sciendo ASIAN BIOMEDICINE DOI 10.1515/abm-2020-0011 — Asian Biomed (Res Rev News) 2020; 14(2):75–81

Brief communication (original) **Open access**

Epidermal growth factor receptor variants in patients from Myanmar with lung adenocarcinoma

Khine San Yin*, Myint Myint Naing, Nitar Khine, May Phyo Maung, Aye Aye Nyunt, Nway Nway Htike, Hnin Hnin Aye, Aye Aye Myint

Abstract

Background: Epidermal growth factor receptor (*EGFR*) sequence variants in patients from Myanmar have not yet been reported.

Objectives: To describe the molecular epidemiology of *EGFR* variants in patients from Myanmar with lung adenocarcinoma.

Methods: Histological diagnosis and categorization of biopsies collected from 66 patients (28–78 years) with lung cancer was conducted using a panel of antibodies including those to: TTF1, P40, synaptophysin, CK7, and napsin-A. Samples from patients with confirmed adenocarcinoma were tested for *EGFR* variants using a cobas EGFR Mutation Test kit and cobas z 480 System (Roche). We conducted a univariate analysis of categorical factors using a χ^2 or Fisher exact test.

Results: Histological types were adenocarcinoma (61%, 40/66), squamous cell carcinoma (24%, 16/66), neuroendocrine carcinoma (9%, 6/66), undifferentiated carcinoma (2%, 1/66), adenosquamous carcinoma (2%, 1/66), small cell anaplastic carcinoma (2%, 1/66), and pleomorphic sarcoma (2%, 1/66). *EGFR* variants were detected in 15 of 40 (38%) cases of adenocarcinoma. Among them, 6 patients (40%) had an exon 19 deletion, another 6 (40%) had exon 21 substitutions, 1 (7%) had exon 20 insertion S768I, and 2 (13%) had compound variations (1 of exon 21 L858R and exon 18 G719X, and 1 of exon 20 S768I and exon 18 G719X). Although limited by small sample size, no significant association was found between the variants and factors including family cancer history, age group, sex, ethnicity, or occupation. However, there was a strong significant association between never-smokers and *EGFR* variants ($P = 0.008$).

Conclusion: Knowledge of *EGFR* variants in patients from Myanmar is encouraging for their effective cancer treatment.

Keywords: adenocarcinoma of lung; EGFR protein, human; ErbB receptors; Myanmar; carcinoma, non-small-cell lung

Cancer is a leading cause of death worldwide, accounting for an estimated 9.6 million deaths in 2018. Lung cancer is one of the most common solid tumors and a leading cause of cancer-related deaths worldwide. Annually, approximately 2.09 million patients are diagnosed with lung cancer of whom 1.76 million die of lung cancer [1]. In Myanmar, where lung cancer is the leading cause of cancer death, the mortality rate of lung cancer is 16.6% in men and boys and 13.8% in women and girls [2].

Adenocarcinoma is one of the most common histological subtypes of non-small-cell lung carcinoma (NSCLC). Molecular profiling of tumor samples from patients with NSCLC

***Correspondence to: Khine San Yin,** Innovative Diagnostics Laboratory, Victoria Hospital, No. 68, Tawin Road, 9 Mile, Mayangone Township, Yangon 11062, Myanmar, e-mail: prof.khinesanyin@innovativediagnosticsmyanmar.com
Innovative Diagnostics Laboratory, Victoria Hospital, No. 68, Tawin Road, 9 Mile, Mayangone Township, Yangon 11062, Myanmar

Open Access. © 2020 Yin et al., published by Sciendo. BY-NC-ND This work is licensed under the Creative Commons Attribution NonCommercial-NoDerivatives 4.0 License.

has identified driver variants that may contribute to early carcinogenesis in >80% of cases of adenocarcinoma, including epidermal growth factor receptor (*EGFR*) sequence variants, which are now considered to be commonly associated with NSCLC tumors. Knowledge of *EGFR* variant status improves predictions of the behavior of lung cancer with adenocarcinoma histology when compared with the use of other prognostic factors, such as TNM staging [3]. A large proportion of patients with NSCLC have variation in exon 19 or 21, which occur in approximately 45% and 40% of patients, respectively, and activate the tyrosine kinase domain in EGFR [4].

