October - November 2017; 6(6): 2796-2799

International Journal of Research and Development in Pharmacy & Life Science
An International open access peer reviewed journal
ISSN (P): 2393-932X, ISSN (E): 2278-0238
Journal homepage: http://ijrdpl.com

Original Article

Epidermal Growth Factor Receptor Mutations detection by Mutant Specific Immunohistochemistry in North Indian Lung Cancer population

Priyanka Gaur [1], Sandeep Bhattacharya [1], Surya Kant [2], RAS Kushwaha [2], Jyotsana Agarwal [3] and Anumesh Kumar Pathak [4]

[1] Department of Physiology, [2] Department of Respiratory Medicine, [3] Department of Microbiology, King George's Medical University, Lucknow, India
[4] Department of Pathology, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, India

Keywords: Lung Cancer, EGFR, Non-small Cell Lung Cancer, Subtype, Diagnosis

Article Information:

Received: August 03, 2017;
Revised: August 27, 2017;
Accepted: September 18, 2017

Available online on:
15.10.2017@http://ijrdpl.com

http://dx.doi.org/10.21276/IJRDPL.2278-0238.2017.6(6).2796-2799

ABSTRACT:
Background: Lung cancer is one of the leading causes of cancer-related deaths due to limited treatment options available for advanced-stage disease. The epidermal growth factor receptor is a trans-membrane glycoprotein having an extracellular epidermal growth factor binding domain and an intracellular tyrosine kinase domain which regulates signaling pathways to control cellular proliferation. **Material and Methods:** Detection of EGFR mutation status of 80 subjects of non-small cell lung cancer of adenocarcinoma subtype had been done by IHC using EGFR mutation specific antibodies. **Results:** 20 cases were positive for Del E746-A750 mutation, 6 for L858R mutation. IHC analysis shows that 30 cases scored 0, 12 cases scored 2+ and 8 cases scored 3+ in Del E746-A750 mutation while 24 cases scored 0, 1 case scored 2+ and 5 cases scored 3+ in L858R mutation. The mean age of mutation positive is 56±9.50 and negative is 53.37±11.83. 65.38% male and 34.61% female were positive for EGFR mutation. The study shows that 8 (30.77%) ex/ current smokers and 18 (69.2%) nonsmokers were positive for EGFR mutations. **Conclusion:** Patients who were positive for EGFR mutation can take gefitinib treatment instead of taking other chemotherapy regimen. This is a rapid method for diagnosis of lung cancer patients and helpful in the treatment decision which improve the burden of the disease.

⇑ Corresponding author at:
Dr. Sandeep Bhattacharya, Department of Physiology, King George's Medical University, Lucknow, India
E-mail address: sbhattachya@gmail.com

INTRODUCTION

Lung cancer is the most common form of cancer worldwide and one of the leading causes of cancer related deaths due to limited treatment options available for advanced-stage disease [1, 2] Non–small cell lung carcinoma (NSCLC) is the major type of lung cancer constituting about 80% of all lung tumors and is classified into adenocarcinoma, squamous cell carcinoma and large cell carcinoma [3, 4]. Non-small cell lung cancer (NSCLC) which comprises of about 75% of lung cancer has been proven difficult to treat due to poorly understood pathological mechanisms. The epidermal growth factor receptor is a trans-membrane glycoprotein having an extracellular epidermal growth factor binding domain and an intracellular tyrosine kinase domain which regulates signaling pathways to control cellular proliferation. The epidermal growth factor receptor binds to its ligand resulting autophosphorylation by intrinsic tyrosine kinase activity and triggers several signaling transduction cascades. Most of the EGFR mutations are found in exons 18 to 21 in the tyrosine kinase domain in which the most common mutation deletions in exon 19 such as delE746-A750 and L858R point mutation in exon 21 [5, 6, 7].

The delE746- A750 mutation in exon 19 and L858R mutation in exon 21 are the most common mutations found in NSCLC accounting for 90% of all EGFR mutations. Immuno-histochemical (IHC) analysis can be used for screening of EGFR mutations, EGFR mutation-specific antibodies are used against EGFR with E746–A750 deletion in exon 19 and L858R point mutation in exon 21.

Since the introduction of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib and its approval for clinical use in the treatment of advanced NSCLC [8], subsequent studies have shown a significant association between the presence of EGFR mutations in lung cancer and their sensitivity to gefitinib and another EGFR tyrosine kinase inhibitor such as erlotinib. Previous studies show that EGFR mutations, especially delE746-A750 and L858R point mutation are closely associated with favorable clinical outcomes in 80% of patients with NSCLC from East Asia [9, 10]. EGFR mutation status is the most valuable indicator for the screening of non-small cell lung cancer patients for tyrosine kinase inhibitor (TKI) therapy. Lung cancer patients are recruiting for the detection of EGFR Mutation This study is also helping in the treatment decision. Since the data from some previous studies indicate that the mutation positive patients have better improvement for tyrosine kinase treatment such as gefitinib instead of other chemotherapy. Hence our study will be helpful for the treatment of lung cancer patients and improving the overall burden of the disease in northern Indian populations.

