



Mutation Specific Antibodies for Immunohistochemical detection of Epidermal Growth Factor Receptor Mutations in Lung Adenocarcinomas

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Abstract

Introduction: *EGFR over expression and mutation play an important role in the carcinogenesis of lung adenocarcinoma. The most common adenocarcinoma-associated EGFR mutations are in-frame deletions in exon 19 (E746_A750del) and the point mutation replacing leucine with arginine at codon 858 in exon 21 (L858R). Antibodies that specifically detect mutant EGFR protein by immunohistochemistry can be used to detect these specific mutations.*

Objectives: *1) To find out the prevalence of EGFR mutations in lung adenocarcinoma patients using mutant specific immunohistochemical markers.*

2) To assess the clinical and biological features associated with EGFR mutation.

Materials and Methods

Study Design: *Descriptive Study*

Study Population: *All cases of Adenocarcinoma Lung diagnosed in the Department Of Pathology, Govt. Medical College, Kozhikode during the study period*

Sample Size: *100*

Results: *Of the 100 cases, 88 were male (88%) and 12 were female (12%) with a mean age of 63 years. Out of 100 cases, 19 cases showed EGFR mutation. 14 patients (13 males; 1 female) showed positivity for del E746-A750 and 9 patients for L858R (9 male, 0 female). 4 patients showed positivity for both. EGFR mutation rate was higher in adenocarcinomas of lepidic and acinar subtypes than of other subtypes(p< 0.05)*

Conclusions: *In this preliminary study, mutation specific IHC was used for assessment of mutation status of EGFR. IHC methodology is a potentially useful tool to guide clinicians for targeted therapies where facilities for molecular analysis are not readily available .*

Keywords: *Adenocarcinoma, Epidermal growth factor receptor (EGFR), exon 19, exon 21, mutation specific antibodies for E746-A750 and L858R.*

Introduction

Lung cancer is the most common type of cancer worldwide with an estimation of 1.82 million new

cancer cases diagnosed. It is the leading cause of cancer related deaths^[1]. It is the commonest cancer and cause of cancer related mortality in men.

Carcinoma lung can be broadly classified into Small cell lung and Non small cell carcinoma. Non small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancers. Adenocarcinoma and squamous cell carcinoma are the two major subtypes of non-small cell lung carcinoma.

Epidermal growth factor receptor (EGFR), is one of several somatic mutations, in NSCLC^[2], which is seen more frequently in certain population groups. This population group is classically described as Asian, non-smoking females with adenocarcinoma^[3,4]. The interest in these mutations is due to the small molecule targeted therapies (such as erlotinib and gefitinib) available and in development, which can have significant prognostic significance^[5,6]

There are many methods to detect the EGFR mutations. The most commonly used methodology is, direct sequencing of PCR products. The main drawbacks of this method are its low sensitivity (20–50%) and the significant risk of contamination involved in handling post-PCR products. Recent advances in molecular techniques have enabled the development of more sensitive methods for detecting mutations with real-time quantitative PCR, using specific probes or amplified refractory mutation system (ARMSTM) technology. Most recently, the development of EGFR mutant-specific antibodies for immunohistochemistry (IHC) has presented a new method for the detection. Antibodies are available against the most common mutant forms of EGFR on EXON 19 and 21.

In our study, we aimed at determining the prevalence of these two EGFR mutations, using IHC specific antibodies among all adenocarcinomas diagnosed in our centre.

Objectives

1. To find out the prevalence of EGFR mutations in lung adenocarcinoma patients using mutant specific immunohistochemical markers.
2. To assess the clinical and biological features associated with EGFR mutation.

Materials and Methods

Type of study: Descriptive study

Study Period: 24 months; January 2017 to December 2018

Study Setting: Dept. Of Pathology, Government Medical College, Kozhikode

Sample Size: 100

Study Population: 100 adenocarcinoma lung patients diagnosed in Dept. of Pathology, Govt. Medical College Kozhikode during the study period

Inclusion Criteria: All lung biopsy samples (Endo bronchial, Trucut, Transbronchial lung biopsies) of clinically and histologically diagnosed lung adenocarcinoma patients irrespective of age and gender received in dept. of Pathology, Govt. Medical College, Kozhikode

Exclusion Criteria: 1. All metastatic carcinomas 2. Those cases where biopsy material is inadequate for IHC studies

