DETECTION OF *EGFR* MUTATION AND ITS ASSOCIATION WITH CLINICOPATHOLOGICAL FEATURES OF NON-SMALL CELL LUNG CANCER PATIENTS

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(Received: June 30, 2022; Accepted: September 19, 2022)

Abstract - Alterations in the epidermal growth factor receptor were confirmed to take a significant role in targeted therapy for patients with lung cancer. Studied 299 non-small cell lung cancer patients to determine the distribution of *EGFR* mutation and its relationship with clinicopathological characteristics of patients. *EGFR* mutations were analysed by allele-specific PCR on the Cobas z480 system, and the association between *EGFR* mutations and patient characteristics was analysed by Pearson Chi-square test and Fisher extract test. The results showed that the frequency of *EGFR* mutation was 38.8%, in which, Ex19Del and L858R substitution were predominant among overall mutation, 45.4% and 29.1%, respectively. There was no difference between *EGFR* mutations with age, specimen type, tumour size and histopathological subtype. However, there was a strong correlation between *EGFR* mutation and gender and tumour status ($p<0.05$). In particularly, Ex19Del and L858R mutation were positively associated with females, while the G719X substitution mainly occurred in male patients.

Key words - Non-small cell lung cancer; *EGFR* mutation; clinicopathological parameter

1. Background

Lung cancer is a significant burden worldwide, with an estimated 2.2 million new cases and 1.79 million deaths in 2020 [1], which non-small cell lung cancer (NSCLC) takes the highest prevalence with approximately 85% of lung cancers [2]. Furthermore, patient with NSCLC often has a poor prognosis and shorter survival time than patients with other forms of cancer [2].

Developments in molecular biology and targeted therapies in lung cancer have revolutionized treatment. To be adapted with targeted therapy, the most important thing is to identify the biomarkers. In NSCLC, the biomarker of great interest is the epidermal growth factor receptor (*EGFR*), which enhances the proliferation, differentiation, mobility and apoptosis inhibitor of the cell [3]. Normally, these processes have controlled through specific interaction of *EGFR*'s ligands to activate the downstream signalling pathways. In the aberrant genetic scenario, *EGFR* activates its downstream signalling pathways without interacting with any ligand. Therefore, the cells proliferate out of control and form somatic NSCLC. Consequently, identifying *EGFR* mutations in lung cancer patients is the top priority in the WHO's 2022 NSCLC mutation diagnosis protocol [4].

In addition, the distribution of *EGFR* mutations significantly changes in different geographical regions. Asia always has a higher rate of *EGFR* mutations than European countries, with approximately 40% vs 15% [5]. Moreover, the therapeutic efficacy of TKIs has also been recognized in good response to patients with NSCLC [6]. Therefore, treatment efforts for NSCLC patients with *EGFR* mutations are gradually gaining more hope. Hence, studies determining the frequency of mutations in these territories become important in the prognosis and treatment selection for NSCLC patients. Therefore, in this study, our research aim is:

To detect *EGFR* mutation utilizing Realtime PCR and Investigate its association with clinicopathological features among Vietnamese NSCLC patients.

2. Materials and methods

2.1. Patients and sample collection

A cross-sectional study was conducted on 299 patients diagnosed with NSCLC at National Cancer Hospital (Hanoi) from March 2021 to June 2022. The sample selection criteria included: (1) NSCLC patients undergoing surgery or biopsy to obtain optimal specimen size for molecular biology and pathology tests; (2) Each specimen contained at least 30% cancer cell. Samples that did not collect enough samples or had undergone treatment were not selected for this study.

2.2. Method

2.2.1. DNA isolation

The DNA stock was isolated from formalin-fixed paraffin-embedded tissue (FFPET) sample by utilising Cobas® DNA sample preparation kit (Roche, Germany) and following the given procedure of manufacture. The concentration of DNA was identified by BioDrop µLITE UV/Vis spectrophotometer (Biodrop, United Kingdom).

2.2.2. EGFR mutation analysis

The Cobas® *EGFR* mutation test kit v2 (Roche, Germany) was used for mutation detection. The test applied allele-specific PCR (AS-PCR) to detect seven *EGFR* mutations (G719X mutation in exon 18; deletion in exon 19; T790M, S768I and insertion in exon 20; L858R and L861Q in exon 21) from exon 18 – 21. The performance of qPCR required a Cobas® z 480 system (Roche, Germany).

