

Frequency of Epidermal Growth Factor Receptor Mutations among Filipino Patients with Non-small Cell Lung Carcinoma in a Private Tertiary Care Setting

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ABSTRACT

Background. Epidermal Growth Factor Receptor (EGFR) mutation status has been shown to have a significant prognostic and predictive role in the management of Non-small Cell Lung Carcinoma (NSCLC), significantly prolonging patients' survival. Thus, EGFR mutational analysis before initiation of treatment is now recommended in several clinical practice guidelines. Although EGFR mutation testing in NSCLC has been a part of clinical care in the Philippines, there is little data on the EGFR mutation spectrum among Filipinos.

Objective. This study aims to determine the frequency of EGFR mutations among Filipino population diagnosed with NSCLC in a private tertiary care setting.

Methodology. A total of 626 tissue samples (444 biopsies, 108 pleural/ascitic fluids, 74 excision/resection), during a 15-month period (January 2015-March 2016) were assessed for the known EGFR driver mutations (exons 18, 19, 20, 21) using the Roche EGFR protocol with the Cobas Quantitative Real Time PCR. Macrodissection was performed as necessary. Available patient demographics were recorded. Statistical analyses were performed using the Fisher's exact test.

Results. In this study, we report the largest EGFR mutation profiling data among Filipino patients with NSCLC, which showed an overall 49.4% EGFR mutation rate. The mutation rates according to histologic types, were as follows: adenocarcinoma (49.9%, n=287/575), squamous cell carcinoma (3.5%, n=9/26), NSCLC NOS (50%, n=10/20), adenosquamous cell carcinoma (66.7%, n=2/3), and adenocarcinoma with neuroendocrine features (50%, n=1/2). Consistent with the literature, we found a significant higher incidence of EGFR mutation among women than men (60.2% vs 39.8%). With regards to individual mutation types, the most common mutations detected were deletions in exon 19 (54.7%, n=168), followed by L858R point mutation in exon 21 (27.4%, n=84).

Conclusion. The incidence of EGFR mutations in NSCLC varies across different ethnicity. In previous reports, the frequency of EGFR mutations is approximately 30% (with a range of 22.2% to 64.2%) among the Asian population compared with 20% among the white population. In the Philippines, the incidence of EGFR mutations is sparsely explored. Here we report the largest EGFR mutation profiling data among Filipinos with NSCLC in a tertiary care setting, with a frequency of 49.4%. This prevalence is almost similar to those reported in Asia. EGFR is differentially mutated among NSCLC patients with different gender, as women have significantly higher incidence than men. Hence, this study establishes relevance of routine EGFR mutation testing for all NSCLC patients as part of initial workup at diagnosis and underscores the significant role of EGFR inhibitors as a treatment option among Filipino population.

Key words: Epidermal growth factor receptor, non-small cell lung carcinoma, Exons 18,19,20 and 21, T790M, polymerase chain reaction

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INTRODUCTION

Lung cancer is the leading cause of cancer mortality in the world and is the second most common cause of cancer deaths among Filipinos. Approximately 85% of lung cancer cases are non-small lung cancers (NSCLC).¹ In patients with NSCLC, platinum-based chemotherapy used to be considered as standard first-line treatment.² During the recent years, there has been continuous development of new and effective targeted treatment modalities for advanced NSCLC. One of these therapeutic agents are tyrosine kinase inhibitors (TKI) which targets mutant epidermal growth factor receptor (EGFR).³⁻⁴ EGFR mutations play an important role in the pathogenesis of multiple carcinoma, including NSCLC. Activating EGFR mutation promotes tumor

growth and progression, stimulates tumor cell proliferation, inhibits apoptosis and produces angiogenic factors.⁵ For patients with advanced NSCLC harboring EGFR mutation, several phase III studies have shown the clinical efficacy of the FDA approved EGFR inhibitors, gefitinib, afatinib and erlotinib, as compared to platinum-based chemotherapy when used as first line of treatment. Also, EGFR mutation status has been shown to have a significant prognostic and predictive role in the management of NSCLC, significantly prolonging patients' survival.⁶⁻⁸ Not all types of EGFR mutation, however, are responsive to the first-generation tyrosine kinase inhibitors. For NSCLC patients with the common EGFR mutations, namely exon 19 deletion and exon 21 L858R, the response rate to TKIs (gefitinib and erlotinib) is approximately 60%. Several studies also suggested that patients with exon 19 deletion mutation might be more sensitive to targeted therapy than with exon 21 L858R. On the contrary, mutation in exon 20 (T790M) have been associated with resistance to first generation TKIs. EGFR T790M mutations usually occurs as a resistance mutation after first generation TKI therapy. For patients with EGFR T790M mutations, treatment with osimertinib may be effective and nazartinib (EGF816) is promising for the majority of them.⁹ Thus, an accurate EGFR mutational analysis before initiation of treatment and repeat EGFR mutation testing at relapse, are now recommended in several clinical practice guidelines.¹⁰

