords: Lung cancer, EGFR gene, exon 21, L858R mutations

Introduction

Lung cancer is the mainly widespread cancer type among cancer types and has the highest mortality rate. The most important factor in the etiology is the use of cigarettes, other environmental pollutants and their genetic predisposition (1,2). Studies have been conducted to investigate whether lung cancer is genetic or environmental causes of a chromosomal alteration event. In people with lung cancer in first-degree relatives, the risk of developing cancer increases 2-4 times. However, this is not entirely due to genetic factors; it is thought that relatives are in the same environment. Chronic carcinogen exposure results in genetic damage.

The underlying cause of cell damage is the change in genes that control cell proliferation. In women with familial history of lung cancer, the risk increases 5-7 times (1-5).

The rate of lung cancer is separated in two major groups as mainly non-small cell and small cell according to the growth rate, extend, timing of metastasis, response to chemotherapy and radiotherapy. Non-small cell lung cancers are subdivided into adenocarcinoma, squamous cell carcinoma and large cell carcinoma. Small cell lung cancer is associated with smoking, mediastinal lymphadenopathy and is the worst prognosis type among the lung cancers.

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Approximately 83% of all lung cancers consist of non-small cell lung cancer and 17% small cell lung cancer (6-10).

The gene mutations that are prognostic value for lung cancer are listed as EGFR 50-80%, Caspase 3 30-73% and KRAS 7-32% respectively. Epidermal growth factor (EGF) is a peptide whose mitogenic effects have been shown to stimulate cell proliferation and differentiation (11,12). Overexpression of EGFR is characterized by improved stage disease, metastatic phenotype development, decreased survival and poor prognosis. The relationship between EGFR and carcinogenesis is due to overexpression of normal EGFR, continuous activation of receptor by the development of mutation at the receptor, impairment of physiological ligand-receptor balance due to over-construction of ligands, decrease in phosphatase activity and heterodimerization (11,12,13). There are many studies on the relationship between EGFR signaling/regulation defects and tumor development (14-19).

Our study included polymorphism analysis of EGFR (exon 21 858. position leucin-arginine change), which is important in the etiology of lung cancer. Information on the association of lung cancer with EGFR gene polymorphisms has become clear in the last few years (20,21,22).

In some developed countries, these analyzes have been included in the coverage of Social Security Institutions or Health Insurance Institutions of the applicable countries in lung cancer. In fact, our study according to the above hypothesis, according to a scientific purpose, will also shed light on the identification of our patients and therefore the determination of the treatment protocol to be followed.

Our secondary goal in this study is to discover the other risk factors for lung cancer in the light of information form to be applied to the disease; age, gender, smoking, alcohol use, BMI, and family history of lung cancer will be evaluated. In this study, frequencies of the polymorphic properties of the gene will be determined on the basis of Mersin sample. This information will make an important contribution to the determination of the frequencies in the Turkish population for these properties. If a relationship with other diseases is detected with these genes, the Turkish population and Mersin sample will contribute to the formation of the risk map of the disease.

Material and Methods

Case Groups

Our study consisted of 178 patients (151 male + 27 female) with a mean age of 59,24 who were diagnosed with lung cancer in Mersin University Medical Faculty Oncology Clinic and a control group of 190 persons with an average age of 57,81 which was formed from healthy individuals considering the same age and gender characteristics. The histopathological diagnosis of the patient group was done by the same clinic. Mersin University Clinical Research Ethics Committee has been approved for our study (Approved Number: 2013/428) and informed consent form has been prepared. Information such as the age, occupation, smoking status and lung cancer stories in their families were recorded of the persons in each group.

Genomic DNA was extracted from the whole blood treated with EDTA using the QIAamp DNA Blood Mini Kit (Maryland, USA), according to the manufacturer's guidelines. The extracted DNA was stored at -20˚C until analysis.