MATERIALS AND METHODS

Patients and samples: This study is conducted at tertiary care hospital in North India. Tissue samples of lung cancer subjects were collected bronchoscopically for the detection of EGFR mutation from the department of pulmonary medicine. The study was approved by the corresponding institutional ethics committees and all participants gave written informed consent. This study included only lung cancer patients. Subjects having other disorders such as COPD, asthma, tuberculosis, interstitial lung disease and other malignancies were excluded from the study. The EGFR mutations were determined by the IHC in non-small cell lung cancer patients.

Immunohistochemistry: Formalin-fixed, paraffin-embedded tissue sections were cut into 4 µm-thick sequential sections. After deparaffinization and rehydration, sections were boiled in citrate buffer (0.01 M, pH 6.0) for antigen retrieval. Sections were then incubated with 3% H_2O_2 and 5% serum to block endogenous peroxidase activity and non-specific binding. IHC with two mutation-specific anti EGFR antibodies: E746-A750del (exon 19) (6B6, 1:25 dilution; Cell Signaling Technology Inc., Danvers, MA, USA) and L858R (exon 21) (43B2, 1:100 dilution; Cell Signaling Technology Inc.) were used for detection of EGFR mutation. The sections were then incubated with biotinylated secondary antibodies and visualized by DAB. Counterstaining was carried out with hematoxylin. The sections were dehydrated in alcohol and mounted with DPX.

Immunohistochemical Scoring: The IHC staining score was based on the staining intensity and percentage positivity (0-100%) of cells in the membrane and/or cytoplasm of tumor cells. IHC evaluation was performed under a microscope. The intensity of the cytoplasmic or membrane staining as well as the percentage of positive cells and staining intensity was recorded. The patterns of staining were applied into scales on % of cells with positive immunostaining as 0=complete absence or negative staining, 1=less than 10 % positive cells, 2=greater than 10% and less than 50 % cells and 3=more than 50% cells positive. In general staining in less than 10% was considered as negative staining and more than 10% was considered positive. The Staining intensity was scored from 0 to 3+. 0/ 1+ score is considered as negative for EGFR Mutation and 2+/3+ as positive for EGFR mutation

Statistical Analysis: Clinical characteristics of all subjects were expressed in mean± SD and in percentage.

RESULTS

Table: 1 Clinical characteristics of lung cancer patients and EGFR mutation status

Variables		EGFR Mutation Positive	EGFR Mutation Negative
Total no of patients (N=80)		**N=26**	**N=54**
Age		56±9.50	53.37±11.83
Sex	Male	17(65.38%)	39(72.22%)
	Female	9(34.61%)	15(27.77%)
Smoking Status	Ex /Current Smokers	8(30.77%)	38(70.37%)
	Nonsmokers	18(69.2%)	16(29.6%)
Histology Types	Adenocarcinoma	26	54
	Squamous Cell Carcinoma	0	0
	Adenosquamous Cell Carcinoma	0	0
	Large Cell Carcinoma	0	0

The Study shows 26 cases were positive for both the mutations and 54 cases were negative for EGFR mutations. The mean age of mutation positive cases is (56±9.50) and negative is (53.37±11.83) The results shows that (65.38%) male and (34.61%) female was positive for EGFR mutation. The study shows that 8(30.77%) ex/current smokers and 18(69.2%) nonsmokers were positive for EGFR mutations.

Immunohistochemistry of 80 non-small cell lung cancer patients of adenocarcinoma subtypes was done for detection of EGFR mutation. This study shows that 26 (32.5%) patients were positive for EGFR mutation including 20 (76.92%) of E746-A750 mutation in exon 19 and 6 (23.07%) patients were positive for a L858R mutation in exon 21.

Table 2: IHC Score of E746-A750 Mutation of exon 19 and L858R Mutation of exon 21 of lung cancer patients

No of patient for EGFR mutation analysis		80		
No of EGFR Mutation positive		26(32.5%)		
EGFR mutation Negative		54(67.5%)		
Type of EGFR Mutation	E746-A750 Mutation	20	0 Score	30
			+1	0
			+2	12
			+3	8
	L858R Mutation	6	0 score	24
			+1	0
			+2	1
			+3	5

IHC analysis of mutation-specific antibodies against EGFR mutants in lung adenocarcinoma:

The mutation specific antibodies against L858R point mutation in exon 21 and E746-A750 deletion in exon 19 were used for the staining of the tumor sections. The mutant-specific antibodies showed cytoplasmic and membranous staining in positive cases. Positive IHC staining of delE746-A750 with 1+ (0 cases), 2+ (12 cases) and 3+ (8 cases) and L858R with 1+ (0 cases), 2+ (1 cases) and 3+ (5cases) were found.