Targeted EGFR-tyrosine kinase inhibitor (TKI) first-line treatment for sensitizing *EGFR* variants results in longer progression-free survival, improved health-related quality of life, and decreased treatment-related severe side effects when compared with those who receive standard chemotherapy [5, 6]. Therefore, clinical guidelines recommend that all patients with sensitizing *EGFR* variants receive first-line treatment with such drugs [7].

Adenocarcinoma histology, female sex, never-smoking status, and Asian ethnicity have been considered the most important factors associated with *EGFR* variants in NSCLC and response to EGFR inhibitors.

The prevalence of *EGFR* variants has been studied in many countries including our neighboring countries, but to our knowledge *EGFR* variants has never been studied in Myanmar. Because lung cancer is at the top of the list of malignancies in Myanmar, it is essential to investigate facilitators in the treatment of this debilitating malignancy. Since 2013, Innovative Diagnostics at Victoria Hospital has been the first laboratory in Myanmar to test for *EGFR* variants using real-time polymerase chain reaction (RT-PCR) analysis in patients with adenocarcinoma of the lung.

The aim of the present study was to determine the *EGFR* variant status in Myanmar patients with adenocarcinoma of the lung. The specific objectives were to categorize the histological types of carcinoma lungs using a panel of antibodies, such as those against thyroid transcription factor-1 (TTF1), P40, synaptophysin, napsin A, and cytokeratin-7 (CK7), to determine the frequency and types of *EGFR* variants in confirmed cases of adenocarcinoma by RT-PCR, to determine the *EGFR* variants and types in both sexes, and to identify any association of family cancer history, age, sex, ethnicity, occupation, and smoking habit with EGFR mutation status.

It is hoped that this study will fill the missing gap in knowledge of such patients to enable those with adenocarcinoma of the lung to benefit from EGFR-TKI therapy, which significantly prolongs the survival of patients. Therefore, it is of great importance to determine the prevalence and frequency of *EGFR* variants. To date, Innovative Diagnostics is the only center providing a service for molecular testing of *EGFR* variants in Myanmar. Hence all the variant tests for lung adenocarcinoma are referred to the Innovative Diagnostics laboratory. Therefore, it is considered that the results obtained will reflect the situation of *EGFR* variant frequency in patients from Myanmar.

Methods

Study design

All biopsy specimens from various hospitals and clinics were received at the Innovative Diagnostics laboratory, Victoria Hospital in 10% neutral-buffered formalin (NBF). Tissue was processed automatically to yield hematoxylin and eosin (HE) sections. Various histological types of lung carcinoma were categorized according to the morphology on the HE sections. After the present study was approved by the Ethics Review Committee on Medical Research Involving Human Subjects, Department of Medical Research, Ministry of Health and Sports, The Government of the Republic of the Union of Myanmar (approval No. Ethics/DMR/2018/051), the investigators randomly selected cases of carcinoma within the study period and contacted the patients or their family members. After describing the research protocol and project, we obtained 66 participants during a 6-month period from March 2018 to August 2018. Informed consent was documented on the consent forms provided, and the proforma were filled. The study was conducted in accordance with the principles of the contemporary revision of the Declaration of Helsinki. Victoria Hospital provided permission to conduct this research. Here we report results using the STrengthening the REporting of Genetic Association Studies (STREGA) reporting guidelines [8].

A panel of monoclonal antibodies and special stains was used as required in all 66 cases of lung cancer. The panel of the antibodies included those against TTF1, P40, synaptophysin, napsin A, and CK7. Of 66 patients with lung cancer, 40 were established as having primary adenocarcinoma of the lung. The patients with confirmed cases of adenocarcinoma proceeded to molecular testing for *EGFR* variant analysis.

EGFR variant analysis

The cobas EGFR Mutation Test is a real-time PCR test for the qualitative detection and identification of variants in exons 18, 19, 20, and 21 of the gene for EGFR in DNA derived from tumor tissue. The cobas EGFR Mutation Test consists of manual specimen preparation using the cobas DNA Sample

Preparation Kit followed by amplification/detection on the cobas z 480 analyzer using a cobas EGFR Mutation Test kit. A single run could include from 1 to 30 specimens and 2 controls per 96 microwell plate. The turnaround time for one test run is about 8 h. The system can detect 42 variants.

