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The Effects of EGFR Exon 19 747–750 Deletion on the Risk of Developing Lung Cancer

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Abstract

Objective: Although smoking is the most significant factor in the etiology of lung cancer, other environmental pollutants and genetic predisposition also play major roles in its development. Histopathologically, lung cancers are divided into two major types, as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The latter accounts for almost 85% of all lung cancers with a very aggressive course and being associated with a high rate of mortality. Among the genetic mutations with prognostic value in NSCLC, the epidermal growth factor receptor (EGFR) mutation is most frequently found in 50 to 80% of cases. The EGFR is a transmembrane glycoprotein with tyrosine kinase activity which is associated with both normal cell growth and malignant transformations.

Material and Methods: In the present study, we aimed to evaluate the effects of exon 19 747–750 deletion in the EGFR gene on the risk of developing lung cancer and to examine its potential relationship with the different histopathological types of lung cancer. The study sample comprised a total of 178 patients diagnosed with lung cancer at Mersin University, Medical Faculty, Oncology Clinics, and 192 age- and sex-matched healthy individuals as the control group. Deoxyribonucleic acid (DNA) isolation was performed using the standard salt-water precipitation method, while the mutation screening and genotyping analyses were carried out with a polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses.

Results: The frequency of mutant EGFR exon-19 deletion in the control group was 15.1%, increasing to 36.9% in the lung cancer group, and increasing the risk of developing lung cancer by 2.64 times (p: 0.014). This increase did not significantly differ between the histopathological types of lung cancer (p: 0.76).

Conclusion: Considering the distribution of lung cancer patients in different age groups, it is obvious that advanced age is a risk factor for the development of EGFR mutation and lung cancers (p<0.001).

Key words: Lung cancer, EGFR gene, Exon 19 deletion, Older age.

Introduction

Lung cancer is the most frequent among all types of cancer and is associated with the highest rate of mortality (1). Lung cancers can be divided into two types based on cell morphology, as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Globally, more than 1.6 million individuals die from lung cancer annually (2), with almost 85% of the total attributable to NSCLC, and the remainder to SCLC. Smoking is the most important factor in the etiology of lung cancer, although other environmental pollutants and genetic predisposition also play major roles in its development (3).

Of note, NSCLC follows a very aggressive course and is associated with a high rate of mortality, being the main type of lung cancer, but incorporating several other histopathological types of lung cancer(4). In adenocarcinomas, the rates of epidermal growth factor receptor (EGFR) gene mutations, Kristen-rat sarcoma oncogene (K-RAS) mutations and mesenchymal–epithelial transition (MET) gene mutations have been reported as 20 to 30%, 30%, and 5%, respectively (5).

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The EGFR is one of the most important proteins playing a role in the cell differentiation and proliferation with an activity that involves its binding to the surface receptors of the EGFR. It is a transmembrane glycoprotein with tyrosine kinase activity which is associated with both normal cell growth and malignant transformations (6). Previous studies have shown an overexpression of EGFR in the presence of lung, head and neck, colon, pancreas, breast, ovary, bladder and kidney cancers, and in gliomas (7,8). Using the immunohistochemical methods, the EGFR overexpression has been found in 39%of adenocarcinomas, 58% of squamous-cell carcinomas, and 38%of large-cell carcinomas (9). This is of prognostic importance, in that it may contribute to the survival, recurrence and selection of appropriate treatment protocols. In the present study, we aimed to evaluate the relationship between the 747–750 deletion mutations in exon 19, the risk of lung cancer development and to examine the co-existence of the relevant mutations with specific histopathological types of lung cancer, as well as its relationship with other risk factors for lung cancer, including smoking, sex, and old age.

Material and Methods

Study population

The study sample comprised a total of 178 patients diagnosed with NSCLC at Mersin University, Medical Faculty, Oncology Clinics and a control group of 192 age- and sex-matched healthy volunteers between 2013 and 2015. All participants were informed about the objective and design of the study and a written informed consent was obtained from each participant. Data including age, occupation, smoking status and family histories of lung cancer were recorded on an information collection form which was designed specifically for the study. The interview response-rates among the eligible patient and control participants were 97.2% and 98.3%, respectively. The study protocol was approved by the Local Ethics Committee of Mersin University, Medical Faculty of Medicine. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Genotype analyses: Peripheral blood samples of 7 to 8 mL were obtained from each participant and placed in 15 mL centrifugation tubes containing 50 mmol/l disodium-ethylenediaminetetraacetic acid (EDTA). Deoxyribonucleic acid (DNA) isolation was carried out through the standard salt precipitation method (10), while the mutation screening and genotyping analyses were performed through polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses. After the obtained PCR/RFLP products were inspected through electrophoresis, the collected data were statistically analyzed.