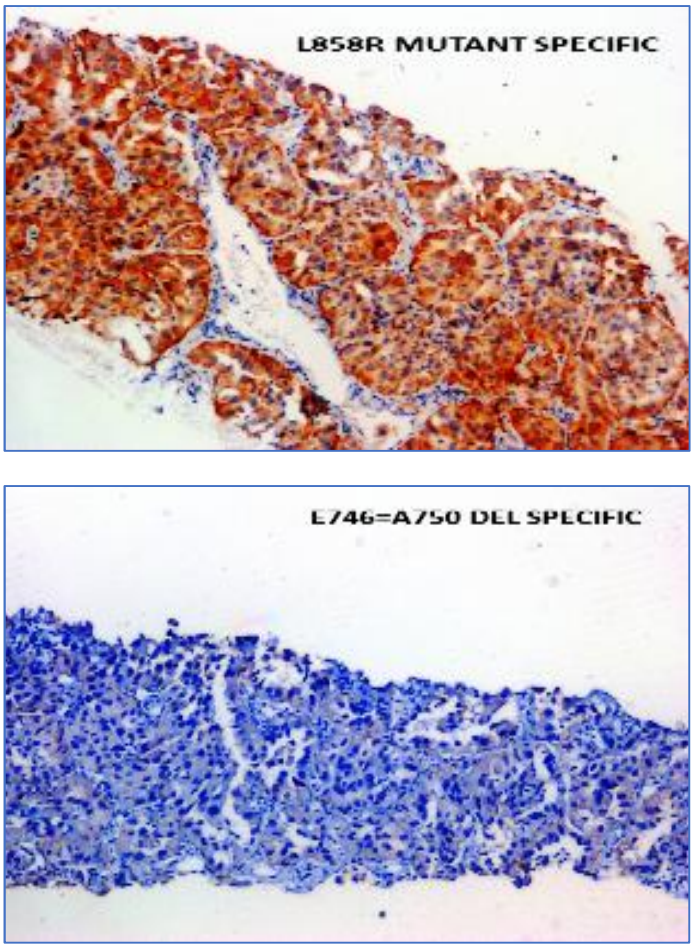

Figure 1: EGFR mutational analysis using monoclonal antibodies: Positive for L858R mutant specific and negative for E746-A750 del specific

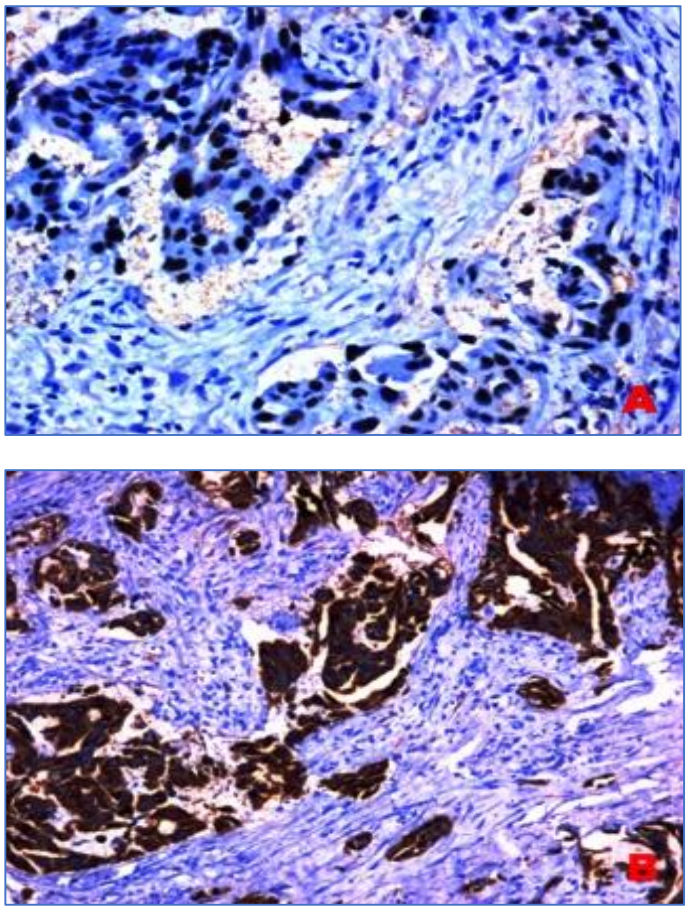

Figure 2: EGFR mutational analysis using monoclonal antibodies: Positive for E746-A750 del specific and negative for L858R mutant specific

DISCUSSION

The testing of patients with adenocarcinoma of the lung for selection of specific therapy is standard of care in clinical practice. Determination of mutational status in pulmonary adenocarcinoma has entered our daily routine and it is now an integral part of the pathological evaluation [11]. Most patients that may get benefited from molecular testing in determining the choice of drugs for target therapy in which lung cancer patients where only small biopsies or cytological material are available [12].

