ORIGINAL ARTICLE

A RETROSPECTIVE, MOLECULAR STUDY OF EGFR AND ALK MUTATIONS IN NON SMALL CELL LUNG CANCER PATIENTS

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ABSTRACT

Background: Epidermal growth factor receptor EGFR/ALK mutations are the strongest response predictors to EGFR tyrosine kinase inhibitors (TKI) and ALK inhibitor respectively, but knowledge of the EGFR/ALK mutation frequency on lung adenocarcinoma is still limited.

Methodology: Our study is a retrospective study of the metastatic non small cell lung cancer patients harboring EGFR/ALK receptors. A total of 94 metastatic non small cell lung carcinoma patients data were evaluated, out of which 74 patient's EGFR&ALK mutation status was known.

Results: All of the patient's data evaluated in this study were in the age group of 30-74.Total 74 patient's EGFR &ALK mutation status was known, out of which 34.2% were positive for EGFR and 4% for ALK respectively. In EGFR positive group 62.96%were male and 37.04% were female. Among males patients 34% were positive for EGFR as compared to 42% of EGFR Positive female patients.

Conclusion: Efforts to obtain tissue samples should be encouraged for *EGFR*&ALK mutation testing in non small cell lung carcinoma patients to provide a molecular basis to treat patients with available targeted therapy.

Keywords: EGFR, ALK, Non Small Cell Lung Cancer

INTRODUCTION

Lung cancer is the most common cancer and cause of cancer related deaths all over the world. The lung cancer represents 13 percent cases of all new cancer cases and 19 percent of cancer related deaths worldwide. In 2012, 1.8 million new lung cancer cases were detected[1].Lung cancer constitutes 6.9 per cent of all new cancer cases and 9.3 per cent of all cancer related deaths in India. It is the most common cancer and cause of cancer related mortality in men.[2] Our understanding of disease biology has evolved over the years. The histological classification is now stretching to molecular classification. Newer molecular targets and driver mutations which play a major role in the pathogenesis have been identified.[3] Metastatic non-small cell lung cancer (NSCLC) patient's treatment historically consisted of systemic combination chemotherapy. Chemotherapy generally kills cells that are growing or dividing; it causes symptomatic improvement, improves quality of life, and improves survival in some patients with NSCLC.[4]

An improved understanding of the molecular pathways of lung is very essential, knowledge molecular pathway has led to the development of agents that target specific molecular pathways in malignant cells at the same time sparing of normal cells, like mutations in the epidermal growth factor receptor (EGFR) or rearrangements of the anaplastic lymphoma kinase (ALK) gene.[5] There are many targeted therapies available in lung cancer against these mutations, most of are administered as orally-available small molecule kinase inhibitors.[6] The identification of these mutation positive patient has led to an ongoing effort to identify biomarkers and treatments that can be used for other subsets of patients with advanced NSCLC. In NSCLC, and in other malignancies, Identification of driver mutation and treatment with targeted therapy specific to that for an individual patient has resulted in significantly improved therapeutic efficacy, often in conjunction with decreased toxicity.[7]

We have done a retrospective record based study of EGFR/ALK mutation status in lung cancer patients.

The primary objective of the study was to assess the overall *EGFR /ALK* mutation frequency. Secondary objectives were to investigate the correlation between *EGFR /ALK* mutation status and demographic and clinical factors**.**

METHODOLOGY

Study design-We Retrospectively evaluated the data of metastatic non small cell lung carcinoma patients

coming to Sri Aurobindo Institute of Medical Sciences(SAIMS) Indore . Study duration was four months from January 2016 to April 2016

All the medical records of patients coming between July 2013 to March 2016 were evaluated for EGFR and ALK mutation status as well as demographic and clinical characteristics of the patients in respect to age , sex, socioeconomic status ,metastatic lesions and smoking history. Patients with unknown receptor status and with incomplete information were excluded from the study.Paraffin embedded tissue blocks obtained by biopsies or surgically resected specimens, from primary tumors as well as from metastatic sites, were analysed for mutation analysis. Genomic deoxyribonucleic acid was extracted and exons 18-21 of EGFR gene were amplified by polymerase chain reaction (PCR).The amplified PCR product was subjected to the direct nucleotide sequencing for the detection of mutations. ALK mutation analysis was done by Fluorescence in situ hybridization (FISH).