2.2.3. Statistical analysis

IBM SPSS ver. 22.0 (IBM Co, NY, USA) was used to analyse the distribution of *EGFR* mutation and its association with clinicopathological features of NSCLC patients. All variables were estimated using Pearson Chi-square or Fisher extract tests. A p-value of <0.05 was considered statistical significance.

3. Results

3.1. Patient characteristics

The clinicopathological characteristics of 299 NSCLC patients are detailed in Table 2. The mean age of patients was 60.1, of which 190 (63.5%) were male and 109 (36.5%) were female. The specimens were collected from biopsy or surgical, 127 (42.5%) and 151 (50.5%) cases, respectively; the other 21 (7.0%) cases were unidentified specimens. Besides, the tumour sample size was measured and classified in forms ≤ 5cm (81.9%) and > 5cm (8.4%), while 29 unidentified specimens account for 9.7%. Among 265 clinical samples from lung cancer patients, 196 (65.6%) were identified as primary tumours and 103 (34.4%) as metastatic tumours. The results of histology showed that the patients in this study were divided into adenocarcinoma (85.6%), squamous cell cancer (13.4%) and large cell cancer (1.0%).

3.2. Prevalence of EGFR mutation analysis

Among 299 FFPET specimens, 116/299 patients were positive for *EGFR* mutation, accounting for 38.8%, in which, 93/116 cases (80.2%) were single mutation, and 23/116 cases (19.8%) were multiple mutations, detailed in Table 2.

Table 1. *EGFR mutation result types from 299 NSCLC patients*

No.	Mutation	Number (%)
	Total	116 (100.0)
1	Ex19Del	52 (44.8)
2	L858R	35 (30.2)
3	Ex20Ins	3 (2.6)
4	G719X	1 (0.9)
5	L861Q	2 (1.7)
6	Ex19Del, Ex20Ins	3 (2.6)
7	Ex19Del, L858R	1 (0.9)
8	Ex19Del, T790M	7 (6.0)
9	L858R, Ex20Ins	3 (2.6)
10	S768I, G719X	6 (5.2)
11	T790M, L858R	1 (0.9)
12	Ex19Del, T790M, Ex20Ins	1 (0.9)
13	L858R, T790M, Ex20Ins	1 (0.9)

Deletion in exon 19 (Ex19del) and alteration at leucine to arginine substitution at position 858 (L858R) in exon 21 were the most common, 52/116 cases (44.8%) and 35/116 cases (30.2%), respectively. Other single mutations were uncommon, with three instances (2.6%) having insertion in exon 20 (Ex20Ins), one case (0.9%) having point mutations at glycine to other residues at 719 position (G719X), and two cases (1.7%) having point mutation that replaces leucine by glutamine at 861 positions of exon 21 (L861Q).

Uncommon *EGFR* mutations frequently co-occur with other Ex19Del and L858R mutations, such as Ex19Del-T790M (6.0%) and Ex19Del-Ex20Ins (2.6%), L858R-Ex20Ins (2.6%), L858R-T790M (0.9%), Ex19Del-T790M-Ex20Ins (0.9%), and L858R-T790M-Ex20Ins (0.9%). S768I and G791X mutations often present with 6 cases and take 4.3% of the whole mutation result.

Seven types of *EGFR* mutations located at the Tyrosine activating region were recorded from 116 NSCLC patients carrying *EGFR*-mutant, detailed in Figure 1. The total *EGFR*-mutant accounts for 141 mutations including 64/141 (45.4%) Ex19Del, 41/141 (29.1%) L858R, 11/141 (7.8%) Ex20Ins, 10/141 (7.1%) T790M, 7/141 (5.0%) G719X, 6/141 (4.3%) S768I and 2/141 (1.4%) L861Q.