EGFR Exon 19(747–750) Deletion Studies: EGFR gene mutation was identified by PCR-restriction fragment length polymorphism in 178 lung cancer patients. The sequences of primers for PCR amplification were as follows:

Forward:

5’- ATCCCAGAAGGTGAGAAAGATAAAATTC -3’

Reverse:

5’- CCTGAGGTTCAGAGCCATGGA -3’

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, Holland). This grade was to present restriction sites of restriction enzyme MseI by primer EGFR. PCR appliance (2720 Thermal cycler, Applied Biosystems) was designed for amplification. The PCR cycling parameters were: one cycle of 95°C for 15 minutes, 40 cycles of 95°C for 20 seconds, 60°C for 30 seconds, and 72°C for 1 minute, followed by one cycle of 72°C for 3 minutes. The product of PCR was 138 bp. PCR product (2 µl) containing 200 ng DNA was digested by MseI (MBI Fermentas, USA) 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 92 bp and 46 bp, and the digested fragments of mutant DNA included 120-129 bp. The principle of the assay is shown in the MseI which was used to digest the TTAA sequence (from first letter of codon 747 to first letter of codon 748) in wild-type genes, which are frequently absent in exon 19 deletion mutants (codons 747–750; Leu Arg-Glu sequence), resulting in the enrichment of deletion-type genes (12,19).

Statistical Analysis: The relationship between positivity in genes and disease were calculated by chi-square test. Distribution of lung cancer in age groups was determined by one sample chi square test. Categorical variables were expressed as frequencies and percentage. Statistical analysis was performed using the STATISTICA (13.3.1) $p<0,05$ was considered statistically significant.

Results

The demographic characteristics and frequencies of alleles/genotypes of EGFR exon 19 deletion (residues 747–750) of the patients with lung cancer and the healthy control sare presented in Table 1.

The mean age in the patient group was slightly higher than that of the control group, being 60.06(±9.75) years vs.58.04(±8.64) years, respectively (p:0.414). Of all the 178 patients with lung cancer, 155(86.6%) were men and 23(13.4%) were women. Lung cancer was more frequent among men (0.001). Smokers accounted for 64.50% and 80.3%of the control and patient groups, respectively ($p<0.001$). When EGFR 19 exon 747–750 deletion genotype rates were analyzed, the rate of mutant genotype was found to be 15.1%in the control group, increasing to 36.9%in the patient group, and a 2.64-times greater risk of developing lung cancer (p:0.014).The distribution of lung cancer patients based on the histopathological types and EGFR exon 19 747–750 deletion rates were as follows: Adenocarcinoma 53.37–52.08%, squamous cell carcinoma 30.89–35.41%, large cell carcinoma 3.93–4.16%, and small cell carcinoma 11.79–8.33%(p:0.76) (Table 2). Agarose gel electrophoresis of PCR products that wild and mutant genotype for EGFR exon 19 was demonstrated in Figure 1.

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Table 1. Distribution of study participants according to cases-control status, demographic characteristics and EGFR exon 19 deletion genotypes

Variables		Cases (n=178) (Mean ± SD)	Controls (n=192) (Mean ± SD)	p-value
Age (Mean ± SD)		60.06±9.75	58.04±8.64	0.414
sex	Male	155 (86.60)	162 (84.40)	0.001
	Female	23 (13.40)	30 (15.60)	
Smoking status	Never smokers	33 (19.70)	68 (35.50)	0.001
	Current smokers	145 (80.30)	124 (64.50)	
Genotype frequencies		112 (63.1)	163 (84.9)	0.014
		66 (36.9)	29 (15.1)	

Table 2. Distribution of EGFR exon 19 deletion (residues 747–750) genotypes based on histopathological types of lung cancer

	Adenocarcinoma		Squamous cell carcinoma		Large cell carcinoma		Small cell carcinoma	
	n	%	n	%	n	%	n	%
Total (n=178)	95	53.37	55	30.89	7	3.93	21	11.79
Wild type (n=130)	70	53.84	38	29.23	5	3.84	17	13.07
Mutant (n=48)	25	52.08	17	35.41	2	4.16	4	8.33

