RESEARCH ARTICLE

Relationship between Epidermal Growth Factor Receptor Gene Mutations and Clinicopathological Features in Patients with Non-Small Cell Lung Cancer in Western Turkey

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Abstract

Background: To investigate epidermal growth factor receptor (EGFR) gene mutations in patients with non-small cell lung cancer (NSCLC) and to analyze any relationship with clinicopathological features and prognosis. Materials and Methods: EGFR gene exons 18-21 in 48 specimens of paraffin-embedded tumor tissue from NSCLC patients were amplified by PCR, followed by direct sequencing and analysis of links to clinicopathological features and prognosis. Results: EGFR mutations were detected in 18 of 48 (42.6%) patients with NSCLC. There were 9 cases of mutations in exon 20, 7 in exon 19 and 2 in exon 21. Mutations were more frequently observed in women (5/7 pts, 71.4%) than in men (13/41 pts, 31.7%) (p=0.086) and in non-smokers (5/5 pts, 100%) than smokers (13/43 pts, 30.2%). There was negative correlation of EGFR mutations with smoking status (p=0.005). EGFR mutations were more frequently observed with adenocarcinoma histology (13/32 pts, 40.6%) than in other types (5/16 pts, 31.3%) (p=0.527). The patients with EGFR mutations had better survival than those with wild-type EGFR (p=0.08). There was no association of EGFR mutations with metastatic spread. Conclusions: EGFR mutations in NSCLC were here demonstrated more frequently in females, non-smokers and adenocarcinoma histology in the western region of Turkey. Patients with EGFR mutations have a better prognosis.

Keywords: EGFR mutations - non small cell lung cancer - clinical features - Western Turkey

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Introduction

Lung cancer is the most important cause of cancer-related death in the world. Each year, it is responsible for 1.6 million newly diagnosed cases, and 1.3 million deaths (Jemal et al., 2011). Non-small lung cancer consists of about 85% of all lung cancer patients. The prognosis of localized NSCLC (stages I, II, and IIIa) ranges from 20% to 60% 5-year survival, depending on stage, while the metastatic stage (stage IV) results in markedly poorer prognosis with median survivals of much less than 1 year. Early-stage NSCLC is treated primarily by surgical resection, with adjuvant chemotherapy for selected patients with stage IB, II, and III disease. Patients with locally advanced (stage III) disease are usually treated with chemo-radiotherapy, while advanced stage NSCLC is an incurable disease and is treated mainly by chemotherapy. (NSCLC Meta-Analyses Collaborative Group, 2008). Although chemotherapy improves survival with the advantage of quality of life in advanced stage NSCLC, treatment outcomes remain disappointing, with 1-year survival less than 50% and 3-year survival less than 25% (NSCLC Meta-Analyses Collaborative Group, 2008; Davidoff et al., 2010; Howlader et al., 2012).

Thanks to recently developed drugs targeted at different pathways within the last decade, important survival advantage has been achieved in the treatment of non-small lung cancer. Nowadays, the important target of these drugs is EGFR. Drugs like erlotinib and gefitinib which target EGFR have achieved higher response rates (Pao et al., 2004; Qi et al., 2012; Saito et al., 2012). In NSCLC, EGFR mutation is identified by typical deletion in exon 19, point mutation in exon 21, and extensive insertion in exon 21, among them the mostly widely known type is exon 19 deletion (Tokumo et al., 2005). Classical EGFR mutations have been mostly detected in women, Asians, never-smokers, and adenocarcinoma histology (Nomura et al., 2007). Despite an impression suggesting their presence only in adenocarcinoma subtype, EGFR mutations have been identified in other subtypes, such as squamous cell histology and other rarely seen types of lung cancers (Lee et al., 2011; An et al., 2012). Frequencies of EGFR mutations in NSCLC have predominantly been determined in East Asian populations rather than European

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populations, showing large differences between these populations. In studies performed in the Western Europe and Asia, EGFR mutations were detected with increasing frequencies (15 vs 30%). Furthermore, it has been reported up to 50% in patients who never-smokers (An et al., 2012).

