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EGFR mutation status in Middle Eastern patients with non-squamous non-small cell lung carcinoma: A single institution experience

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ABSTRACT

Background: Epidermal growth factor receptor (EGFR) represents an important molecular target in the treatment of lung adenocarcinomas; many anti-EGFR therapies are approved as first line and second-line treatment in patients having metastatic lung adenocarcinomas. The occurrence of this mutation varies in terms of race; it is around 10% in Caucasians and can reach 30% in Asians. Its prevalence in our Middle Eastern region is not well known.
Methods: Patients diagnosed with non-squamous non-small cell lung carcinomas between March 2013 and March 2015 were included. This study was conducted at Hôtel-Dieu de France University Hospital, a tertiary medical center in Lebanon. EFGR mutations were analyzed using real time PCR technique on the Rotor-Gene Q using Scorpions and ARMS technologies. The following data was collected: the patients' characteristics (age, gender, smoking status, stage), the samples' characteristics (histology subtype, TTF-1 and Napsin A immunostainings, the site and the adequacy and the type of the sample), and the mutational EGFR status (presence and type of mutation). These variables were analyzed using SPSS 20.
Results: 201 patients were included. The mean age was 65.2 years [31–87]; 40.2% were females. 78.1% of the included patients were smokers or ex-smokers. 12.9% of patients had a localized disease, 17.4% a locally advanced disease and 69.7% a metastatic disease. Adenocarcinoma was the main histologic subtype found in 90.5% of patients, followed by large cell carcinoma (3.5%), adenosquamous carcinoma (3.0%) and non-small cell carcinoma not otherwise specified (3.0%). 11.9% of patients had an EGFR mutation: 48% of them presented a deletion on exon 19, 40.0% a L858R mutation on exon 21, 4.0% a G719X mutation in exon 18, 4.0% an insertion in exon 20, and 4% a T790M mutation in exon 20. The presence of an EGFR mutation was significantly associated with the female gender (two-third) ($p < 0.05$) and the non-smoking status (two-third) ($p < 0.05$).
Conclusions: The prevalence of EGFR mutation (11.9%) detected in our Lebanese population is similar to that observed in the Caucasian population. This mutation is also significantly more frequent in females and non-smokers.

1. Introduction

Lung cancer is among the most frequently diagnosed cancers in the Lebanese population. It ranked 3rd in male patients, after prostate and bladder cancer, and accounted for 582 new male cases in 2008. It also ranked 3rd in female patients, after breast and colon cancer, and accounted for 255 new female cases in 2008 [1]. The high incidence has been related to high prevalence of smoking among the Lebanese population. The most recent smoking prevalence rates among adults in Lebanon shows that 50–60% of men are smokers and 35% of women [2].

Platinum-based chemotherapy regimens were considered for years the standard of care for all histologic subtypes of lung cancer. In the era of targeted therapy, the evolution of molecular classification of lung cancer was remarkable and had major therapeutic impact especially in adenocarcinomas. The molecular classification of adenocarcinomas is based on different detected mutations and rearrangements like KRAS, EGFR, ALK and ROS1 [3]. EGFR (epidermal growth factor receptor) was the first and most frequently targetable mutation in this malignancy, knowing that

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KRAS is the most commonly detected mutation. The occurrence of EGFR mutation varies in terms of race; it is around 10% in Caucasians and can reach 30% in Asians [4]. EGFR tyrosine-kinase inhibitors were approved in first and second line treatment of advanced non-small cell lung carcinoma (NSCLC) [5,6]. Various mutations of this receptor have been described representing either sensitizing mutations or resistance-conferring mutations [7]. The guidelines of the College of American Pathologists recommend testing for the EGFR mutations in adenocarcinoma, adenosquamous carcinoma, large cell carcinoma and other NSCLC NOS, but not in squamous cell carcinoma [8].

The aim of this study is to report the prevalence of the EGFR mutation in Hôtel-Dieu de France University Hospital, a tertiary medical center where almost 20% of the Lebanese cancer patients are referred (unpublished data), and to assess the similarities and/or differences in patient and tumor characteristics compared to other countries.

2. Material and methods

2.1. Sampling and data collection

204 patients with NSCLC referred to Hôtel-Dieu de France Hospital for EGFR testing over a 2-year period, between March 2013 and March 2015, were included in the study. Almost 75% of samples originated from patients treated at Hôtel-Dieu Hospital, whereas the remaining 25% were referred from multiple hospitals and laboratories in different regions nationwide (Beirut, Mount-Lebanon, South, North, and Bekaa).