The incidence of EGFR mutations in NSCLC varies across different ethnicities. In previous reports, the frequency of EGFR mutations is approximately 30% (range of 22.2% to 62%) among the Asian population compared with 20% among the white populations.^{11,12} In the Philippines, although EGFR mutation testing in NSCLC has been a part of clinical care setting, there is little data on the EGFR mutation spectrum among Filipinos. Here, in this study we determine the EGFR mutation status in 626 NSCLC patients of Filipino ethnicity and correlate with different variables like age, gender, and histologic types.

METHODOLOGY

This is a retrospective study of Filipino patients diagnosed with NSCLC who were referred to our institution for EGFR mutation testing from various hospitals across the Philippines over a 15-month period (January 2015-March 2016). The EGFR mutational status is performed when a diagnosis of adenocarcinoma, non-small cell carcinoma favors squamous cell carcinoma, adenosquamous carcinoma and non-small cell carcinoma, NOS are rendered. The histopathological diagnoses were mostly made based on the histomorphology with or without immunohistochemical staining performed with TTF-1, p63, Napsin A, CK7, CK20. In distinguishing adenocarcinoma from squamous cell carcinoma, or a lung primary vs. metastatic, 2 or more immunohistochemical stains were used. 78 of 626 patients were diagnosed as primary lung carcinoma using panel of immunohistochemical stains (positive for CK7, TTF1 and Napsin A; negative for CK20). 44 cases were diagnosed using positivity with TTF-1 only. The remaining cases were diagnosed as primary lung carcinoma based on clinical and radiologic correlation (absence of other organ involvement). The patient demographic data (including age, nationality, gender) if available, and histologic diagnosis were recorded. This study was approved by the Institutional Scientific Review Board and Ethics Committee of St. Luke's Medical Center-Quezon City. Since this was a retrospective analysis, the ISRB and the IERC waived the need for an informed consent.

Inclusion criteria

All Filipino patients diagnosed as Non-small cell Lung Carcinoma from St. Luke's Medical Center-Global City and other hospitals from the Philippines.

Exclusion criteria

Formalin-fixed paraffin embedded (FFPE) samples from patients diagnosed with NSCLC containing less than 5% viable tumor cells were excluded from this study. Patients with incomplete histopathologic report were also excluded from this study.

Collection of patient samples

The patient's FFPE blocks and H&E slides, together with histopathological report, were sent to the Cellular Immunology Section of the Institute of Pathology-St. Luke's Medical Center. The hematoxylin and eosin stained slides were viewed under the microscope to confirm that the tumor cells constitute more than 5% of the tissue mass. Macrodissection was performed on cases with less than 50% tumor cells in the tissue block/slide. A total of 626 tissue samples (444 biopsies, 108 pleural/ascitic fluids, 74 excision/resection) were assessed for EGFR mutation.

DNA extraction

DNA was isolated from the FFPE samples after deparaffinization and extraction of 3-5mm thick paraffin sections in xylene. The Cobas Roche DNA Sample Preparation Kit (Roche Diagnostics, USA) and the Cobas cfDNA Sample Preparation Kit (Roche Diagnostics, USA) were used for manual sample preparations, which were based on nucleic acid binding to glass fibers.

Mutation analysis by PCR and Sanger sequencing

After the extraction of DNA, the target DNA was then amplified and detected on the Cobas Z 480 analyzer (Roche Diagnostics, USA) using the amplification and detection reagents provided in the Cobas Roche EGFR Mutation Test v1 kit (Roche Diagnostics, USA). A mutant control and negative control were included in each run to confirm the validity of the run.