In the manual sample preparation, initially unmounted 5 μm sections of formalin-fixed paraffin-embedded tissue (FFPET) was deparaffinized using xylene and ethanol. DNA was prepared from the deparaffinized tissue in microcentrifuge tubes by adding DNA tissue lysis buffer and proteinase K, with one extra tube prepared as a negative control. DNA was then isolated with protein binding buffers, isopropanol, wash buffers, and elution buffer. The concentration of DNA in the isolated stock solution was quantified using a Nanodrop 2000 spectrophotometer and an average of 2 consistent readings was calculated. The minimum DNA stock concentration required was 2 ng/μL. If the DNA stock concentration is exactly 2 ng/μL, it can be used without dilution. For concentrations of 2–36 ng/μL and >36 ng/μL, the stock was diluted with DNA Specimen Diluent by calculating with the equations provided. Consequently, working EGFR master mixes (MMX-1–3), mutant control, and negative control were prepared. Next, the controls and the DNA specimen diluents were transferred into a defined microwell plate to which the working MMX-1–3 had already been added in the areas of the plate specified by Roche Diagnostics. Finally, the plates were covered and sealed with sealing foil from the kit and PCR was conducted using a cobas z 480 real-time PCR analyzer (Roche Diagnostics). The results were generated automatically by the cobas system [9].

Statistical analysis

IBM SPSS Statistics for Windows (version 25.0) was used for all analyses. The absolute and relative frequencies of the quantitative variables were calculated as percentages. We conducted a univariate analysis of categorical factors using a χ^2 test or Fisher exact test as appropriate, with no correction for multiple comparisons. $P < 0.05$ was considered significant in the two-tailed tests.

Results

Histological types of lung carcinomas

The histological types of lung carcinomas were categorized by using a panel of antibodies. Of 66 cases of lung carcinoma, there were 40 cases (61%) of adenocarcinoma. In order of frequency, this was followed by squamous cell carcinoma 16 cases (24%), neuroendocrine carcinomas 6 cases (9%), undifferentiated carcinoma 1 case (2%), adenosquamous carcinoma 1 case (2%), small-cell anaplastic carcinoma 1 case (2%), and pleomorphic sarcoma 1 case (2%).

Using a panel of antibodies, the adenocarcinoma cases showed 85% immunoreactivity with TTF1, 87% with napsin A, and 95% with CK7 (**Figure 1**).

Frequency and types of *EGFR* variants in patients with adenocarcinoma

Of the 40 cases of confirmed adenocarcinoma of the lung, *EGFR* variation was detected in 15 (38%), but not in 25 (63%). Among the cases detected, 6 (40%) had exon 19 deletion, 6 (40%) had exon 21 substitution, 1 (7%) had exon 20 insertion, and 2 (13%) had compound variations (1 case of exon 21 L858R and exon 18 G719X, and 1 case of exon 20 S768I and exon 18 G719X).

EGFR variant types by sex

Among the 15 cases of *EGFR* variants, 5 (26%) patients were male and 10 (48%) were female. No significant association was seen between *EGFR* variants and sex ($P = 0.17$) Nor was there any significant association between types of *EGFR* variant and sex ($P = 0.69$) (**Figure 2**).

Relationship between factors and *EGFR* variant status

All the cases of variants were found in patients with Bamar ethnicity. The most distinctive finding was seen in relation to the smoking history. Of 15 patients with a variant, 10 were never-smokers and a significant association was found ($P = 0.008$; **Table 1**).

Discussion

Of the 40 cases of adenocarcinoma of the lung, *EGFR* sequence variants were detected in 15 accounting for 38%. To date, *EGFR* variant status has been determined in 151 studies worldwide. There is substantial variation between studies even when grouped by geographical region. The Asia-Pacific NSCLC/adenocarcinoma subgroup had the highest frequency of *EGFR* variation (47%) and the lowest occurred in the Oceania NSCLC/ adenocarcinoma subgroup (12%). Among