RESULTS

Our study was a retrospective study from July 2013 to March2016. Total 94 patients were registered out of which 74 patients were evaluated for EGFR/ALK mutation analysis.Overall demography/clinical characteristics for the Patient population are summarized in **Table 1**.

Table 1: Key Demographic and Clinical Characteristics

Variable	No. (%)
Age,Median (range)	57(30-74)
Sex %	
Male	50 (68.00)
Female	24 (32.00)
Smoking History	
Smoker	45 (61.00)
Non smoker	29 (39.00)
Metastasis	
Liver	20 (27.00)
Brain	10 (14.00)
Bones	54 (73.00)
Others	34 (46.00)
Exposure while cooking(Females)	
Exposure	10 (42.00)
No Exposure	14 (58.00)
Back ground	
Rural	40 (54.00)
Urban	34 (46.00)

Demographic and clinical data shows that median age was 57 years (Range, 30–74y).out of total patients ,32% patients (24 of74) were female, Total 61% patients had a smoking history, interestingly in female subgroup 42% females had a history of exposure to smoke while cooking food. Background data shows, 58% patients with rural as compared to 42 % of patients with urban background. Most of the patients had bone mets (74%) followed by liver (27%).

Total 34.2% **(Table.2)** patients were positive for EGFR mutation and 4% were positive for ALK mutation. On sub group analysis of EGFR Mutation positive patients, 66% patients were non smoker. In the subgroup of ALK Mutation patients, only 1 patients (33%) was smoker.

Table 2: Stratification of EGFR /ALK Mutation

Variable	EGFR POSIITVE	ALK POSITIVE
Median age (range)- 57(30-74)	34.2%	4%
Sex%		
Male	17(34%)	2(67%)
Female	10(42%)	1 (33%)
Cooking exposure	5(19%)	0
Smoking history		
Smoker	9(33%)	1(33%)
Never smoker	18(67%)	2(67%)
Occupational exposure %	8(30%)	0

DISCUSSION-

Diagnostic work-up for NSCLC includes driver mutation screening, and the information obtained by such diagnostic workup is useful in choosing standard therapy according to the mutation status. Upfront targeted therapies in driver mutation positive NSCLC, whereas conventional chemotherapy in the absence of driver mutation. In a French study of lung cancers by molecular profiling, 50 percent of tumors exhibited a genetic alteration, which led to use of targeted agent as first-line therapy in half of these cases.[8] Advanced NSCLCs containing characteristic mutations in EGFR/ALK are highly sensitive to EGFR /ALK TKIs. Erlotinib has shown better response rates and PFS as compared to conventional chemotherapy for first line treatment in EGFR mutation positive advanced NSCLC.[9-10]

Crizotinib, a tyrosine kinase inhibitor targeting ALK, has shown a response rate of 65 per cent in previously treated patients of NSCLC that harbour ALK rearrangement and has been approved for this indication.[11-12] Multiple reliable techniques are available to assay for EGFR/ALK mutations, and these are feasible on formalin fixed tissue.[13] Mutations in the EGFR tyrosine kinase are observed in approximately 15 percent of NSCLC adenocarcinoma in the United States and occur more frequently in women and nonsmokers. In Asian populations, the incidence of EGFR mutations is substantially higher. In our study the EGFR mutation rate was 34.2%, which is higher compared to EGFR mutation rate in western lung cancer population.In the PIONEER study, which is

an Asian study.[14] The incidence of EGFR mutations ranged from 22 to 62% (51.6%).Although EGFR mutations were more common in nonsmokers, still the incidence was 37 percent in regular smokers. The frequency of such mutations was higher in women than in men. EGFR mutation rate in our study falls in the range of Asian PIONEER study. Among non smokers, 67 percent were positive for EGFR mutation in our study. Whereas 60.7 percent non smoker patients were positive for EGFR mutation in PIONEER study. So majority of the patients were non smoker in the EGFR mutation positive group.

