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Frequency of EGFR Mutation and EML4-ALK fusion gene in Arab Patients with Adenocarcinoma of the Lung

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Abstract: Introduction: Improvement in the clinical outcome of lung cancer is likely to be achieved by identification of the molecular events that underlie its pathogenesis.

The frequency of epidermal growth factor receptor (EGFR) mutations is ethnicity-dependent, with a higher proportion in Asian populations than in whites, while the incidence of EML4-ALK (echinoderm microtubule-associated-protein like 4–anaplastic lymphoma kinase) fusion gene ranged from 1.6% to 16.4% in patients with NSCLC and these individuals were distinct from those harbouring mutations in the epidermal growth factor receptor gene.

This study was conducted to determine the frequency of EGFR mutation and EML4-ALK fusion gene in our population and to determine the effect of different clinicopathological features on the expression of those mutations in patients with lung adenocarcinoma.

Results: EGFR mutations were detected in approximately 33% of our patients in this series; the most frequently detected mutation was exon 19 deletion. EML4-ALK fusion gene was detected in 7.3% of patients.

Conclusion:

Our population exhibited the incidence of EGFR mutation approximately similar to that reported in East Asia and Japanese patients, higher than that recorded in USA, and Australia. However, more studies with larger patients' numbers are needed to verify this finding.

Keywords: *NSCLC • adenocarcinoma • EGFR • EML4-ALK*

Introduction

Although lung cancer remains the leading cause of cancer-related mortality for both men and women worldwide accounting alone for a quarter of all cancer deaths. Recent years have seen advances in the understanding and treatment of this difficult malignancy. Knowledge about molecular subsets of lung cancer defined specific oncogenic aberrations like epidermal growth factor receptor (EGFR), Kirsten Rat Sarcoma viral oncogene (KRAS) and anaplastic lymphoma kinase (ALK) that control the proliferation of cancer cells. This renders some cancers sensitive to therapeutic inhibitors targeting the mutated pathway for example; gefıtinib, erlotinib and afatinib targeting EGFR-mutated NSCLC and crizotinib targeting ALK-rearranged NSCLC. [1, 2]

EGFR is a member of the Erb-B family of transmembrane receptor tyrosine kinases involved in signal transduction pathways that regulate proliferation and apoptosis. Activating EGFR mutations such as exon 19 deletions and exon 21 (L858R) substitutions are pertinent to NSCLC [3]. Lung cancers harbouring EGFR mutations have a high sensitivity to EGFR-tyrosine kinase inhibitors (TKI) because these tumours are dependent on the EGFR signalling pathway for their survival and proliferation [4].

Certain groups of patients with NSCLC, such as those with adenocarcinoma histology, women, Asian ethnicity and nonsmokers are reported to be more likely to have tumour responses to EGFR-TKIs than other groups [5].

Data from 13 phase III trials in which an EGFR-TKI were compared with platinum-based chemotherapy were summarized in a meta-analysis that included data of 2620 patients (1475 EGFR mutation positive and 1145 mutation negative). Time to progression was significantly prolonged in EGFR-TKI group (hazard ratio (HR) 0.43,

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95% CI 0.38–0.49), while no effect on OS was observed (HR 1.01, 95% CI 0.87–1.18) [6].

ALK mutations have been identified in several types of cancer, including anaplastic large-cell lymphoma, neuroblastoma, inflammatory myofibroblastic tumour and NSCLC. [7] In NSCLC, the potential driver mutation is a fusion of an intrachromosomal inversion on the short arm of chromosome 2 that joins exons 1–13 of the Echinoderm microtubule-associated-protein like 4 gene (EML4) to exons 21–29 of ALK [7,8]. The resulting EML4-ALK is a fusion of the N-terminal portion of the protein encoded by EML4, with the intracellular signalling portion of the receptor tyrosine kinase encoded by the ALK gene [7, 9, 10].

EML4-ALK is uncommon, occurring in 2% to 7% of all NSCLC, and is more prevalent in patients who have never smoked or who have a history of light smoking and in patients with adenocarcinomas. [11] EGFR, ALK are mutually exclusive in patients with NSCLC, and the presence of one mutation in lieu of another can influence response to targeted therapy. Therefore, testing for these mutations and tailoring therapy accordingly is widely accepted as standard practice.

The tests of genes mutation (EGFR, ALK and ROS1) were adapted in many countries to determine the appropriate treatment. However, the reported incidences of gene mutations among adenocarcinoma lung patients in our populations are still lacking.

The aim of our study is to report the frequency and pattern of EGFR mutations as well as ALK gene rearrangement in patients attending Medical Oncology out-patient clinic, Kuwait Cancer Control Center (KCCC) with lung adenocarcinoma.