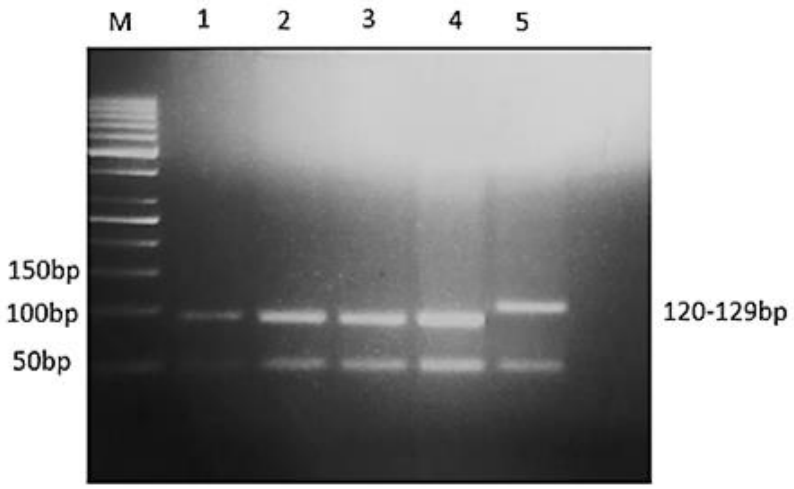

Figure 1: Agarose gel electrophoresis of PCR products for sequencing EGFR exons 19 gene by RFLP method. M: DNA marker; 1-4: patients wild type DNA sample; 5: patient mutant type DNA sample.

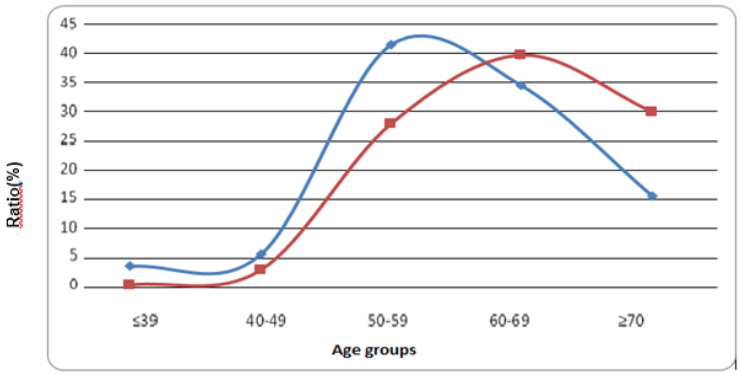

Figure 2. Blue line: Distribution of ratio of Lung cancer patients with respect to age. Red line: Distribution of ratio of Lung cancer patients with respect to older age in the Mersin sample.

The mean age of the 178 patients in lung cancer group was 60.06(±9.75) years with a distribution of patients according to the age groups being 3.3% in <39 years age group, 5.0% in 40–49 years age group, 39.2% in 50–59 years age group, 33.7% in 60–69 years age group, and 18.7% in >70 years age group. To identify whether old age represented a risk factor, the distribution of the respective age groups in the total Mersin population was initially estimated. As the population was not distributed evenly between the all age groups, the ratios of all lung cancer patients in all age groups were calculated. These ratios were as follows: 0.4% in the ≤39 years age group, 2.93% in 40-49 years age group, 27.9% in 50-59 years age group, 38.6% in 60-69 years age group and 29.9% in ≥70 years age group. When the ratio of the overall population and lung cancer patients in the relevant age groups was compared, old age was found to be a risk factor for the development of lung cancer (p:0.001).

To evaluate the increased risk of lung cancer in the older age groups, case numbers and ratios of the relevant age groups were estimated within the Mersin population (Fig. 2). We found no relationship between being in an older age group and the risk of lung cancer (Fig.2, blue line), although the ratio of lung cancer patients increased with older age in the Mersin sample (Fig.2, red line). Consistent with the literature, lung cancer was directly associated with smoking.

Discussion

In this population-based case-control study, we investigated whether the exon 19 deletion (residues 747–750) in the EGFR gene was related to the risk of lung cancer in a Turkish population, and whether there was any relationship between the EGFR exon 19 deletion genotypes and the histopathology types of lung cancers. We further investigated whether demographic risk factors influenced lung cancer development in our study sample. Based on our study results, the EGFR exon-19 747–750 deletion mutant genotype and older age were the main risk factors for the development of lung cancer.