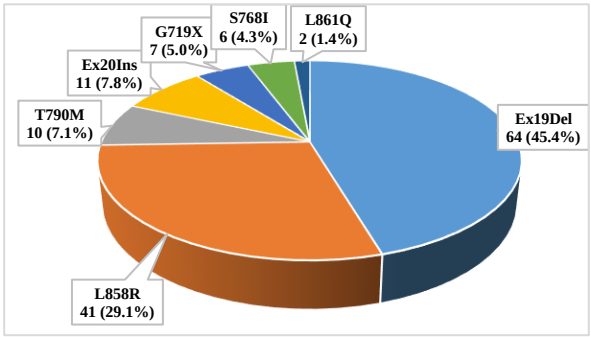

Figure 1. *Distribution of EGFR mutations*

3.3. Association of EGFR mutation with clinicopathological parameters among Vietnamese NSCLC patients

The association of *EGFR* mutation was evaluated with clinicopathological features; including gender, age, size and type of specimen, tumour status and histological type of NSCLC patients. Gender and tumour status have a critical association with *EGFR* mutation, as described in Table 2.

Table 2. *Association of EGFR mutation with clinicopathological features of NSCLC patients*

Characteristics		**N**	**Yes (%)**	***p-value***
		299	116 (38.8)	
Gender				***<0.001***
	Male	190	49 (25.8)	
	Female	109	67 (61.5)	
Age (60.1 ± 9.0)				***0.488***
	< 60.1	126	46 (36.5)	
	> 60.1	173	70 (40.5)	
Specimen type				***0.917***
	Biopsy	127	51 (40.2)	***0.678***
	Surgical	151	57 (37.7)	***0.707***
	Other	21	8 (38.1)	***0.946***
Size				***0.615***
	≤ 5cm	245	93 (38.0)	***0.527***
	> 5cm	25	12 (48.0)	***0.324***
	Unknown	29	11 (37.9)	***0.920***
Tumour status				***0.047***
	Primary	196	84 (42.9)	
	Metastasis	103	32 (31.1)	
Histological type				***0.168***
	AD	256	104 (40.6)	***0.113***
	SCC	40	12 (30.0)	***0.220***
	LCC	3	0 (0.0)	***0.166***

The correlation established between *EGFR* mutation and gender feature, p<0.001. The prevalence of *EGFR* mutations in female patients was 61.6% (67/109). Meanwhile, among male patients, this incidence was 25.8% (49/190). Consequently, the *EGFR* mutation frequently occurred among female patients.

Two states of NSCLC were recorded, primary and metastasis. The alteration of *EGFR* commonly found in primary tumours, such as lung and bronchi, which accounting for 42.9% (84/112 cases). Whilst, the *EGFR*

mutation in patients whose tumours metastasized to other organs, including liver, bone, brain and connective tissue, had a lower prevalence rate of 31.1% (32/103). Consequently, *EGFR* mutations of NSCLC patients are usually detected in primary tumour with p=0.047.

Otherwise, the other clinicopathological features were not detected any association with *EGFR* mutation, included age, type and size of specimen, as well as histological type.

Moreover, the specific association of *EGFR* mutations, seven groups of *EGFR* mutations were examined to detect their relationship with clinical features, detailed in Table 3. Ex19Del and L858R substitution were positively correlated with a female, p<0.001 and p=0.014. In contrast, G719X substitution was associated with male patients, p=0.043.

Table 3. *Types of EGFR mutation and their association with clinicopathological parameters.*