Patient and methods

According to KCCC policy specimens from 110 cases diagnosed with adenocarcinoma of lung origin between June 2010 till June 2014 were sent to lab 21 London for EGFR testing, samples which were found negative to EGFR were subjected to ALK testing. The median time required for such analysis was 10 days (range: 7–14) from the time the sample arrived at the laboratory until the results were reported.

Genomic DNA was isolated from formalin-fixed paraffin-embedded tumour tissues. Genetic analysis of the EGFR gene was performed using Therascreen EGFR PCR kit. Twenty-eight different mutations in EGFR gene were studied with three targets being mutation specific (L858R, L861Q, S7681). The other 25 targets were detected if present but were grouped into 3 types (deletion 19, G719x and insertion 20). ALK FISH gene rearrangement testing was performed using the CE-marked Vysis LSI ALK Break Apart Rearrangement Probe Kit. Interpretation of the results was based on the review by Lindeman et al. [13]

Data of the patients was then retrospectively analysed.

Results

Specimens from 110 patients with NSCLC (adenocarcinoma) received at KCCC out-patient clinic between June 2010 and June 2014 were collected and sent to laboratory 21, London, UK, for EGFR and ALK testing. Mean age of the patients was 61.37±10.45. Males represented 63.3% from the patients and females 36.4%. Forty-one patients (37.3%) were smokers. All patients were of Arab ethnicity out of which 57 (51.8%) were Kuwaitis. Demographic characteristics of the patients are shown in table 1.

Thirty-six patients (32.7%) carried EGFR mutation. The most frequently detected mutation was exon 19 deletion in 18 patients (50%), followed by exon 21 (L858R) substitution in 14 patients (39%) then G719X mutation and Insertion 29 in two patients (5.5%) each.

Table 1: Demographic characteristics of all patients.

Patients characteristics	Patients number, N= 110	
	n	(%)
Mean age	61.37±10.45	
Age group		
≤60	51	(46.4)
>60	56	(53.6)
Sex		
Male	70	(63.6)
Female	40	(36.4)
Smoking		
Smoker	41	(37.3)
Nonsmoker	69	(62.7)
Nationality		
Kuwaiti	57	(51.8)
Non-Kuwaiti	53	(48.2)
Histology	Adenocarcinoma	
Stage at diagnosis	IV	

Table 2: Frequency of EGFR mutation types.

Mutation type	N=110	
	n	(%)
EGFR negative	74	(67.3)
EGFR positive	36	(32.7)
Exon 19 deletion	18	(16.4)
Exon 21 (L858R) substitution	14	(12.7)
G719X mutation	2	(1.8)
Insertion 29	2	(1.8)
ALK gene mutation	8	(7.3)

Table 3: Patient's characteristics by EGFR status.

Characteristic	EGFR Mutated, N=36		EGFR Wild, N=74		P Value
	n	(%)	n	(%)	
Mean age at diagnosis	61.6 ±9.9		61.3±10.8		0.18
Age					0.186
≤60	14	(38.9)	37	(50)	
>60	22	(61.1)	37	(50)	
Sex					0.004
Male	16	(44.4)	54	(73)	
Female	20	(55.6)	20	(27)	
Smoking					0.06
Nonsmoker	27	(75)	42	(56.8)	
smoker	9	(25)	32	(43.2)	
Nationality					0.5
Kuwaiti	17	(47.2)	40	(54)	
Non-Kuwaiti	19	(52.8)	34	(46)	

ALK gene rearrangement was detected in eight patients (7.3%), table 2.

Incidence of EGFR mutation was found to be higher in females. Out of the 36 patients harbouring EGFR mutations, females represented 55.6% and males represented 44.4% while in EGFR wild population females represented 27% and males represented 73%, a difference which was statistically significant, P = 0.004.

The frequency of EGFR mutation was found to be higher in never smokers than smokers. In our patients series the frequency was (75% versus 25%; *p* = 0.06) a difference which is nearly significant.

Table 4: Patient's characteristics by ALK status.

Patient's characteristic	ALK negative, N=102		ALK positive, N=8		P Value,
	n (%)		n (%)		
Mean age at diagnosis	62.2±10.1		51.6±10.1		0.006
Age					0.015
≤60	44	(43.1)	7	(87.5)	
>60	58	(56.9)	1	(12.5)	
Sex					0.4
Male	66	(64.7)	4	(50)	
Female	36	(35.5)	4	(50)	
Smoking					0.46
Nonsmoker	63	(61.8)	6	(75)	
Smoker	39	(38.2)	2	(25)	
Nationality					0.53
Kuwaiti	52	(51)	5	(62.5)	
Non-Kuwaiti	50	(49)	3	(37.5)	

The mean age did not differ between EGFR positive and negative patients (p = 0.18), and there was no difference in the incidence of EGFR mutations in Kuwaitis and patients from other Arab countries, table 3.