Since Turkey is located between Asia and Europe, we aimed to evaluate the frequency of EGFR mutation and it's relationship with clinicopathological features and prognosis in the western region of Turkey.

Materials and Methods

Study design: The study approval was obtained from the Ethics Committee of Noninvasive Investigations of Dokuz Eylul University on 04.19.2012 with decree #2012/15-17, and protocol #578-GOA. In this study, 48 patients with diagnoses of NSCLC who had EGFR mutation analyses, and followed up in the Division of Medical Oncology, Dokuz Eylul University, Faculty of Medicine between 2005 and 2012 were retrospectively analyzed. Staging was performed according to AJCC Cancer Staging System 7th edition. Their clinicopathological characteristics, age, gender, and smoking status of the patients were recorded.

EGFR mutation analysis

Tissue samples were cut from appropriate paraffin blocks which best represented NSCLC characteristics obtained from Archives of Dokuz Eylul University Department of Pathology. Samples used were retrieved from blocks containing only adequate number of samples. After cleansing microtome with alcohol swaps, a 10-μ thickness sections were cut, and placed in sterile Eppendorf tubes provided by the Department of Basic Oncology. In the Laboratory of Basic Oncology spin column method was applied on tissue samples in tubes using kits which extracted DNA from paraffin blocks. After quantification of DNA by spectrophotometer, and assessment of its adequacy, known EGFR mutations (in 18-21 exons) were evaluated by molecular microarray methods. As a gold standard, DNA sequencing methods were used.

Statsitical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) software (version 10.0 for Windows). Survival curves were estimated using the Kaplan-Meier method and log-rank test was used for comparisons. Comparisons were made with parametric (Student's t test) and nonparametric (Mann-Whitney) tests. Comparisons between groups were performed with chi-square test. All differences associated with a chance probability of 0.05 or less were considered statistically significant.

Results

Patient characteristics: The study population consisted of 41 men, and 7 women. Median age of all patients was 63.2 (range 41 to 80) years. The patients comprised of never-smokers (n=5), those who quitted smoking at least 6 months before the diagnosis of NSCLC (n=16), and smokers (n=27) at the time of diagnosis. Mean pack-years was 40 (range 10 to 90) pack-years in smokers. ECOG performance status scores were 0 in 37, 1 in 9, and 2 in 2 patients. The patients had metastatic (n=41) or locally advanced (n=7) disease, adenocarcinomas (n=32), squamous cell carcinomas (n=7), non-small cell carcinomas (n=4), and carcinomas of other histopathological types (n=5). Demographic characteristics of the patients are shown in Table 1.

In patients with metastatic disease, single (n=19), two (n=10), three (n=10), four (n=1), and five (n=1) metastatic foci were detected. Most frequently metastases were encountered in bones, and contralateral pulmonary lobes. Metastatic characteristics of the tumours are given in Table 2.

EGFR mutation status

In all patients EGFR somatic mutations (exon 19 deletion, exon 20 T790M mutation, exon 21 L858R point mutation) were investigated in all patients, and mutations were detected in 18 patients (37.5%). In these 18 patients, exon 20 mutations (n=9), exon 19 mutations (n=7), and exon 21 mutations (n=2) were found. In 30 patients any evidence of mutation could not be found, and reported as wild- type.

Outcomes of correlations between clinical, and pathological characteristics

EGFR mutation status was detected in 5 of 7 female (71.4%), and 13 of 41 male (31.7%) patients without any statistically significant difference between genders (p=0.086). Besides, in all of 5 (100%) nonsmokers, but only 13 of 43 smokers (30.2%) EGFR mutation was observed without any statistically significant difference between these two groups (p=0.005).