The frequency of EGFR mutation positive rate was higher in females (42%) compared to males (34%),which is also comparable with PIONEER study. Where 61% females were positive for EGFR mutation as compared to 44 % male population in PIONEER study. In female EGFR positive sub group analysis,19 percent females had a history of exposure to smoke while cooking food in rural areas. Occupational exposure was associated with 30 percent of EGFR Positivity. Generally, female sex, adenocarcinoma histology, never-smoking status, and Asian ethnicity are considered the most important factors associated with *EGFR* mutation and response to EGFR-TKIs.[15]

In unselected NSCLC populations, the ALK rearrangement is a relatively rare event. The overall incidence of ALK gene rearrangements in subsequent series has been about 4 percent. Except in rare cases, the presence of ALK gene rearrangements in NSCLC tumors tends to occur independent of epidermal growth factor receptor (EGFR) or KRAS mutations. Similar frequencies of ALK gene rearrangements have been reported in Asian and Western populations.[16]In our study out of 74 patients, only 4% were positive for ALK Mutation. Among ALK Positive patients majority (67%) were males and Non smoker (67%) which is comparable to other studies.Our study is the first study in central India highlighting the *EGFR /ALK* mutation status.

In summary, the observed frequency of tumor *EGFR and ALK* mutation in demographic and clinical subgroups of patients in our study suggests that *EGFR*&ALK mutation testing should be done not only in female and non smoker patients but also in males and smokers, particularly in Asian populations Such an approach should help ensure the optimal identification and treatment of patients whose tumors harbor *EGFR* /ALK mutations.

CONCLUSION

EGFR &ALK mutation testing should be encouraged upfrontly in metastatic non small cell lung cancer patients to provide a molecular basis to treat patients with available targeted therapy.

REFERENCES

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. Lyon, France: International Agency for Research on Cancer; 2013.GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11.
2. Indian Council of Medical Research; 2013. National Cancer Registry Programme. Three Year Report of Population Based Cancer Registries: 2009-2011.
3. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. Lancet Oncol. 2011;12:175–80.
4. G. D'Addario, M. Früh, M. Reck, P. Baumann, W. Klepetko,E. Felip. Metastatic non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up.On behalf of the ESMO Guidelines Working Group*
5. Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors. Ann Oncol (2010) 21 (suppl 5):v116-v119.
6. Míriam Méndez,* A J na Custodio,* and Mariano Provencio. Thorac Dis. 2011 Mar; 3(1): 30–56.New molecular targeted therapies for advanced non-small-cell lung cancer.
7. Barlesi F, Mazieres J, Merlio JP, et al. Routine molecular profiling of patients with advanced non-small-cell lung cancer: results of a 1-year nationwide programme of the French Cooperative Thoracic Intergroup (IFCT). Lancet 2016; 387:1415.
8. Ferlay J, Shin HR, Bray F, *et al* Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893–917.
9. Zhou C, Wu Y-L, Chen G, Feng J, Liu X-Q, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol. 2011;12:735–42.
10. Rosell R, Carcereny E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol.2012;13:239–46.
11. Camidge DR, Bang Y-J, Kwak EL, Iafrate AJ, Varella-Garcia M, Fox SB, et al. Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a phase 1 study.LancetOncol. 2012;13:1011–9.
12. Shaw AT, Kim D-W, Nakagawa K, Seto T, Crinó L, Ahn M-J, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med. 2013;368:2385–94.
13. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. Arch Pathol Lab Med 2013; 137:828.
14. Shi Y, Au JS, Thongprasert S, et al. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). J ThoracOncol 2014; 9:154.
15. Bareschino MA, Schettino C, Rossi A, et al. Treatment of advanced non small cell lung cancer. J Thorac Dis. 2011;3:122–133
16. Solomon B, Varella-Garcia M, Camidge DR. ALK gene rearrangements: a new therapeutic target in a molecularly defined subset of non-small cell lung cancer. J ThoracOncol 2009; 4:1450.