Epidermal growth factor Receptor Mutation status is valuable indicator for the screening of non-small cell lung cancer patients for tyrosine kinase inhibitor (TKI) therapy. The result of this study and some other studies suggest that scoring criteria based on the staining intensity of the membrane or cytoplasm of tumor cell and the percentage of staining was divided into four grades such as 0, +1, +2 and +3. The primary antibody E746-A750 del specific detects EGFR Mutation in exon 19 and the L858R mutant specific detects EGFR mutation in exon 21. This study shows that out of 80 lung cancer subjects of adenocarcinoma subtypes, 26 (32.5%) are mutation positive. This study may be helpful in optimizing the therapeutic option for the treatment of lung cancer. The patients having EGFR mutation positive are treated with EGFR -TKI such as gefitinib as the 1st line treatment instead of other chemotherapy regimen and show better improvement in the disease. This study proposed IHC as an initial, rapid and cost-effective tool, especially in our diagnostic set-up and peripheral laboratory services where facilities for molecular analysis are limited. However, more studies are needed to validate these results further in many patients and to evaluate their utility in management of patient.

ACKNOWLEDGEMENTS

We are thankful to the entire faculty of the Department of Pulmonary Medicine, King George's Medical University, Lucknow, U.P., India, Department of Physiology, King George's Medical University, Lucknow, U.P. We are also grateful to all the subjects participated in this study.

REFERENCES

1. Jamal A, Thomas A, Murray T, Thun M. Cancer statistics, 2002. CA *Cancer J Clin* 2002; 52:23-47.
2. Sasaki H, Endo K, Okuda K, Kawano O, Kitahara N, Tanaka H, *et al*. Epidermal growth factor receptor gene amplification and gefitinib sensitivity in patients with recurrent lung cancer. *J Cancer Res ClinOncol 2008*; 134:469-77.
3. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004, 350(21):2129–2139.
4. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004, 304(5676):1497–1500.
5. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, Singh B, Heelan R, Rusch V, Fulton L, Mardis E, Kupfer D, Wilson R, Kris M, Varmus H: EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl AcadSci U S A* 2004, 101(36):13306–13311.
6. Sharma SV, Bell DW, Settleman J, Haber DA: Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer 2007*, 7(3):169–181.
7. Bai H, Wang Z, Chen K, Zhao J, Lee JJ, Wang S, Zhou Q, Zhuo M, Mao L, An T, Duan J, Yang L, Wu M, Liang Z, Wang Y, Kang X, Wang J: Influence of chemotherapy on EGFR mutation status among patients with non-small-cell lung cancer. *J ClinOncol 2012*, 30(25):3077–3083.
8. Bai H, Mao L, Wang HS, Zhao J, Yang L, An TT, Wang X, Duan CJ, Wu NM, Guo ZQ, Liu YX, Liu HN, Wang YY, Wang J: Epidermal growth factor receptor mutations in plasma DNA samples predict tumor response in Chinese patients with stages IIIB to IV non-small-cell lung cancer. *J ClinOncol 2009*, 27(16):2653–2659.
9. Mitsudomi T, Kosaka T, Endoh H, et al. Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. *J ClinOncol* 2005; 23:2513–20.
10. Takano T, Ohe Y, Sakamoto H, et al. Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. *J ClinOncol* 2005; 23:6829–37.
11. Yong Hannah Wen, Edi Brogi, Adnan Hasanovic, Marc Ladanyi, Robert A Soslow, Dhananjay Chitale, Jinru Shia and Andre L Moreira. Immuno histochemical staining with EGFR mutation-specific antibodies: high specificity as a diagnostic marker for lung adenocarcinoma. *Modern Pathology (2013)* 26, 1197–1203.
12. Guiyang Jiang, Chuifeng Fan, Xiupeng Zhang et al. Ascertaining an Appropriate Diagnostic Algorithm Using EGFR Mutation-Specific Antibodies to Detect EGFR Status in Non-Small-Cell Lung Cancer. *PLo One. 2013*;8(3): e59183.

How to cite this article:
Gaur P, Bhattacharya S, Surya Kant, Kushwaha RAS, Agarwal J and Pathak AK. Epidermal Growth Factor Receptor Mutations Detection by Mutant Specific Immunohistochemistry in North Indian Lung Cancer population. *Int. J. Res. Dev. Pharm. L. Sci.* 2017; 6(6): 2796-2799. Doi: 10.13040/IJRDPL.2278-0238.6(6). 2796-2799.