In a previous study by Dearden et al. (11), the frequency of EGFR mutations in the Western populations was found to be 19.2% in patients with adenocarcinoma and 3.3% in patients with squamous cell carcinomas. These rates are higher in the Asian population, reported to be 47.9% in patients with adenocarcinomas and 4.6% in patients with squamous cell carcinomas. Asano et al. (12) identified exon 19 mutations in the EGFR gene in 52% of 108 patients with lung cancer, including mutations identified by direct sequencing in 16 (15%) of the 108, from a fine-needle lung biopsy sample in four (22%) of 18, and from a pleura fluid sample in three (15%) of 20 patients. In another population-based study performed by Shiau et al. (13) in Ontario State, Canada, EGFR exon 19 deletion rates in 166 histology and 73 cytology samples were reported as 239 (53.6%). The EGFR mutations were positive in 220 women (23.1%), 98 men (13.5%) and 79 Asian (48.8%) patients. In another study reported by Sandra et al. (14), EGFR exon 19 deletions were present in 53%of 2,142 patients with an early lung adenocarcinoma, while this rate was 61%among patients with an advanced adenocarcinoma. The frequency of EGFR mutations in the NSCLC tumor tissue samples was reported as 19% in men and 26% in women. In a study by Baek et al. (15) involving a Korean population of 1,738 patients, the efficacy of EGFR tyrosine kinase inhibitors in NSCLC patients with exon 21 L858R and exon 19 deletion mutations of the EGFR gene was examined. In the aforementioned study, 88 patients (5.1%) were positive for rare or complex mutations and 54 were treated with tyrosine kinase inhibitors. Rare and complex mutations were identified in 33 and 21 patients, respectively. In another study, Bircan et al. (16) investigated EGFR exon 19 and exon 21 mutations in 25 patients, including 14 with an adenocarcinoma and 11 with a squamous cell carcinoma. The EGFR mutations were identified 11 (44%) patients with NSCLC, while exon 19 and exon 21 mutations were identified in eight (32%) and five (20%) patients, respectively. Two patients had both mutations concomitantly. Of the six (35.7%) patients with adenocarcinomas, three had exon 19 and the remaining three had exon 21 mutations. Of the seven (54.5%) patients with squamous cell carcinomas, five had exon 19 and two had exon 21 mutations. In their study investigating the EGFR mutations in a Chinese sample of 157 individuals, Li et al. (17) detected exon 19 and exon 21 mutations in 22 and 35 individuals, respectively. Quan et al. (18) found that the total rate of somatic EGFR mutations was 48.02% in the Chinese population, including 354 patients with NSCLC. Of those mutations, 27.40% were in exon 19 and 25.99% were in exon 21. The most common mutations in exon 19 and exon 21 were identified as E746-A750del (8.47%) and L858R (10.17%) mutations, respectively. Of all the patients with EGFR mutations, 60.13% were women and 38.81% were men[adjusted odds ratio (OR), 1.93, 95% confidence interval (CI), 1.07–3.51, P:0.029]. The distribution of patients by age groups was 58.62% in the <60 years age interval and 40.67% in ≥60 years age interval (adjusted OR, 1.87; 95% CI, 1.20-2.92; P:0.006). The distribution of the EGFR mutation rates according to histological tissue type was 52.76% for adenocarcinoma and 26.56% for non-adenocarcinoma cases. The mean age of the patients in the study was 62 years.

We found that the rate of the EGFR exon 19 (747–750) deletion mutant genotype was higher among lung cancer patients, and although the frequencies differed, our results are overall consistent with those previous studies (12, 14–18). The mean age of the patient group in the present study was similar to those reported by Li et al. (17) and Quan et al. (18). In the study by Baek et al. (15), the rates of adenocarcinoma and non-adenocarcinoma cases were 88.9 and 11.1%, respectively, while in the study of Bircan et al. (16), these rates were 56% for adenocarcinoma and 44% for squamous cell carcinoma. In the study by Li et al.(17), the rates were as follows: squamous cell carcinomas: 8.3%, adenosquamous carcinoma: 50%, mixed adenocarcinomas with bronchiole alveolar components and bronchiole alveolar adenocarcinomas: 17.6% and other adenocarcinomas: 58.8%. The distribution according to histological tissue types was 81.92% for adenocarcinoma, 16.95% for squamous cell carcinoma and 1.13% for large cell carcinoma in the study by Quan et al. (18). The

distribution of lung cancer cases in terms of the major histological tissue types in the present study was adenocarcinoma, squamous cell carcinoma, and small cell carcinoma, followed by large cell carcinoma. This finding is also consistent with the aforementioned studies, indicating that the male sex, smoking, and old age are greater risk factors for the development of lung cancer. This is the first study, however, to provide proof of the link between old age and the risk of developing lung cancer by presenting statistical analyses based on the distribution of a sample population according to age groups, and secondly, by calculating the ratio of lung cancer patients in each age group.