ASIAN BIOMEDICINE

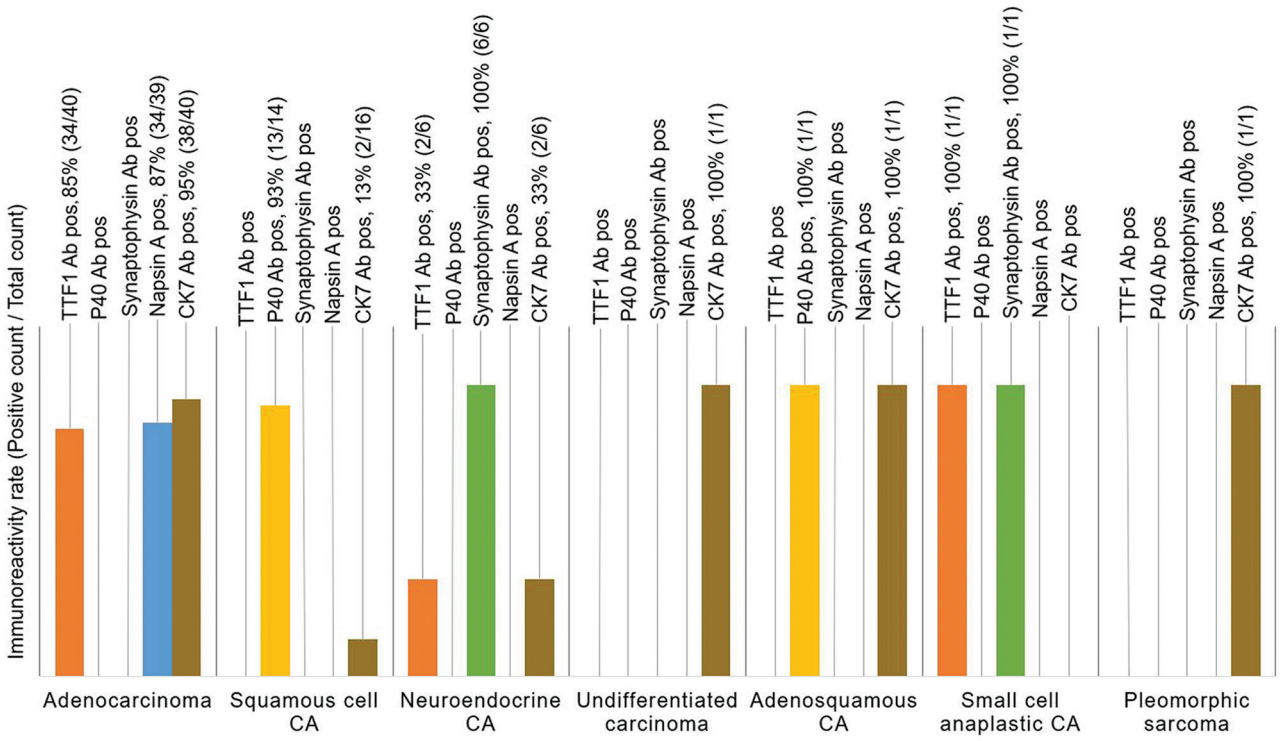

Figure 1. Immunoreactivity for a panel of antibodies in various lung carcinoma. The panel included antibodies to thyroid transcription factor-1 (TTF1; orange), P40 (yellow), synaptophysin (green), napsin A (blue), and cytokeratin-7 (CK7; brown). Ab, antibody; CA, carcinoma.

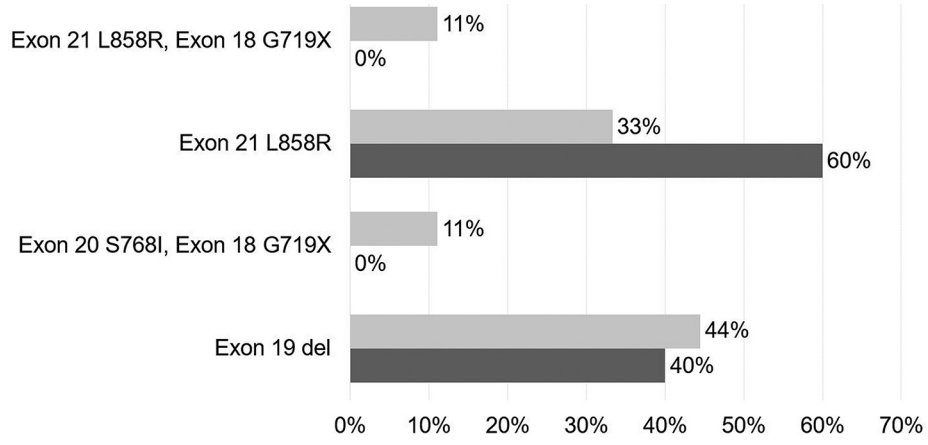

Figure 2. Frequency of epidermal growth factor receptor sequence variation types by sex in 40 cases of confirmed adenocarcinoma of the lung. Female patients (light gray) male patients (dark gray).