When correlations between histological features and EGFR mutations were evaluated, EGFR mutations were detected in 13 (40.6%) of 32 patients with adenocarcinomatous histology, and in 5 (31.3%) of

Table 1. Patient Characteristics

Male/female		41/7
Age (median, interquartile range)		63.2 (41-80)
Smoking status (%)		89.6
ECOG performance status	0	37
	1 ve 2	11
Grade (n)	Locally advanced disease	7
	Metastatic disease	41
Histopathological type	Adenocarcinoma	32
	Squamous cell carcinoma	7
	Large cell carcinoma	4
	Other hystopathological types	5

*4: Non-small cell carcinoma, 1: pleomorphic carcinoma, ECOG= Eastern Cooperative Oncology Group

Table 2. Metastatic Features of the Patients

	n (%)
Bone metastases	21 (43.8%)
Contralateral pulmonary metastases	18 (37.5%)
Brain metastases	11 (22.9%)
Adrenal gland metastases	10 (20.8%)
Pleural metastases	8 (16.7%)
Liver metastases	7 (14.6%)

16 cases with other histological findings, without demonstrating any statistical significance between these groups (p=0.527). EGFR mutations were detected 2 patients (28.5%) with squamous cell carcinoma, 1 patients (25%) with large cell carcinoma, and 2 patients (50%) with nonsmall cell carcinoma histology.

Cerebral metastases were observed in 7 (38.8%) of 18 patients with EGFR positivity, and only in 4 (13.3%) of 30 patients with wild-type, without any statistically significant difference between two groups (p=0.074). Pleural metastasis was detected in 3 (16.6%) of 18 patients with EGFR mutation positivity, and 5 (16.7%) of 30 cases with wild-type without statistically significant difference between both groups (p=1.0). Contralateral pulmonary metastatic lesions were detected in 8 (44.4%) of 18 patients with EGFR mutation positivity, and 10 of 30 patients with wild- type without any statistically significant intergroup difference (p=0.44). Bone metastases were detected in 9 (50%) of 18 patients with EGFR mutation positivity, and 12 (40%) of 30 patients with wild-type without any statistical significant difference between them (p=0.49). Liver metastases were found in 3 (16.6%) of 18 patients with EGFR mutation positivity, in 4 (13.3%) of 30 patients with wild-type without any statistically significant intergroup difference (p=1.0). Surrenal metastasis was noted in 3 (16.6%) of 18 patients with EGFR mutation positivity, and 7 (23.3%) of 30 cases with wild-type, still without any statistically significant difference between groups (p=0.72).

Survival analysis

Median follow-up period of the patients was 14 (range 1 to 67) months. One and 2-year survival rates of all patients were 84.7, and 69%, respectively, while mean survival time was estimated as 33.7±5.1 months. Mean survival times in the groups with EGFR mutation positivity, and wild-type were 46.8±8.1, and 22.3±3.8 months, respectively without any statistically significant intergroup difference (p=0.08). When results of survival were evaluated based on types of EGFR mutation, mean survival times in cases with mutations in exons 19, and 21 combined, and also in exon 20 were detected to be 49.3±10.1 and 13.8±1.8 months, respectively, without any statistically significant difference between both groups (p=0.71). Four patients with exon 19 and 21 mutations used an EGFR inhibitor, while all patients with exon 20 mutations did not receive any EGFR inhibitor. Mean survival curves of the patients, and survival curves according to state, and type of mutations are shown in Figures 1 and 2 respectively.