		Ex19Del		L858R		T790M		Ex20Ins		G719X		S768I		L861Q	
		yes (%)	p-value	yes (%)	p-value	yes (%)	p-value	yes (%)	p-value	yes (%)	p-value	yes (%)	p-value	yes (%)	p-value
N	**299**	**64**		**41**		**10**		**11**		**7**		**6**		**2**	
Gender			***<0.001***		***0.014***		***0.116***		***0.519***		***0.043***		***0.061***		***0.690***
Male	190	22 (11.6)		19 (10.0)		4 (2.1)		8 (4.2)		7 (3.7)		6 (3.2)		1 (0.5)	
Female	109	42 (38.5)		22 (20.2)		6 (5.5)		3 (2.8)		0 (0.0)		0 (0.0)		1 (0.9)	
Age (60.1 ± 9.0)			***0.562***		***0.145***		***0.609***		***0.396***		***0.426***		***0.659***		***0.226***
< 60.1	126	29 (23.0)		13 (10.3)		5 (4.0)		6 (4.8)		2 (1.6)		2 (1.6)		0 (0.0)	
≥ 60.1	173	35 (20.2)		28 (16.2)		5 (2.9)		5 (2.9)		5 (2.9)		4 (2.3)		2 (1.2)	
Specimen type			***0.701***		*0.972*		***0.649***		***0.650***		***0.622***		***0.774***		***0.256***
Biopsy	127	26 (20.5)	*0.736*	18 (14.2)	*0.842*	5 (3.9)	*0.624*	5 (3.9)	*0.839*	4 (3.1)	*0.427*	3 (2.4)	*0.706*	2 (1.6)	*0.099*
Surgical	151	32 (21.2)	*0.928*	20 (13.2)	*0.812*	5 (3.3)	*0.974*	6 (4.0)	*0.785*	3 (2.0)	*0.682*	3 (2.0)	*0.980*	0 (0.0)	*0.152*
Other	21	6 (28.6)	*0.406*	3 (14.3)	*0.937*	0 (0.0)	*0.377*	0 (0.0)	*0.353*	0	*0.462*	0 (0.0)	*0.496*	0 (0.0)	*0.697*
Size			***0.637***		***0.940***		***0.622***		***0.541***		***0.572***		***0.577***		***0.801***
≤ 5cm	245	50 (20.4)	*0.371*	33 (13.5)	*0.795*	9 (4.1)	*0.500*	10 (4.1)	*0.431*	6 (2.4)	*0.793*	5 (2.0)	*0.929*	2 (0.8)	*0.505*
> 5cm	25	6 (24.0)	*0.741*	4 (16.0)	*0.728*	0 (0.0)	*0.331*	1 (4.0)	*0.929*	1 (4.0)	*0.567*	1 (4.0)	*0.458*	0 (0.0)	*0.668*
Other	29	8 (27.6)	*0.393*	4 (13.8)	*0.989*	1 (3.4)	*0.974*	0 (0.0)	*0.268*	0 (0.0)	*0.380*	0 (0.0)	*0.417*	0 (0.0)	*0.642*
Tumour status			***0.366***		***0.145***		***0.292***		***0.610***		***0.741***		***0.954***		***0.642***
Primary	196	45 (23.0)		31 (15.8)		5 (2.6)		8 (4.1)		5 (2.6)		4 (2.0)		1 (0.5)	
Metastasis	103	19 (18.4)		10 (9.7)		5 (4.9)		3 (2.9)		2 (1.9)		2 (1.9)		1 (1.0)	
Histological type			***0.211***		***0.588***		***0.898***		***0.857***		***0.478***		***0.944***		***0.844***
AD	256	59 (23.0)	*0.091*	37 (14.5)	*0.364*	9 (3.5)	*0.688*	10 (3.5)	*0.610*	5 (2.0)	*0.279*	5 (2.0)	*0.872*	2 (0.8)	*0.561*
SCC	40	5 (12.5)	*0.140*	4 (10.0)	*0.463*	1 (2.5)	*0.750*	1 (2.5)	*0.670*	2 (5.0)	*0.232*	1 (2.5)	*0.811*	0 (0.0)	*0.577*
LCC	3	0 (0.0)	*0.364*	0 (0.0)	*0.488*	0 (0.0)	*0.746*	0 (0.0)	*0.734*	0 (0.0)	*0.788*	0 (0.0)	*0.803*	0 (0.0)	*0.886*

4. Discussion

Among 299 NSCLC patients in this study, the incidence of *EGFR* mutation was recorded in 116/299 patients, accounting for 38.8%, as described in Table 2. The prevalence of *EGFR* mutations among Asian patients is approximately 30-50% [7]. In China, a high prevalence of *EGFR* mutations was recorded from 21,324 FFPET specimens for a duration of 10 years (2009 – 2018), accounting for 45.1% [8]. In 2018, a high frequency of *EGFR* mutations was detected in 156/318 (49.1%) FFPET specimens from Thai NSCLC patients [9]. These proportions were quite similar to other studies investigating the prevalence of *EGFR* mutation among the Vietnamese population. Also, Anh-Thu Huynh Dang et al. described the prevalence of six common mutations among Vietnamese NSCLC patients utilizing NGS and *EGFR* mutation was the most frequently with 35.4% [10]. Consequently, the prevalence of *EGFR* mutation in 299 NSCLC patients is relevant to the other published studies.