The Cobas Roche EGFR Test is designed to detect the following mutations:

- Exon 18: G719X (G719A, G719C, and G719S)
- Exon 19: deletions and complex mutations
- Exon 20: S768I, T790M, and insertions
- Exon 21: L858R and L861Q

Statistical analysis

The Fischer's exact test was performed to reveal any significant correlation between the mutation status and gender, gender and specific mutation type.

RESULTS

626 Filipino patients diagnosed with lung cancer were tested for common EGFR mutation subtypes by real time PCR using TaqMan primer probes for point mutations in exons 18, 20 and 21 and in frame deletion in exon 19. As shown in Table 1, of the 626 patients tested, 52% (n=325) were males and 48% (n=301) were females. The median age was 64 years (with a range of 13-94 years old). The overall EGFR mutational analysis result was positive in 49.4% (n=309), negative (wild type) in 50% (n=313) and invalid in 0.6% (n=4). Invalid results were secondary to DNA degradation, resulting to failure of PCR amplification or testing. The presence of EGFR mutation was significantly higher in females (60.2%) as compared with males (39.8%) (two

Table 1. Patient demographics (n=626)	
Variables	
Age	13-94
Median Age	64
Gender	
Male	325
Female	301
Histopathology	
Adenocarcinoma	575
Squamous cell	26
Adenosquamous	3
NSCLC, NOS	20
Adenocarcinoma with neuroendocrine features	2

tailed p test, $p < 0.0001$) (Table 2). As to histologic classification with EGFR mutation, there were 575 cases of adenocarcinoma, 26 cases of squamous cell carcinoma, 20 cases of NSCLC NOS, 3 cases of adenosquamous cell carcinoma and 2 cases of adenocarcinoma with neuroendocrine features. EGFR mutations were identified in 49.9% of adenocarcinoma (n=287), 3.5% of squamous cell carcinoma (n=9), 50% of NSCLC NOS (n=10), 66.7% of adenosquamous cell carcinoma (n=2), and 50% of adenocarcinoma with neuroendocrine features (n=1) (Table 1).

In our study, there were 26 patients less than 40 years old (male n=11, female n=15), 221 patients between 40-60 years old (male n=114, female n=107) and 379 patients older than 60 years old (male n=200, female n=179). Although not statistically significant, the EGFR mutation rate was higher in patients with age >60 (Roche Diagnostics, USA) years as compared to < 60 years (61.4% vs. 38.5%) ($p=0.0661$). With respect to gender, mutation rate in females was higher in older individuals (>60 years) as compared to 40-60 years (66% vs. 32%; n=123 vs. 59), which was statistically significant ($p=0.022789$). In males, there was no statistical significance as to age group between 40-60 years and >60 years (54% vs. 41%; n=67 vs. 51; p value= 0.05313) (Tables 3a and 3b).

Among the 309 EGFR mutated cases, 54.7% (n=168) have deletions in exon 19, 27.4% (n=84) with point mutations in exon 21 L858R, 7.2% (n=22) with exon 20 insertion, 1.3% (n=4) with point mutation in exon 18 G719X, 0.7% (n=2) with point mutation in exon 18 L861Q and 0.3% (n=1) with point mutation in exon 20 T790M. Dual mutations in exons 19, 20 and 21 were found in 8.4% (n=26) of the cases. Dual mutations involving exon 19 in combination with others was seen in 15 (4.9%) patients; exon 21 L858R point mutations in combination

with others was seen in 11 (3.6%) patients. (Figure 1). Out of the 26 cases of squamous cell carcinoma, 9 cases (34.6%) showed EGFR mutation, which include: exon 19 deletion (55.6%, n=5/9), point mutations in exon 21 L858R (33.3%, n=3/9) and exon 20 insertion (11.1%, n=1/9). Exon 19 deletion is found to be the most common mutation among both males and females. But, the prevalence of mutations in exon 19 deletion ($p=0.0069$) and exon 21 L858R ($p < 0.0001$) were significantly higher in females as compared with males (Table 4 and Figure 2). Between different age groups (<40 years, 40-60 years and >60 years), we did not find any statistical significance in terms of difference in the prevalence of EGFR mutation (p value=0.4815) (Figure 3).