Younger patients exhibited a higher incidence of ALK mutation (87.5% versus 12.5% p = 0.015) in patients ≤60, and those >60, respectively. There was no difference in the incidence of ALK mutation between males and females, smokers and nonsmokers and finally Kuwaitis and non-Kuwaitis, table 4.

Discussion

In our study we examined 110 patients with adenocarcinoma for gene mutations, in which 36 (32.7%) patients exhibited EGFR mutations and 8 (7.3%) exhibited ALK gene rearrangement. The observed EGFR mutations frequency in our study were approximately similar to that reported in East Asia and Japanese patients, higher than the recorded in USA, Australia and India. Vietnam, Taiwan and Thailand showed the highest incidence. The reported EGFR mutation frequency in Arab region was from Moroccan patients. They reported a 21% incidence which is still lower than ours [13, 14], table 5.

Table 5: Molecular epidemiology status of EGFR mutation [16].

Region	Incidence	Mutation rate
Vietnam	77/120	64.2%
Japan	71/263	27%
East Asia	107/361	30%
Taiwan	108/174	62.1%
Thailand	63/117	53.8%
Philippines	34/65	52.3%
China	372/741	50.2%
Hong Kong	76/161	47.2%
United States of America	11/80	14%
Australia	6/83	7%
India Chennal	16/72	22.2%
India (Tata Memorial Hospital)	202/780	26%

Among subtypes of EGFR mutation exon 19 Del was detected in 50%, exon 21 (L858R) substitutions in 39%, D719x mutation and insertion in 5.5%. Our results are consistent with those reported by Sharma et al. where exon 19 Del was detected in 45% of patients, exon 21 (L858R) substitution in 40–45% of patients and D719x in 5% of patients. [15] Similar incidence was observed by Anuradha et al., 50% deletions in exon 19, 42% exon 21 (L858R) substitution and 7% of the mutation in exon 18. [14] The same was reported in IPASS trial. [18] Hassan et al. reported EGFR mutations to be mainly detected in exon 19 (69%) followed by exon 21 (21%) and exon 20 (7%), whereas mutations in the exon 18 were rare (3%) that slightly differ than what is seen in the current study. [14] Another report released by Ismaili et al. in Moroccan population showed the overall frequency of the EGFR mutation to be 21%. Mutations were mainly detected in exon 19 (69%), followed by Exon 21 (21%) and exon 20 (7%), whereas mutations in the exon 18 were rare (3%). This percentage is slightly higher than our study but with the same pattern of distribution among different type of mutation [16].

In our study incidence of EGFR mutations were higher in females than males (55.6%, 44.4%) respectively. Females represented only 27% of EGFR wild population and males represented 73%, a difference which was statistically significant, P= 0.004. A finding which was recorded in different studies [17, 14].

In this study there was no significance difference in mean age between EGFR mutated and nonmutated patients (p = 0.18). Similar results were reported by other studies. [18]In this study no difference in incidence of EGFR mutations in Kuwaitis and patients from other Arab countries.

It has been reported that exon 18 EGFR mutations are detected more frequently in younger patients, which was also noted in our study [19].

In a review by Shigematsu and Gazdar, 45% of never smokers had EGFR mutations, whereas only 7% of smokers had EGFR mutations [20]. The high frequency of EGFR mutations in never smokers is consistent across different ethnic and geographic groups. Similar result was detected in this study. The frequency of EGFR mutation in never smokers was significantly higher than smoker patients (75% versus 25%; p = 0.06). Furthermore, it has been reported that the frequency of EGFR mutations is inversely associated with the amount of exposure to tobacco smoke, for both passive and active smoking [21].

The overall frequency of ALK fusion oncogene in the general NSCLC population is low 2 to 7%. (4 introduction). The frequency in our study was 7.3%. Similar frequencies have been reported in Asian and Western populations [22]. One study showed a 13% incidence in metastatic NSCLC in Western populations and a 22% in never/light smokers and among never or light smokers who did not have an EGFR mutation, the frequency was 33%. Also, ALK fusion oncogene-positive lung cancer is observed in younger and adenocarcinoma patients.[8] Data which are consistent with ours where ALK gene rearrangement was higher in younger patients (87.5% versus 12.5% p = 0.015), in patients ≤60 and those >60, respectively. There was no difference in incidence of ALK mutation between males and females, smokers and nonsmokers and finally Kuwaitis and non-Kuwaitis.

Conclusion

Our population exhibited incidence of EGFR mutation approximately similar to that reported in East Asia and Japanese patients, higher than that recorded in USA, and Australia. Incidence of ALK gene rearrangement is approximately similar to that reported by other trials. However, more studies with larger patient's numbers are needed to verify these finding.

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