Ex19Del and L858R were commonly presented, with 45.4% and 29.1%, respectively (Figure 1). Thanh Ha Vu et al. reported the proportion of *EGFR* mutation among 44 Vietnamese NSCLC patients; Ex19Del and L858R also presented the highest percentage, 46% and 18%, respectively [11]. Zineb Benbrahim et al. reported an average proportion of *EGFR* mutation in the Middle East and African patients [12], in which, Ex19Del and L858R substitution took the highest rate, with respectively 57.2% and 23.4% on average. In Europe, Susanne Gahr et al. [13] also reported a high incidence of Ex19Del (61.9%) and L858R (33.1%) in their research. As a result, Ex19Del and L858R mutations frequently occur among NSCLC patients who carry *EGFR*-mutant worldwide.

Additionally, the other *EGFR* mutation types of tyrosine kinase active sites in our research (G719X – 5.0%, T790M – 7.1%, Ex20Ins – 7.8%, S768I – 4.3% and L861Q – 1.4%) presented with low incidence. These *EGFR* mutations are well known as uncommon mutations, with approximate 10% of incidence among NSCLC patients [14]. Xiuzhi Zhou et al. reported a low frequency of uncommon *EGFR* mutation among the Chinese population [15]. Particularly, the distribution of G719X, L861Q and Ex20Ins appeared with low incidence, 2.7%, 4.5% and 1.8%, respectively. However, the prevalence of S768I was not detailed in this study. Also, Grainne O'Kane et al. reported a low prevalence of uncommon *EGFR* alterations in a review, including G719X (3%), Ex20Ins (10%), S768I (1%) and L861Q (2%) [16]. Therefore, the distribution of uncommon *EGFR* mutations from 299 Vietnamese NSCLC patients is relevant to previously published worldwide.

Furthermore, the *EGFR* multi-mutation results also presented some potential outcomes. The resistance mutant T790M tends to co-exist with Ex19Del and L858R [17,18]. T790M substitution is well known as a secondary mutation that frequently occurs in NSCLC patients who received treatment with TKIs [19]. The T790M alteration is one of

the *EGFR* mutations that resisted the activation of the TKIs drug by changing the affinity of *EGFR* for ATP [20]. The distribution of T790M mutation in our research tends to co-exist with Ex19Del. However, several worldwide reported that T790M mutation frequently combined with L858R in exon 21 rather than Ex19Del in exon 19 [18]. Although several studies on the distribution of *EGFR* mutation in NSCLC patients have been described in Vietnam, data on the co-existence of T790M and other compound mutations were limited [10, 11]. Therefore, our observation of T790M substitution has shown a difference from previous scientific publications when the T790M tends to co-exist with Ex19Del rather than L858R substitution.

Interestingly, G719X and S768I substitutions were contemporary presents. Among NSCLC patients carrying G719X substitution in our research, 6/7 cases (5.2%) co-existence G719X and S768I mutation. This result was relevant to the outcome of Yuankai Shi et al. [21]. Furthermore, Yangyang Cai et al. [22] reported the success in treating NSCLC patients carrying uncommon compound *EGFR* G719X and S768I mutations by applying a certain regimen by TKIs. Therefore, the prevalence of compound G719X and S768I in Vietnamese NSCLC patients is related to previous scientific publications and promises a suitable treatment regimen for NSCLC patients positive with this *EGFR* mutation type.

EGFR mutation is frequently found in particular subjects related to clinicopathological such as female patients with a light or non-smoking history [23, 24]. Table 2 described a similar outcome: the *EGFR* mutation was commonly diagnosed in female NSCLC patients, with 61.5% (67/109 cases). The correlation presented between *EGFR* mutation and females with $p<0.001$. Several scientific studies have been conducted to clarify the link between *EGFR* mutations and female patients with NSCLC. Zhihuang Hu et al. established a study to explore the relationship between hormone receptor expression and *EGFR* mutation. As a result, a significantly high expression of estrogen receptors and progesterone receptors was recorded among *EGFR*-positive patients ($p<0.05$) [25]. Consequently, the correlation between *EGFR* mutation and female have been confirmed worldwide, although lacking understandable relation to the molecular mechanism of this phenomenon.