our neighboring countries, *EGFR* variant frequency was found to be 48% in China, 45% in Japan, 54% in Thailand, 64% in Vietnam, 52% in Philippines, and 45% in Malaysia [10]. The highest frequency was seen in Vietnam (64%) and lowest in Singapore (40%). The overall frequency was 47%. These figures in the Asia-Pacific countries appear consistent with the frequency detected in our patients from Myanmar. The high frequency in these Asia-Pacific countries highlights the need for EGFR testing in all patients with NSCLC/ adenocarcinoma. In Europe, the frequency is much lower, e.g., 15% in France, 12% in the UK, and 15% overall. In North America, the frequency is 22%. The frequency in the Indian subcontinent (26%) was also found to be lower than it was in Myanmar. In Africa, the frequency is 21% [10].

Table 1. Association between *EGFR* variation and clinical features

Characteristic		*EGFR* variation		
	Total	Detected (%)	Not detected (%)	*P*
Family cancer history				
Present	7	2 (29%)	5 (71%)	0.69
Absent	33	13 (39%)	20 (61%)	
Age group				
<40 years	1	1 (100%)	0 (0%)	0.35
40–59 years	11	5 (45%)	6 (55%)	
≥60 years	28	9 (32%)	19 (68%)	
Sex				
Male	19	5 (26%)	14 (74%)	0.17
Female	21	10 (48%)	11 (52%)	
Ethnicity				
Bamar	38	15 (39%)	23 (61%)	0.52
Kayin	2	0 (0.0%)	2 (100%)	
Occupation				
Unemployed	18	9 (50%)	9 (50%)	0.60
Service and sale workers	7	1 (14%)	6 (86%)	
Officers/managers	5	1 (20%)	4 (80%)	
Professionals	3	1 (33%)	2 (67%)	
Armed forces occupations	3	2 (67%)	1 (33%)	
Technicians	1	0 (0%)	1 (100%)	
Transporters	2	1 (50%)	1 (50%)	
Agricultural, forestry, and fishery workers	1	0 (0%)	1 (100%)	
Smoking habit				
Regular smokers	4	0 (0%)	4 (100%)	0.008*
Ex-smokers	21	5 (24%)	16 (76%)	
Never-smokers	15	10 (67%)	5 (33%)	
Chemical/dust exposure				
Exposure present	9	1 (11%)	8 (89%)	0.12
No exposure	31	14 (45%)	17 (55%)	

**P* < 0.05. *EGFR*, gene for epidermal growth factor receptor.

The most frequent variant types found in this study were exon 19 deletion and exon 21 L858 R substitution each accounting for 6 cases (40%). This finding was similar to that in a study in mainland China that found the most common variants to be exon 19 deletion and L858R substitutions [11]. In that study, *EGFR* variant frequency was 50%. A similar finding was reported by Li et al. in which 71% of patients had exon 19 deletions and 29% had exon L858R substitutions [12]. In Japan, Kobayashi et al. found exon 19 deletion (43%) and L858R substitutions (30.5%) as the most prevalent variant types [13]. These 2 variants account for about 90% of all *EGFR* variants in NSCLC. Screening for these variants in patients with NSCLC can be used to predict which patients will respond to EGFR-TKIs. Different types of *EGFR* variants may lead to different levels of tyrosine activation and associate with different oncogenic potential. Common *EGFR* variants that are found at high frequencies such as L858R substitution and exon deletions, as in our study, might be more oncogenic and thus highly selected for therapy [14].

One case of exon 20 S7681 insertion was found in our study. There were also cases of double variation, i.e., 1 case of exon 21 L858R and exon 18 G719X, and 1 case of exon 20 S7681 and exon 18 G719X. Both cases of double variants in our study were in female patients. In a study in China, double variants were also found to be more common in female patients [15]. The oncogenic potential of the less common variants and double variants is largely unknown, and there is a suggestion that these types confer resistance to therapy such as gefitinib [14].