DISCUSSION

Because EGFR mutation status has been shown to have a significant prognostic and predictive role in the management of NSCLC, many countries have recommended an accurate EGFR mutational analysis before initiation of treatment to determine appropriate treatment with EGFR inhibitors. Several studies suggested that clinical response to TKIs with exon 19 del

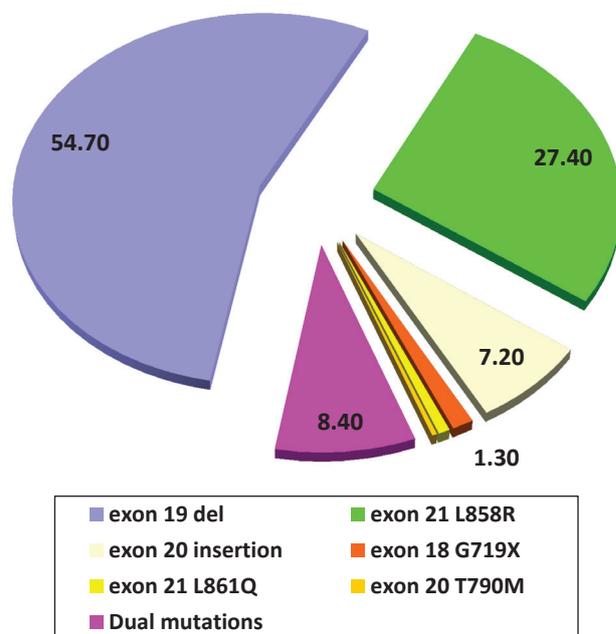


Figure 1. EGFR mutation status among NSCLC Filipino patients.

Table 2. Frequency and overall mutation rates in NSCLC				
	EGFR Positive	EGFR Negative	Percentage of EGFR Mutation	Significance
Male (n=325)	123	202	39.8%	< 0.0001
Female (n=301)	186	115	60.2%	

Table 3a. EGFR mutation status with respect to age and gender				
Age	Male (n=123)	Female (n=186)	Total	Significance
< 40 years	5	4	9	0.0661
40-60 years	51	59	110	
> 60 years	67	123	190	

Table 3b. EGFR mutation status with respect to age and gender						
Age	Male (n=123/325)		Significance	Female (n=186/301)		Significance
	EGFR positive	EGFR negative		EGFR positive	EGFR negative	
< 40 years	5	6	p=0.05313	4	11	p= 0.022789
40-60 years	51	63		59	48	
> 60 years	67	133		123	56	

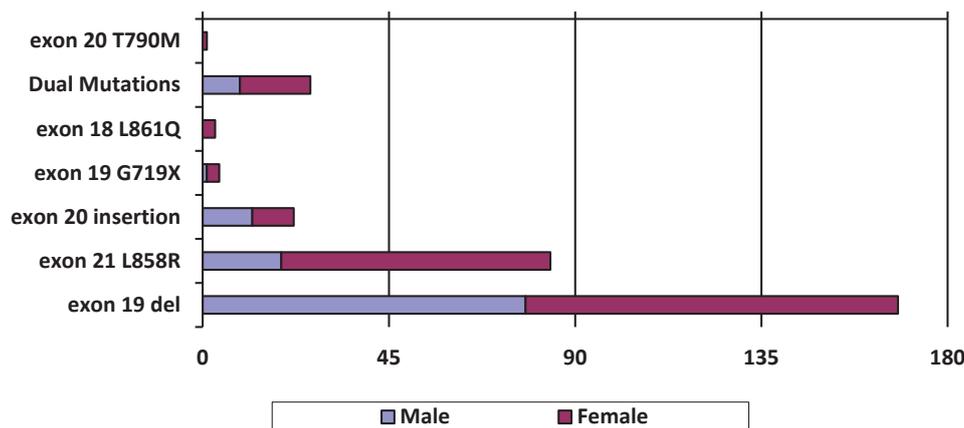


Figure 2. EGFR mutation status with respect to gender.