EGFR Exon 21 L858R Mutation Studies

EGFR gene mutation was identified by PCR-restriction fragment length polymorphism in 178 lung cancer patients. The 20 µL PCR system of the first run contained 20 ng DNA, 1.5 mmol/L MgCl2, 1×PCR buffer, 200 nmol deoxynucleotide triphosphates, 200 nmol/L PCR primers and 0.2 U TaqDNA polymerase (Qiagen). This grade was to present restriction sites of restriction enzyme BstXI and XcmI by primer EGFR

Forward, 5'-GCAGCATGTCAAGATCACAGATT-3' and Reverse, 5'-CCTCCTTCTGCATGGTATTCTTTCT-3'. PCR appliance (2720 Thermal cycler, Applied Biosystems) was designed for amplification. PCR cycling condition was as follow: initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing in 55°C for 30 s, and extension at 72°C for 30 s. There were a total of 35 run and a final extension for 5 min at 72°C. The product of PCR was 166 bp. PCR product (2 µL) containing 200 ng DNA was digested by MscI (MBI Fermentas) and PvuII (New England Biolabs) at 37°C for 2 h. The digested 10 µL products were examined on a 3% agarose gel electrophoresis and staining with ethidium bromide. The results were analyzed by a UV imaging system. The digested fragments of wild-type DNA included 157, 97, and 60 bp, and the digested fragments of mutant DNA included 97 and 60 bp (23-27).

Statistical Analysis

Selected characteristics were compared between cases and controls by using the Student's T-test for continuous variables and the Chi-square test for categorical variables. Allele and genotype frequencies between cases and controls were calculated and deviation from Hardy-Weinberg equilibrium was examined by the Chi-square test. We calculated odds ratios and 95% confidence intervals by using unconditional binary logistic regression. Results are reported as the mean±SD. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS for Windows v.22.0). p value less than 0.05 was accepted as statistically significant. Lung cancer patients are analyzed with respect to age groups and their ratios in Mersin sample using Kruskal Wallis test. Total Mersin population is 1.727.255.

Results

When the genotype ratios for the EGFR exon-21, L858R mutation were examined, the wild genotype in the control group was 48.33%, the mutant genotype was 51.66%, the wild genotype 45%, the mutant genotype 55% in lung cancer (p:0.605)(Figure 1). The other risk factors were diagnosed for lung cancer, smoking, sex, and the age of cancer. Smoking rate was 68.33% in control group and 87.5% in lung cancer (p: 0.04). 85% of the lung cancer are male and 15% are female (p: 0.0001) (Table 1).

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When the distribution ratios of lung cancer according to age groups are examined in the experimental group, > 49 ages patients: 15%, 49-59 ages patients: 40%, 59-69 ages patients: 30% and 69 years old patients were 15% (p:0.0045). In our country, especially in our region, because of the short duration of life due to the shortage of old age, lung cancer has been examined by age group in the population of Mersin in order to not to mislead us of the low rate of old age (Table 2). Distribution of lung cancer among the Mersin population according to age groups; > 49 ages 13%, 49-59 ages 26%, 59-69 ages 33% and 69 ages and over 22% respectively (p:0.0001).

The distribution of patients with lung cancer according to histopathological types was 53.93%, squamous cell carcinoma 30.33%, large cell carcinoma 3.93% and small cell carcinoma 11.79% (p: 0.76). On the basis of histopathological types of lung cancer, heterozygous or homozygote carrying EGFR-21 mutation was proportionally compatible (p:0.90). This indicates that the EGFR-21 L858R mutation is not associated with histopathological types of lung cancer.

Table 1: General results of EGFR gene exon-21, L858R mutation genotype ratios and other risk factors for lung cancer

	Control		Lung Cancer		p Value
Genotype	N	%	N	%	
TT	92	48.3	80	45	0.605
TG	98	51.66	98	55	
Gender					
Male	165	86.7	151	85	0.0001
Female	25	13.3	27	15	
Smoking					
Smokers	130	68.33	156	87.5	0.04
Non- Smokers	60	31.67	22	12.5	

Table 2: Distribution of lung cancer by age group in the experimental group

Age Groups	Lung Cancer (N)	Lung Cancer (%)	Distribution of population by age groups (N)	Lung Cancer in total population (%)	p Value
< 49	27	15	1.328.787	0.0013	
49-59	71	40	186.367	0.0258	
59-69	53	30	110.367	0.0326	0.045
>69	27	15	80.581	0.0223	
Total	178	100	1.705.774	0.082	