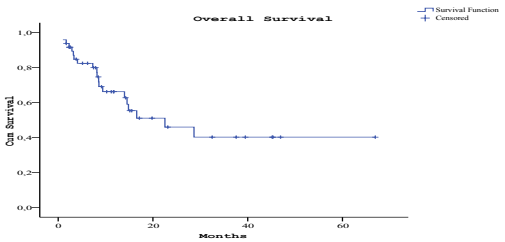

Figure 1. Mean Survival Curve of the Patients

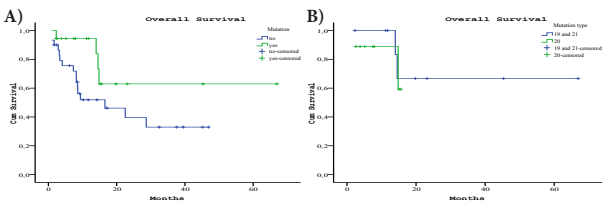

Figure 2. Survival Curve According. A) Mutation Status and B) Type of Mutation

Discussion

To the our knowledge, this investigation is the first study performed in Turkey on EGFR mutation, its clinicopathological correlation, and prognosis in lung cancer. In our study, the frequency of EGFR mutation was detected to be 37.5%. Studies performed in other countries have reported varying results, and marked differences were noted based on ethnic characteristics. Especially studies conducted in yellow race in Asia detected higher rates (40.8%) of EGFR mutations (Wu et al., 2008), conversely a Latin American study revealed lower (33.2%) incidence of EGFR with remarkable differences between countries. For example, incidence of EGFR mutations was 19% in Argentina, and 67% in Peru (Arrieta et al., 2011). An investigation among African- Americans found a lower incidence (11%) of EGFR mutation (Cote et al., 2011). Similarly, a study conducted in the UK, its incidence was revealed as 11% (Leary et al., 2012). Generally, in studies performed in Asia, lower incidence of EGFR has been observed when compared with those found in Europe. However we thought that higher incidence of EGFR mutation detected in our study, stems from conduction of our study mainly among selected patient population. Besides it might be associated with our country's geographic position which is situated at the junction of Europe, and Asia. Available data is inadequate to arrive at a definite conclusion on this issue.

Nowadays, gender is one of the most important, and known clinical predictors for epidermal growth factor receptor mutations, and female gender appears to be more predominant (Toyooka et al., 2007). Most typically, in Asian studies, higher incidence of EGFR mutation in female gender has been demonstrated (Wu et al., 2008). Studies in the Western world have provided results similar to those reported in Asian investigations, and female gender was detected as one of the most important predictors of EGFR (Arrieta et al., 2011; Cote et al., 2011; Leary et al., 2012). Also in our study higher rates of EGFR mutation were found in the female gender in parallel with the literature data. However, because of scarce number of female patients in our study, its incidence did not reach to a statistically significant level.

A strong correlation was reported between absence of smoking history, and the presence of EGFR mutation (Toyooka et al., 2007). In a study investigating the association between EGFR mutation, and smoking status, individuals smoking more and less than 15 pack-years were compared, and significantly higher rates of EGFR mutation were detected in the latter group (Huang et al., 2011). Similarly, in another study, lower rates of EGFR mutation were noted in individuals smoking less than 25 pack-years when compared with heavier

smokers (Lee et al., 2010a). Besides in both of these studies, higher incidence of EGFR mutation was noted in nonsmokers. In another important study, lower rates of EGFR mutation were detected in nonsmokers exposed to second-hand smoke relative to nonsmokers who were not passive smokers (Lee et al., 2010b). In a meta-analysis investigating the association between smoking history, and EGFR mutation, a 4.8-fold increase in EGFR mutation was reported in nonsmokers compared with smokers (Ren et al., 2012). When all relevant studies were evaluated, it has been noted that EGFR mutation is seen most frequently in never-smokers, and in even nonsmokers environmental exposure to second-hand smoke intensifies with increased number of cigarettes smoked by others in the vicinity. One of the reasons for relatively lower rates of EGFR mutation in smokers has been explained with more prevalent k-ras mutation in this group of patients. Since k-ras and EGFR mutations were mutually exclusive, EGFR mutation is observed less frequently in smokers. In our study, in accordance with literature findings, EGFR mutation was detected in the nonsmoker group as a whole which was significantly more frequently encountered when compared with the smoker group.