In our patients with *EGFR* variants, 5 (26%) were male and 10 (48%) were female. The difference was not statistically significant, but, in the present study, variants were more common in female than male patients. This finding is consistent with the literature reports of high *EGFR* variant frequency in Asian women. In Japan, *EGFR* variants were more frequently observed in female patients (76.3%) [16]. In the regions where data were available, *EGFR* variant frequency in NSCLC/adenocarcinoma is higher in women than men: Europe 22% vs. 9%; Asia-Pacific 60% vs. 37%; Indian subcontinent 31% vs. 23%; Africa 48% vs. 8%; and North America 28% vs. 19%. Only in Bangladesh, is the frequency higher in men (26% vs. 14%) [10].

EGFR variants were most frequently seen in those aged ≤40 years, but we found no significant association with age. Cancer of the lung in general occurs most often between ages 40 and 70 years, with a peak incidence in people in their 50s or 60s. Only 2% of all cases appear before the age of 40 years. It is noteworthy that *EGFR* variants tend to occur around this younger age.

There were altogether 38 patients from the Bamar ethnic group with cases of adenocarcinoma and *EGFR* variants were seen in 15 (39%). There were 2 patients with Kayin ethnicity and they showed no *EGFR* variants.

There was a higher frequency of *EGFR* variants in never-smokers and ex-smokers, and *EGFR* variants were not detected in regular smokers from our group. This is consistent with a systemic review [10], which found that *EGFR* variant frequency is higher in never-smokers compared with ever-smokers: Europe 35% vs. 8%; Asia-Pacific 64% vs. 33%; Indian subcontinent 32% vs. 17%; Africa 41% vs. 6%; and

North America 28% vs. 19%. However, in Bangladesh and Taiwan, *EGFR* variation was slightly higher in ever smokers compared with nonsmokers (24% vs. 22% in Bangladesh and 57% vs. 53% in Taiwan). There was also no significant association between nonexposure to chemicals or dust and *EGFR* variants. In the present study, *EGFR* variants were detected in 14 of 31 patients with adenocarcinoma of the lung (45%) who were not exposed to chemicals.

Limitations of our study include the limitations of the EGFR Mutation Test [9] and the small sample size compared with other studies. The logical validity of χ^2 tests is limited by sample size. Nevertheless, we were able to obtain sufficient data to present a preliminary study of *EGFR* variant status in patients from Myanmar.

Conclusions

Adenocarcinoma dominates the histological types of lung cancer of the patients from Myanmar whom we tested. *EGFR* sequence variants were detected in 38% cases, and among them 80% had exon 19 or 21 variants, 7% had an exon 20 insertion, and 13% had compound variants. There were more female than male patients with *EGFR* variants, but we found no significant association in the small sample size included in the present study. A significant association was found between never-smokers and *EGFR* variants. Knowledge of *EGFR* variant status in patients from Myanmar is encouraging for their effective cancer treatment. Further studies are warranted to support clinicians to reduce the mortality and morbidity of patients with lung cancer that remains a major health concern in Myanmar.

Author contributions. KSY, MMN, and NK contributed substantially to the conception and design of the study. NK, MPM, AAN, and NNH contributed substantially to the acquisition of data and KSY, MMN, MPM, AAN, HHA, and AAM contributed substantially to its analysis and interpretation. All authors contributed to drafting the manuscript and KSY, MMN, HHA, and AAM critically revised it. All authors have approved the final version and take responsibility for the statements made in the published article.

Acknowledgments. We thank our surgeon colleagues for their interest and collaboration in our study. We thank Miss May Soe Thu, our health data scientist for her invaluable help with data analysis, Dr. Thi Thi Myint for her help with data collection, Dr. Chan Nyein Maung, Deputy Regional Public Health Director, Mandalay Regional Public Health Department, Department of Public Health, Ministry of Health and Sports, Myanmar for the review of statistical analysis. We are grateful to The Government of the Republic of the Union of Myanmar, Department of Medical Research (DMR), External Grant Committee for the research grant [DMR external grant serial number 4/2017] that funded this study. We thank Roche Myanmar for their continuous support to Innovative Diagnostics whenever required and Victoria Hospital for their assistance and permission to conduct this research.