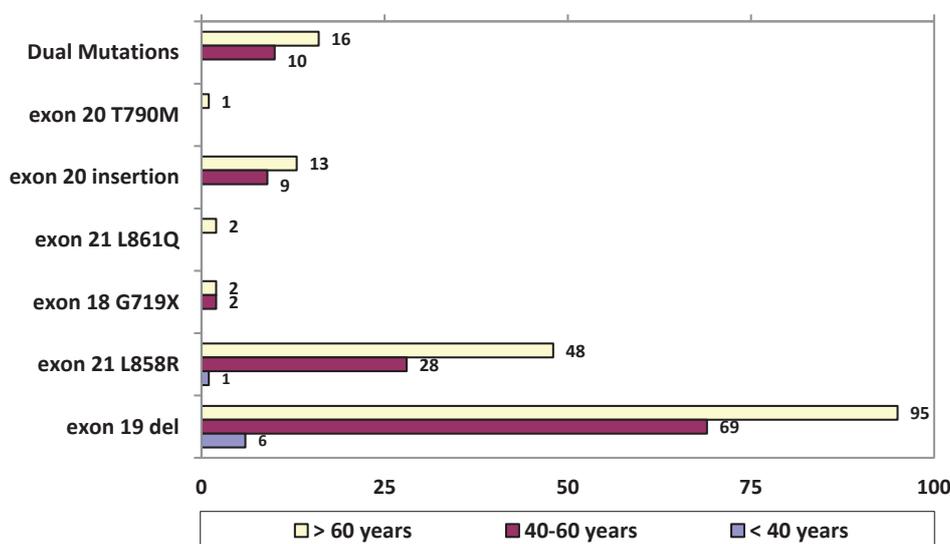


Figure 3. Specific EGFR mutation with respect to age group.

mutations might be more sensitive than with exon 21 L858R. In one study by Jiang-Yong Yu et. al, of the 453 patients with mutations in Exon 19 del and Exon 21 L858R, the response rate of TKIs in patients with Exon 19 del was significantly higher than that with exon 21 L858R mutations (55.2% vs. 43.7%).¹³ For rare genotypes, recent studies showed that these could be targetable if appropriate TKI are selected. For example, mutations with G719X, Del18, E709K, insertions in exon 19, S768I or L861Q

have moderate sensitivities to gefitinib or erlotinib with response rate of 30%– 50%.⁹ In up to 60%–80% of patients treated with TKIs, the tumor regresses dramatically, but after a median time of 9-12 months, all patients develop acquired resistance to the targeted therapy. A secondary mutation, *T790M*, in the exon 20 of the EGFR gene is the most frequent cause of acquired resistance, which is found in 50%–60% of relapsed cases. Among other causes of acquired resistance are: target-independent mechanisms such as MET amplification (4%), Human EGFR type 2 (*HER2*) amplification (8%–13%), *PIK3CA* mutation (2%), *BRAF* mutation (1%), histological transformation from NSCLC to SCLC (6%), or epithelial–mesenchymal transition (1%-2%). Unknown mechanism of acquired resistance is noted in 18% of cases.¹⁴⁻¹⁶

Table 4. EGFR (Exon 18-21) mutation types			
	Female N=186	Male N= 121	Significance
Exon 19 deletion	90	78	0.0069
Exon 21 L858R	65	19	0.0001
Exon 20 insertion	10	12	
Exon 18 G719X	1	3	
Exon 21 L861Q	2	0	
Exon 20 T790M	1	0	
Dual mutations			
Exon 19 deletion + Exon 20 insertion	5	2	
Exon 19 deletion + Exon 20 T790M	2	0	
Exon 19 deletion + Exon 18 G719X	2	0	
Exon 19 deletion + Exon 21 L858R	1	1	
Exon 19 deletion + Exon 21 L861Q	2	0	
Exon 21 L858R + Exon 20 insertion	3	5	
Exon 21 L858R + Exon T790M	2	0	
Exon 21 L858R + Exon 20 S768I	0	1	

In the Philippines, the incidence of EGFR mutations is sparsely explored. In the PIONEER study, the authors have demonstrated that approximately half (51.4%) of the patients with NSCLC from seven regions of Asia harbored EGFR mutations. As with regards to Asian regions, Vietnam (64.2%) has the highest incidence while India has the lowest incidence (22.2%). Frequency of EGFR mutations was significantly higher among women (61.1%) than men (44%) (Table 5).^{17,18} In previous study from the Philippines, the EGFR mutation rate was also reported to be 42%.¹⁹

Table 5. Prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER)(adapted¹⁷)

	Number of Patients	EGFR Mutation Rates
Country/Region		
China	741	372 (50.2%)
Vietnam	120	77 (64.2%)
Taiwan	174	108 (62.1%)
Philippines	65	34 (52.3%)
Thailand	117	63 (53.8%)
Hong Kong	161	76 (47.2%)
India	72	16 (22.2%)
Gender		
Male	822	362 (44%)
Female	626	384 (61.1%)