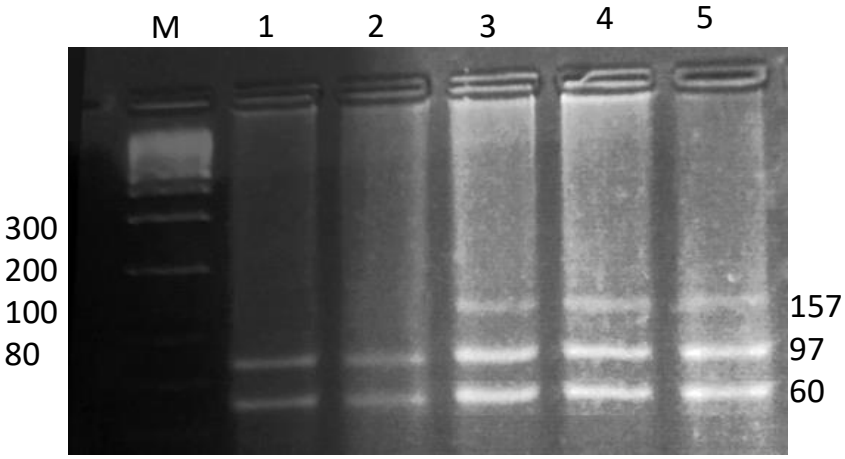

Figure 1: Detection of the L858R polymorphisms of EGFR exon 21 gene by RFLP method. M: DNA marker; 1-2: patients wild type DNA sample; 3-5 patients mutant type DNA sample

Discussion

Lung cancer is the major widespread cancer type among both man and women in cancer types and the incidence is increasing all over the world (3,4,5).

Lung cancer is the expression used to explain the increase of unusual cells inside layer the air passages in the lung tissue. These cells separate and increase more quickly according as normal cells and merge to appearance a group, or tumor. There are two major types of lung cancer: Non-small cell lung cancer (NSCLC) and Small-cell lung cancer (SCLC). NSCLC is the mainly widespread type of lung cancer, comprising of 80-85% of lung cancer cases. NSCLC might be additional separated inside numerous dissimilar subtypes so are identified by the kinds of cells and the position of the tumor. Each subtype needs to be treated differently. The major prevalent subtypes are adenocarcinoma, squamous cell carcinoma and large cell carcinoma (6,7,9,28).

Adenocarcinoma both the major widespread type of lung cancer and the most common form of NSCLC, improve within the mucus producing cells in the coating of the airways. Squamous cell carcinomas develop in the squamous cells extending along the airways and are inclined to extend locally. It is frequently owing to smoking and has restricted dealing options. Large cell carcinoma named after the large, circular cells that are observed as checked microscopically. It is occasionally recognized as 'undifferentiated carcinoma' and has a high propensity to extend to other parts of the body (6,7,9,10).

SCLC about 15% of all lung cancers is small cell lung cancer. In this group, cancer cells are small cells dominated by nuclei. SCLC is generally correlated with smoking and commonly spreads rapidly at early periods. Owing to its thrusting character there are merely two phase of SCLC: restricted and extensive disease and prognosis are frequently reduced. Cigarette smoking is the primary reason of most lung cancer contributing to nearly 90% of cases in high-income countries. Another reason contains extended contact through radon gas, asbestos or certain other chemicals. Previous non-malignant lung diseases as well enlarge the risk of lung cancer (6,7,29).

Current treatments include surgery, radiotherapy and chemotherapy. Chemotherapy increases the survival rate of patients with lung cancer, but the median 5-year survival rate is still 15%. Smoking especially in NSCLC has a significant role in etiology and the 5-year survival rate of patients is less than 5%. Many aspects of the molecular biology of lung cancer have not been elucidated and studies have been increasing in this area over the past 20 years (6,11,28,30).