Patient characteristics were collected from physicians' reports, and the variables analyzed were age, gender and smoking status (current or former smoker versus never-smoker). Tumor characteristics were collected from the Pathology department database, and included disease stage (localized, locally advanced or metastatic), biopsy site (lung or metastatic lesion in: lymph node, brain, liver, bone, adrenal gland, pleura, peritoneum and skin), tumor histopathological subtype (adenocarcinoma, adenosquamous carcinoma, large cell carcinoma or other NSCLC NOS) and immunohistochemical phenotype (positivity or negativity for the TTF-1 and or Napsin A staining). Data concerning the status of the EGFR mutation (presence or absence of a mutation) and the different types detected was also collected.

2.2. Inclusion and exclusion criteria

All newly diagnosed NSCLC cases diagnosed at our hospital as well as cases referred from other hospitals and laboratories, were submitted, free of charge, for EGFR mutation testing. Prior to carrying out the testing, cases lacking sufficient material for the analysis and squamous cell carcinoma cases were excluded. 204 cases were submitted for EGFR mutation testing. After the EGFR mutation testing, 3 cases were excluded, 2 of them due to missing clinical data and 1 for insufficient material.

2.3. Techniques

Histological sections of 4-μm thickness were prepared from formalin-fixed paraffin-embedded (FFPE) tissue blocks. Immunomarker detection was performed using antibodies against TTF-1 (thyroid transcription factor-1) (4:100 dilution; BioGenex; Clone BGX-397A), Napsin A (1:400 dilution; Novocastra; Clone IP64). The immunomarkers were considered positive if at least 10% of the tumor cells stained. Appropriate positive and negative controls were included. Immunostaining for TTF-1 antibody was performed using the Ventana Benchmark GX Autostainer, and that of Napsin A was performed using the Dako Autostainer Link 48.

Four 10-μm FFPE tissue ribbons were obtained from each paraffin block. DNA was extracted using the QIAamp DNA FFPE Tissue Kit according to the manufacturer's protocol. Extracted DNA was stored at 4 °C. The detection of EGFR mutations was made using the real time Polymerase Chain Reaction (PCR) on the Rotor-Gene Q. While SPSS Statistics version 22.0 was the software used for the establishment of the statistical results.

3. Results

201 patients diagnosed with non-squamous NSCLC were included in this study. The mean age of patients was 65.2 years ± DS (extremes: 31–87 years). 61.2% (123) were males with a male/female sex ratio of 1.6. 69.7% (140) of the cases had a metastatic disease, 17.4% (35) a locally advanced disease and 12.9% (26) a localized disease. 78.1% (157) of the patients were smokers (Table 1).

Among the 201 included patients, 90.5% (182) had adenocarcinoma, 3.5% (7) large cell carcinoma, 3% (6) adenosquamous carcinoma and 3% (6) NSCLC NOS. 75.6% (152) of the samples were of pulmonary origin and 24.4% (49) from metastatic extra-pulmonary sites. The latter included the following sites: lymph nodes (17), pleura (11), bone (6), brain (7), liver (2), adrenal gland (2), peritoneum (2) and skin (2). The sample type corresponded to a biopsy in 70.1% of case (141), a surgical specimen in 24.9% (50) and a cytology specimen (cell blocks) in 5% (10). TTF-1/Napsin A immunostaining was positive in 79% (131/165) of the evaluated samples (Table 2).

11.9% (24) of the patients had an EGFR mutation. Adenocarcinoma was the histologic subtype of all these patients. A single mutation was found in all twenty-four patients except for one patient having 2 mutations: an exon 19 deletion and an exon 20 T790M mutation. A total of 25 mutations were therefore detected in 24 patients and distributed as follow: 48% (12) exon 19 deletions, 40% (10) exon 21 L858R mutation, 4% (1) exon 18 G719X mutation, 4% (1) exon 20 insertion and 4% (1) exon 20 T790M mutation (Table 3).

Table 1
Patient characteristics and repartition according to gender, smoking status and disease stage.

Patient characteristics	Variables studied	Percentage of patients (*N*)
Gender	Female	38.8 (78)
	Male	61.2 (123)
Smoking status	Current or former smoker	78.1 (157)
	Never-smoker	21.9 (44)
Disease stage	Localized	12.9 (26)
	Locally advanced	17.4 (35)
	Metastatic	69.7 (140)
Age	Mean ± DS	Extremes
	65.2 ± 10.4	[31.0; 87.0]

Table 2
Tumor samples characteristics.

Tumor characteristics	Variables studied	Percentage of cases (*N*)
Tumor site	Pulmonary	75.6 (152)
	Extra-pulmonary	24.4 (49)
Sampling method	Biopsy	70.1 (141)
	Surgery	24.9 (50)
	Cytology	5.0 (10)
Histological type	Adenocarcinoma	90.5 (182)
	Large cell carcinoma	3.5 (7)
	Adenosquamous carcinoma	3.0 (6)
	NSCLC NOS	3.0 (6)

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