In this study, we report the largest EGFR mutation profiling data among Filipino patients with NSCLC from a tertiary private hospital, which showed an overall 49.4% EGFR mutation, mostly were adenocarcinoma. This prevalence is similar to those reported in Asia.¹⁷ Consistent with the literature, we found a significantly higher incidence of EGFR mutation among women than men (62% vs 39.8%). Among patients with squamous cell lung carcinomas, there have been few studies within East Asians with conflicting results: two study from China reported EGFR mutation rate of 21% (3/14) and 13.3% (4/30) compared to a Korean based study with a mutation rate of 7.3% (3/41).²⁰⁻²² We found EGFR mutation rate of 35% (9/26) in small cohort of 26 patients with squamous cell carcinoma. In which 4 of these cases were confirmed by immunohistochemical staining as histologically squamous cell carcinoma. Of these positive specimens, 6 came from FNAB/CT-guided biopsy of lung, 2 from bronchial biopsy and 1 lobectomy specimen. We cannot entirely rule out an adenocarcinoma component of Adenosquamous carcinoma in the cases where no immunohistochemical staining was performed. Nevertheless, rare occurrence of EGFR mutation among cases diagnosed as SQCC histologically warrants inclusion of these cases for EGFR mutation screening in routine practice.

With regards to individual mutation types, the most common mutation in these studies detected was deletion in exon 19 (54.7%) followed by L858R point mutation in exon 21 (27.4%), similar to that described in IPASS study. The IPASS study showed 53.6% had exon 19 deletions and 42.5% had a mutation at exon 21 (L858R).²³ The Pioneer study showed the following mutation rates: 24.6% for exon 19 deletion and 22.8% for L858R point mutation.

We found dual mutations in exons 19, 20 and 21 in 8.4% of the cases. In the Pioneer study, dual mutations were also seen; exon 19 deletion in combination with others comprising 24.3% [352 of 1450] and L858R point mutation in exon 21 alone in combination with others were 22.9% [332 of 1450].¹⁷ But in the IPASS study, patients that were dually mutated were 4.2% (11/261).²³ The variability of the incidence of dual mutations identified in these studies may be secondary to different diagnostic sensitivities of the different molecular platforms utilized.

Major limitations of this study are the following: correlation of EGFR mutation patterns with other clinical characteristics (e.g. smoking history, patient’s family history, grading and staging of NSCLC), complete information on targeted treatment received, response to targeted therapy and overall survival data in the Filipino population. Since the cases were collected from 2015-2016, survival data of these patients were far from maturity.

In summary, western population shows a mutation rate of 20%^{11,12} and Asian population show a heterogeneous mutation rate of

22–64%.¹⁷⁻¹⁹ EGFR mutation rate of 49.4% among Filipino population in our cohort is similar that of East Asian patients. EGFR mutations were significantly higher in females than males. Although EGFR mutation is common among younger patients, interestingly, we found mutation rate among females to be higher among older individuals (>60 years). This study warrants validation in a larger prospective study.

CONCLUSION

The incidence of EGFR mutations in NSCLC varies across different ethnicity. In previous reports, the frequency of EGFR mutations among the Asian population are approximately 30% (with a range of 22.2% to 64.2%) compared with the western population (20%). In the Philippines, the incidence of EGFR mutations is sparsely explored. Here we report the largest EGFR mutation profiling data among Filipinos with NSCLC in a private tertiary care setting, with a frequency of 49.4%. This prevalence is almost similar to those reported in Asia. EGFR is differentially mutated among NSCLC patients with different gender, as women have significantly higher incidence than men. With regards to individual mutation types, the most common mutations detected were deletion in exon 19 followed by L858R point mutation in Exon 21. Also, rare occurrence of EGFR mutation among cases diagnosed as squamous cell carcinoma histologically warrants inclusion of these cases for EGFR mutation screening.

Hence, this study establishes relevance of routine EGFR mutation diagnostics for NSCLC patients in the clinical setting and emphasizes effectiveness for adoption of EGFR inhibitors as a treatment among Filipino population. Further studies to correlate EGFR mutation patterns with other clinical characteristics (patient’s family history, smoking history, grading and staging of NSCLC), response to targeted therapy and overall survival in the Filipino population is warranted.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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