A strong correlation was also found between adenocarcinomatous histology, and an epidermal growth factor receptor mutation (Wu et al., 2008). Also many case reports, and case series have indicated the presence of EGFR mutations in other histological types of NSCLC. A large cell neuroendocrine carcinoma was also reported as a case report (De Pas et al., 2011). In a Chinese study where 1195 patients were evaluated, EGFR mutation was detected in cases with adenocarcinomatous histology, squamous cell carcinoma, and non-small cell carcinomas in 38, 4, and 2 % of the patients, respectively (Huang et al., 2011). EGFR mutations were also reported in rarely seen types of NSCLC such as pleomorphic (15%), and also adenosquamous carcinomas (38%) (Jia and Chen, 2011; Lee et al., 2011). A South Korean study detected EGFR mutations in 24% of the cases with adenocarcinomas (Jang et al., 2009). EGFR mutations were also found in 9 of 33 cases with sarcomatoid carcinoma which is a rarely seen subtype (Jiang et al., 2012). Generally, EGFR was noted in 15-35% of adenocarcinomas, while it was rarely (3-5%) seen in cases with other types of NSCLC. In our study, it was more frequently encountered in cases with adenocarcinomatous histology without any statistical significance.

In the literature different remarkable results have been reported related to correlations between epidermal growth factor receptor mutations, and metastatic sites. In a study, relatively more frequent EGFR mutations were seen in cases with hepatic metastases, in this study, cases with EGFR mutation positivity, and those with EGFR, k-ras, and EML-AKT mutation negativity (triple negativity) were compared (Doebele et al., 2012). While in a study performed on 125 patients in South Korea, a significant correlation was found between EGFR mutation, and pleural metastases (Na et al., 2011). In a study conducted with scarce number of patients, a significant correlation was found between extensive pulmonary metastases, and exon 19 mutations (Laack et al., 2011). In a large scale study, significant correlation between extensive pulmonary metastases, and EGFR mutation was confirmed (Togashi et al., 2011). In our study, cerebral, and contralateral pulmonary metastases were more frequently detected in the mutation group without reaching any statistical significance which might be due to scarcity of the study population. Larger scale studies are required to investigate the correlation between metastatic region, and EGFR mutation.

Correlations between epidermal growth factor receptor mutation, and improved prognosis, and longer survival in NSCLC have been reported. Especially with administration of drugs inhibiting EGFR pathway, survival advantage has become more prominent. When compared with wild-types, mutation positive cases demonstrate better prognosis, while prognosis and survival rates differ according to exon mutation sites (Jackman et al., 2006; Wu et al., 2008; Li et al., 2011; Sun et al., 2011). Exon 20 mutation was associated with a poor prognosis, while exon 19, and 21 mutations were correlated with a good prognosis, and longer survival (Jackman et al., 2006; Wu et al., 2008; Li et al., 2011; Sun et al., 2011). At the same time, exon 19, and 21 mutations were found to be robust predictors of improved response rates for anti-EGFR treatments, on the contrary, exon 20 mutation was reported as an inducer of resistance to therapy. Besides, differences between exon 19, and 21 mutations were indicated as for responsiveness to anti-EGFR treatment, and higher response rates were reported in patients with exon 19 mutations. Also in our study, outcomes consistent with the literature were obtained. Accordingly, longer survival rates were achieved in patients with wild- type relative to those with mutations, and improved survival rates were obtained in cases with exon 19, and 21 mutations when compared with individuals with exon 20 mutations. However, statistical significance could not be reached due to scarcity of patients.

In conclusion, EGFR mutation demonstrates correlations with certain clinical and pathological characteristics in NSCLC. The presence of mutation is also a favorable prognostic indicator for survival. Exon 19, and 21 mutations are favorable indicators for predicting response to anti-EGFR treatments, while exon 20 mutation indicates refractoriness to therapy. Our results demonstrate similarities to the literature findings in this respect, and a significant correlation was not detected between metastatic regions, and EGFR mutations. Since our country is situated at the junction of Asia, and Europe, larger scale clinical series performed in our country will provide important information about the frequency, and distribution of mutations, treatment responses, and survival.

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