Study Procedure

- Approval from Ethics committee and the Research Board of the Institution was obtained.
- All cases clinically diagnosed as carcinoma of lung from the Department of Chest disease and which were diagnosed as Adenocarcinoma lung (TTF 1 positive cases) from the Department of Pathology was collected.
- The clinical history was collected from the case sheets as well as Pathology requisition forms.
- H &E slides studied and histological sub typing done
- Paraffin embedded blocks of the same were taken, cut at 5 micrometre, IHC staining(using standardised procedure) was done with exon 19 deletion mutation antibody and one for exon 21 point mutation antibody ((Primary antibodies used: delE746-A750 mutation specific monoclonal antibody (6B6) and L858R mutation specific monoclonal antibody (43B2);Cell signalling technology, Danvers, MA,USA)

Intensity of staining was scored as following: 0= no staining; 1+ =faint membrane and/or cytoplasmic staining in >10 per cent of tumour cells in x 40 objective magnification; 2+ = moderate membrane

and/or cytoplasmic staining in x 10 to x 20 objective magnification; 3+ = strong membrane and/or cytoplasmic staining x 2 to x 4 objective magnification.

Scores 1+, 2+ and 3+ were considered as positive cases.

Data Management and Analysis: The data was entered in Microsoft Excel and Statistical analysis was done using SPSS 18 software. Chi square test was used and a p value <0.05 was considered as statistically significant.

Results

- The present study was carried out to analyse the two common EGFR mutations (exon 19 deletion and exon 21 point mutation) in lung adenocarcinoma by immunohistochemistry using mutant specific antibodies.
- Among the 100 cases studied, 88(88%) were males and 12(12%) were females. The mean age of cases was 63 years.

Table 1: Age group wise distribution of the cases

Age group	Frequency	Percentage
40-49	4	4%
50-59	27	27%
60-69	46	46%
70-79	19	19%
80-89	4	4%

- 56% of the patients were smokers and 44% were never smokers.

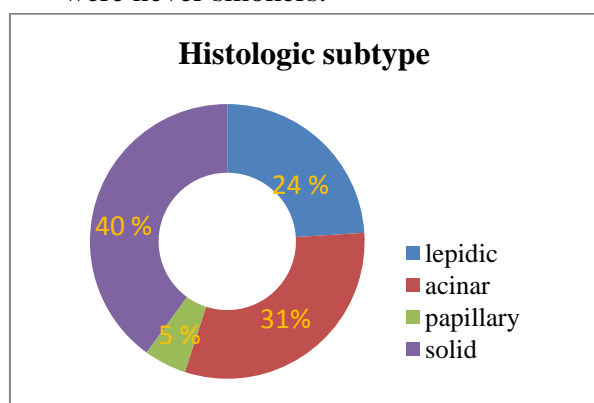


Figure 1: Distribution of cases on the basis of Histological Subtype of the tumor

Table 2: Stage wise distribution of cases

Stage	No. of cases	Percentage
2	22	22%
3	68	68%
4	10	10%

Table 3: EGFR IHC expression among the cases

Total no. of cases:100	
Positive cases:19	
Cases with double mutation:4	
Total mutations	23
Exon 19 Deletion	14/23 (61%)
Exon 21 point mutation	9/23 (39%)

Discussion

In our study, 19% of the patients were found to harbor an EGFR mutation (exon 19 Del=14 and exon 21 mutation=9) among the 100 patients tested.4 patients had concurrent mutation in these exons.

Gaur P et al, in their study among North Indian lung cancer patients, obtained a prevalence of 34%(73) using mutant specific antibodies by immunohistochemistry. 79.17% of the mutations were in exon 19 and 20.83% in exon 21. In our study, 60.86% were exon 19 mutations and 39.13% were exon 21 mutations^[7]. Another study by Jain D^[8] et al, using both immunohistochemistry and PCR on 206 adenocarcinomas reported a prevalence of 26.6% .Of the positive cases,60% were exon 19 deletions and 40% were exon 21 point mutations.