Even if cytotoxic agents provide an improvement in value of life in lung cancer treatment, further therapeutic approaches are considered necessary to achieve disease-related survival benefit. Factors considered to play a role in the etiology of lung cancer include: smoking habits, genetic factors, age, gender, race and geographical distribution, diet-related factors and air pollution. Various molecular alterations have been suggested to play an important role in the pathogenesis of lung cancer. These factors are considered to play a role in the etiology of lung cancer and are largely accepted: tobacco habit, genetic factors, age, gender, ethnic and geographical distribution, dietary dependent factors and air pollution (30,31,32,33).

Lung Cancer constitutes a large rate of 80%, and EGFR mutations in etiology are an important diagnostic criterion (25,26,32,34). With these mutations, characterization of lung cancer types sheds light on the treatment protocol to be followed. Our main goal in this study is to investigate the association of EGFR 21 exon L858R mutation with lung cancer. The results we obtained will shed light on the treatment protocol.

When the genotype ratios of L858R mutation of EGFR gene exon 21 were examined, it was determined that it was not a risk factor for lung cancer. In the control group, the wild genotype ratio was 48.33%, the mutant genotype ratio was 51.66%, the wild genotype ratio in lung cancer was 45% and the mutant genotype ratio was 55%.

Mitsudomi et al. found that the mutation rate in EGFR exon 21 in lung cancers was 43% (12). In an immunohistochemical study conducted by David M. et al. on non-small cell lung cancer patients, the mutation rate for the same gene region was 57% (35). Sandra P. D'Angelo et al found that 218 patients with adenocarcinoma in 218 patients (20%) and 285 patients (27%) with late phase EGFR mutations. Among early stage patients, EGFR detected a rate of exon 21 mutation of 47%, in the late phase was 39% for Exon 21 (36). Lee JS, et al. found the EGFR mutation rate as 50.5% in cases of surgically removed pulmonary adenocarcinoma (37).

In another study Kawada et al. was detected EGFR mutation in 109 lung cancer patients 41% of adenocarcinoma cases and 8% of nonadenocarcinoma cases. In the same study, EGFR ratio was found to be 45.5% in younger patients and 22.2% in older patients (38).

Other risk factors for lung cancer were smoking, male gender, and older age were significant risk factors. In addition to the presence of lung cancer in the family history, if there is a history of smoking, the risk increases by a factor of 30 and increases by 15 times, if there is only a cigarette story without family history (39,40,41,42).

EGFR is involved in the proliferation, apoptosis, angiogenesis and tumor invasion of cells. This mutation is particularly found in the region of EGFR, exon 19 and exon 20. This mutation causes sequential signal generation and activation of the ACT. This mutation identified in EGFR has also led to significant recuperations in the therapy of lung cancer. Inhibitors of tyrosine kinase, like erlotinib and gefitinib, targeting the EGFR pathway have taken place since the first stage of therapy (43,44,45).

Interestingly, this mutation in EGFR is more widespread in female patients with Asian, non-smokers and adeno-cancers. While EGFR mutation analysis is significant in starting therapy by tyrosine kinase inhibitor in the choice of first line treatment, EGFR mutation analysis is not required in the second line treatment plan because successful results can be obtained with tyrosine kinase inhibitor in patients

with non-EGFR mutated lung cancer. Sequential mutations could play a role in the success of this group of patients with no EGFR mutation (46,47,48).

Conclusion

EGFR gene exon-21, L858R mutation genotype ratios were not found to be risk agents for lung cancer. Other risk factors for lung cancer have been demonstrated to be important risk factors for smoking, male gender, and age. In the experimental group, the risk of increasing advanced age lung cancer seems to have not increased in the expected period after 59-69 and 69 years of age. Considering the fact that the reason is due to the low proportion of the population aged 59-69 and 69 years - the number of people in these age groups in Mersin population and the number of lung cancer are evaluated, lung cancers ratios are high.

Acknowledgments: This study has been approved by the Mersin University Clinical Research Ethics Committee (Number: 2013/428).

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Author's Contribution: EDE, AA, RBA, EN, AA, NE, EA: Research concept and design; data collecting, Patient examination, DNA extraction, RFLP, **DDY:** Statistics **EDE:** Preparation of article, and Revisions. All authors approved the final version of the manuscript

Ethical issues: All Authors declare, Originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities.

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