The association of *EGFR* mutation was also found in patients with primary tumours ($p=0.047$). Table 2 describes *EGFR* mutations that were frequently detected in primary tumours (84/109 cases – 42.9%) rather than metastatic tumours (32/103 – 37.9%). In 2016, the relevant results were also shown in a study that investigated the association of *EGFR* mutations among Korean NSCLC patients by Jaeyoung Cho et al. [26]. However, the other studies showed the relationship with different stages of NSCLC patients. Lynette M. Sholl et al. reported that *EGFR* mutations were significantly associated with stage IV when lung tumours were already metastasized [27]. As a result, our observation supposes that *EGFR* mutation is associated with the primary stage of NSCLC as well as the formulation of lung tumours, however, this result conflicts to worldwide available reports. The other clinicopathological features have not generated any relationship with *EGFR* mutation, including age, specimen type and tumour size. Also, these outcomes were not recorded in reports of Barbara Melosky et al. [7] as well as Zineb Benbrahim et al. [12].

Interestingly, gender factor has a significant relationship with 3/7 mutation types investigated in the research, including Ex19Del, L858R and G719X, in which, Ex19Del and L858R mutations were positively associated with female patients, $p<0.001$ and $p=0.014$, while G719X substitution was linked with male patients with $p=0.043$ (Table 3). Ex19Del and L858R were considered common *EGFR* mutations and confirmed in the high response with TKIs [28]. However, no significant association existed between Ex19Del, L858R and clinicopathological features recorded worldwide. Weiwei Hong et al. did not explore any significance between Ex19Del, L858R and gender features among Chinese NSCLC patients [29]. Also, Priyanka Gaur et al. did not describe any significant link between common *EGFR* alterations and females [24]. In contrast, G719X was in the group of rare *EGFR* mutations. Recent reports were limited in illustrating the association of uncommon *EGFR* mutations due to their rarity. However, TKIs in NSCLC patients possessing the G719X substitution trials have shown certain potential. Kaidi Li et al. reported that G719X substitution had shown an intermediate response with specific target therapy [30]. Therefore, associations of three identified *EGFR* mutations were significant for treating *EGFR* mutation among Vietnamese NSCLC patients.

Findings in this study have to be seen in light of some limitations. There are two major limitations that could be addressed. Study design is the first one. Particularly, the limit of data collection time in the duration of one year might be a negative impact for the investigation of this research. Furthermore, lack of diversity of clinicopathological parameters was considered as the second major limitation. There are some clinicopathological features of NSCLC patient that unavailable to collect, including smoking status, treatment status and stage of tumour. Thus, there are some suggestions for the improvement of this study, including: (1) expand the data collection time; and (2) collect more clinicopathological features. As a result, these improvements might prevent the current limitations and develop meaningful knowledge related to NSCLC patient in Vietnam.

Overall, the distribution of *EGFR* mutation among Vietnamese NSCLC patients in this study was 38.8% (116/299 cases). Almost the distribution of *EGFR* mutations in our results was meaningful in treating *EGFR* mutation in Vietnamese NSCLC patients by clearly describing their distribution. For further study, the sample size should be increased, and specific clinical information (including smoking history and tumour stage) and treatment information have to collect for a widen observation among NSCLC patients in Vietnam.

5. Conclusion

In this study, the distribution of *EGFR* mutation among 299 Vietnamese NSCLC patients was 38.8%. The *EGFR* mutation frequently occurred in female and commonly diagnosed in NSCLC patients with primary tumour status.

Furthermore, Ex19Del and L858R alterations were positively associated with female, while G719X substitution linked with male patients. Our work confirms and extends the previously reported findings regarding the distribution of *EGFR* mutations and clinicopathologic features. In addition, these finding provide suggestion in the application of TKIs among NSCLC patients with *EGFR*-positive in Vietnam.

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