Comparing with these studies, we got a prevalence of 23 % (23 mutations among 100 patients) by using immunohistochemistry alone. The frequency on the lower side in the present study can be explained by the method of detection that is IHC which detects only two most common mutations.(Exon 19 deletion and exon 21 point mutation)

Table 4: comparison between mutated and non mutated EGFR cases

	Non mutated EGFR(n=81)	Mutated EGFR(n=19)	P value
Age(in years)			
mean	63.57	60.58	0.54
Sex			
Male	70(86%)	18(95%)	0.315
Female	11(14%)	1(5%)	
Smoking history			
Current or Ex smoker	43(53%)	13(68%)	0.226
Never smoker	38(47%)	6(32%)	
Stage at presentation			
1&2	17(21%)	5(26%)	0.55
3&4	64(79%)	14(74%)	
Histological subtype			
lepidic	18(22%)	6(32%)	0.006
acinar	20(25%)	11(57%)	
Papillary	5(6%)	0	
Solid	38(47%)	2(10%)	

Previous studies^[7-10] have reported a high prevalence of EGFR mutation in non smokers and in females. But in our study, no significant association was obtained between gender, smoking habitus and mutation status. The population of females were only 12 % in our study and majority of the study population were smokers. Since no correlation between the presence of EGFR mutations and the stage of disease was found in this study EGFR mutation may be considered as an early event that plays an important role in the pathogenesis of lung adenocarcinoma.

We observed a significant association between histologic subtype and EGFR mutation status with a p value of 0.006(<0.05). 33% of the lepidic and 57 % of the acinar subtypes showed EGFR mutation. This was in concordance with the previous studies^[11] which showed high rate of EGFR mutation in lepidic and acinar subtypes compared to less occurrence of the mutation in papillary, micro papillary and solid subtypes.

Photomicrographs

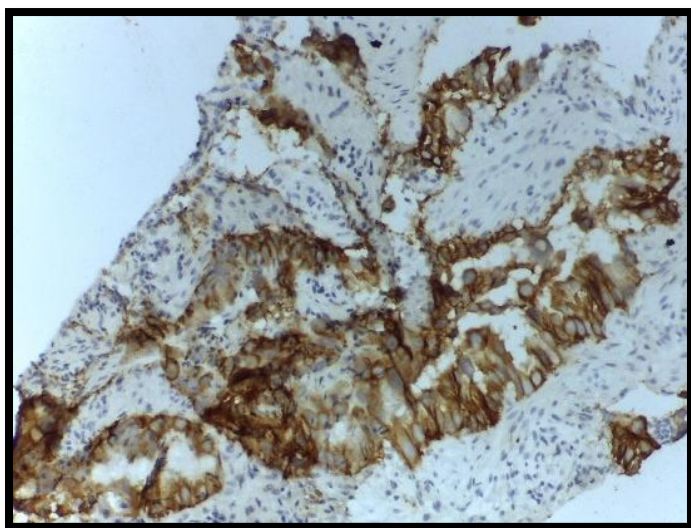


Figure 2: EGFR (L858R) (point mutation exon 21) IHC showing 3+ membranous positivity (20X)

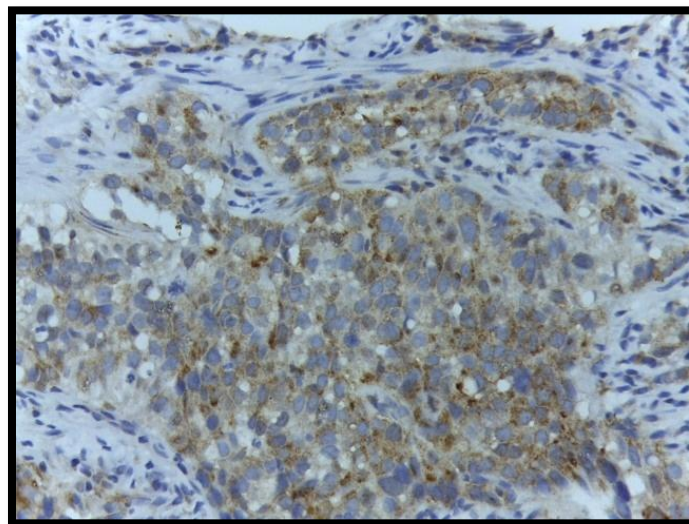


Figure 3: EGFR (E746-A750) (EXON 21 Point mutation) IHC showing 2+membranous and cytoplasmic positivity (20 X)

Conclusion

Epidermal Growth Factor Receptor over expression and mutation play an important role in the carcinogenesis of lung adenocarcinoma. Studies have shown that immunohistochemical (IHC) staining using epidermal growth factor receptor (EGFR) mutation specific antibodies, is an easy and cost-effective, screening method compared with molecular techniques.

Limitations

One major drawback for the use of IHC to detect EGFR mutations is that these are specific only to their target mutations (E746-A750 deletion in exon 19 and L858R in exon 21). Other mutations such as TKI resistance exon 20 mutations cannot be detected by these two antibodies. However, it can be used as a screening tool especially in Indian setting.

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