Conclusion

In conclusion, the EGFR exon-19 747–750 deletion mutant genotype represents a risk factor for the development of lung cancer with individuals with this mutation being 2.64 times more likely to develop the disease. According to our study results, the EGFR exon-19 747–750 deletion mutant genotype frequencies did not differ significantly between the histological tissue types, while smoking, male sex, and old age were found to be the most significant risk factors for the development of lung cancer. Considering the distribution of lung cancer patients across the different age groups, it is evident that older age represents a risk factor for the development of lung cancer. Nonetheless, further large-scale, comprehensive studies are needed to establish a conclusion.

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References

1. Wong MCS, Lao XQ, Ho KF. Incidence and mortality of lung cancer: global trends and association with socioeconomic status. Sci Rep 2017 Oct 30;7(1):14300.
2. http://www.who.int/cancer/en/July 16, 2016
3. Dela Cruz CS, Tanoue LT, Matthay RA. Lung Cancer: Epidemiology, Etiology, and Prevention. Clin Chest Med 2011 Dec;32(4):605-44.
4. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. Mayo Clin Proc 2008 May; 83(5):584-94.
5. Shtivelman E, Hensing T, Simon GR, et al. Molecular pathways and therapeutic targets in lung cancer. Oncotarget 2014 Mar 30;5(6):1392-433.
6. da Cunha Santos G, Shepherd FA, Tsao MS. EGFR mutations and lung cancer. Ann Rev Pathol 2011 Feb 29;6:49–69.
7. Siegelin MD, Borczuk AC. Epidermal growth factor receptor mutations in lung adenocarcinoma. Lab Invest 2014 Feb;94(2):129–37.
8. Mitsudomi T, Yatabe Y. Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. FEBS Journal 2010 Jan;277(2):301–8.
9. Lee SM. Is EGFR expression important in non-small cell lung cancer? Thorax. 2006 Feb; 61(2):98-9.
10. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988 Feb 11;16(3):1215.
11. Dearden S, Stevens J, Wu YL, Blowers D. Mutation İncidence and Coincidence in Non Small-Cell Lung Cancer: Meta-Analyses by Ethnicity and Histology (mutMap). Ann Oncol 2013 Sep;24(9):2371–6.
12. Asano H, Toyooka S, Tokumo M, et al. Detection of EGFR Gene Mutation in Lung Cancer by Mutant-Enriched Polymerase Chain Reaction Assay. Clin Cancer Res 2006 Jan 1;12(1):43-48.
13. Shiau CJ, Babwah JP, da Cunha Santos G, et al. Sample features associated with success rates in population-based EGFR mutation testing. J Thorac Oncol 2014 Jul; 9(7):947-956.
14. D'Angelo SP, Pietanza MC, Johnson ML, et al. Incidence of EGFR Exon 19 Deletions and L858R in Tumor Specimens From Men and Cigarette Smokers With Lung Adenocarcinomas. J Clin Oncol 2011 May 20;29(15):2066-70.
15. Baek JH, Sun JM, Min YJ, et al. Efficacy of EGFR tyrosine kinase inhibitors in patients with EGFR-mutated nonsmall cell lung cancer except both exon 19 deletion and exon 21 L858R: A retrospective analysis in Korea. Lung Cancer 2015 Feb; 87(2): 148-154.
16. Bircan S, Baloglu H, Kucukodaci Z, Bircan A. EGFR and KRAS mutations in Turkish non-small cell lung cancer patients: a pilot study. Med Oncol 2014 Aug;31(8):87.
17. Li M, Zhang Q, Liu L, et al. The different clinical significance of EGFR mutations in exon 19 and 21 in non-small cell lung cancer patients of China. Neoplasma 2011; 58(1):74-81.
18. Quan X, Gao H, Wang Z, et al. Epidermal Growth Factor Receptor Somatic Mutation Analysis İn 354 Chinese Patients With Non-Small Cell Lung Cancer. Oncology Letters 2018 Feb;15(2):2131-8.
19. Kawada I, Soejima K, Watanabe H, et al. An alternative method for screening EGFR mutation using RFLP in non-small cell lung cancer patients. J Thorac Oncol 2008 Oct;3(10):1096-103.