Conflicts of interest statement. All authors are employees of Innovative Diagnostics, a private company and diagnostic laboratory at Victoria Hospital, a private hospital in the Myanmar healthcare system. The authors declare that they had full access to all of the data in this study and take complete responsibility for the integrity of the data and the accuracy of the data analysis. The authors have each completed and submitted an International Committee of Medical Journal Editors Uniform Form for Disclosure of Potential Conflicts of Interest. None of the authors has any potential conflict of interest to disclose in relation to the present article.

Data sharing statement. Any data produced in this study will be shared by the authors on reasonable request after deidentification of data from any individual patient. The present study has been registered at the U.S. National Library of Medicine National Center for Biotechnology Information BioProject Accession No. PRJNA612731. Available from *https://www.ncbi.nlm.nih.gov/bioproject/612731*

References

[1] World Health Organization. Cancer; 2018 [cited 2019 Aug 07]; Available from: http://www.who.int/news-room/fact-sheets/detail/cancer

[2] World Health Organization. Cancer country profiles 2014. [cited 2019 Aug 07]; Available from: http://www.who.int/cancer/country-profiles/en/

[3] Fang S, Wang Z. EGFR mutations as a prognostic and predictive marker in non-small-cell lung cancer. Drug Des Dev Ther. 2014; 8:1595–611.

[4] Sgambato A, Casaluce F, Maione P, Rossi A, Rossi E, Napolitano A, et al. The role of EGFR tyrosine kinase inhibitors in the first-line treatment of advanced non small cell lung cancer patients harboring EGFR mutation. Curr Med Chem, 2012; 19:3337–52.

[5] Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al.; North-East Japan Study Group. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med. 2010; 362:2380–8.

[6] Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al.; West Japan Oncology Group. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor

(WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol. 2010; 11:121–8.

[7] Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol. 2013; 31:3327–34.

[8] Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, von Elm E, et al. STrengthening the REporting of Genetic Association Studies (STREGA)—an extension of the STROBE statement. Genet Epidemiol. 2009; 33:581–98.

[9] Roche Molecular Systems. cobas EGFR Mutation Test (v1); 2013 April [cited 2019 Aug 07]. Available from: https://www.accessdata.fda.gov/cdrh_docs/pdf12/P120019c.pdf

[10] Midha A, Dearden S, McCormack R. EGFR mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: a systematic review and global map by ethnicity (mutMapII). Am J Cancer Res. 2015; 5:2892–911.

[11] Shi Y, Li J, Zhang S, Wang M, Yang S, Li N, et al. Molecular epidemiology of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology – mainland China subset analysis of the PIONEER study. PLoS One. 2015; 10:e0143515. doi: 10.1371/journal.pone.0143515

[12] Li AR, Chitale D, Riely GJ, Pao W, Miller VA, Zakowski MF, et al. EGFR mutations in lung adenocarcinomas: clinical testing experience and relationship to EGFR gene copy number and immunohistochemical expression. J Mol Diagn. 2008; 10:242–8.

[13] Kobayashi S, Canepa HM, Bailey AS, Nakayama S, Yamaguchi N, Goldstein MA, et al. Compound EGFR mutations and response to EGFR tyrosine kinase inhibitors. J Thorac Oncol. 2013; 8:45–51.

[14] Tam IY, Leung EL, Tin VP, Chua DT, Sihoe AD, Cheng LC, et al. Double EGFR mutants containing rare EGFR mutant types show reduced in vitro response to gefitinib compared with common activating missense mutations. Mol Cancer Ther. 2009; 8:2142–51.

[15] Wei Z, An T, Wang Z, Chen K, Bai H, Zhu G, et al. Patients harboring epidermal growth factor receptor (EGFR) double mutations had a lower objective response rate than those with a single mutation in non-small cell lung cancer when treated with EGFR-tyrosine kinase inhibitors. Thorac Cancer. 2014; 5:126–32.

[16] Sonobe M, Manabe T, Wada H, Tanaka F. Mutations in the epidermal growth factor receptor gene are linked to smoking-independent, lung adenocarcinoma. Br J Cancer. 2005; 93:355–63.