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STUDY OF EXPRESSION OF EGFR AND ALK BY IMMUNOHISTOCHEMISTRY IN NON-SMALL CELL LUNG CARCINOMA

Medical Science

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ABSTRACT

Background: Epidermal growth factor receptor (EGFR), a cell surface protein, is overexpressed in 27-44% of non-small cell lung carcinoma (NSCLC) cases of Indian Ethnicity. First line treatment with EGFR tyrosine kinase inhibitors, in combination with conventional radiotherapy or chemotherapy, has shown uncontested benefits by increasing overall survival.

A fusion gene between echinoderm microtubule-associated protein-like 4 (EML4) and anaplastic lymphoma kinase (ALK), named EML4-ALK, has been identified in a subset of non-small-cell lung carcinoma cases, and, when inhibited by ALK inhibitors, has shown response rates of more than 60%.

Aim: The objective of this study was to find out the prevalence of EGFR and ALK expression in histologically diagnosed cases of NSCLC, to evaluate their clinical characteristics.

Material and methods: A total of 67 histopathololgically diagnosed cases of non-small cell lung carcinoma were included. Formalin fixed, paraffin-embedded blocks were evaluated for EGFR and ALK expression by immunohistochemistry and their expressions were compared with clinico-pathological variables such as age of presentation, sex, smoking habit, histological type, stage etc. Spearman correlation test has been used to find the significance of study parameters on categorical scale between two or more groups using statistical software MedCalc V 11.3.0.0.

Results: The incidence of EGFR positivity (42%) in this study population is close to the reported incidence in Asian patients. Here we found a positive correlation of EGFR expression with female gender, and non- smoker patients. The positivity for ALK IHC in our study is 4%, and demonstrates a positive correlation with younger age at presentation.

Conclusion: As the high likelihood of response to EGFR and ALK tyrosine kinase inhibitors can improve the overall survival of the patients and avoid the unnecessary side effects of conventional chemotherapy, it emphasizes the importance of detection of EGFR and ALK expression in non-small cell lung carcinoma cases.

KEYWORDS

non-small cell lung carcinoma, EGFR, ALK

INTRODUCTION

According to GLOBOCAN 2012, Lung cancer is the most common cancer diagnosed worldwide contributing 1.6 million total cases and 1.38 million total cancer deaths per year. The incidence of lung cancer in India is about 5.6 per one lakh, leading to most common cancer related mortality in male. In female although the incidence of lung cancer in the developed countries is quite high, in India it is low with a rising trend (1).

Despite improvements in therapeutic methodology, including surgery, chemotherapy and radiotherapy, the average prognosis of lung carcinoma still remains unsatisfactory and the five year survival rate is merely 15% (2). Among those lung cancer patients, non-small cell lung cancer (NSCLC) accounted for approximately 80%-85% of all lung cancer cases (3). At the time of diagnosis, about 60% of patients present with an advanced stage of disease (3). In this scenario, the recent discovery of novel molecular signal alterations may be involved in defining a therapy, which may turn out to be more effective and with less side effects than conventional treatment.

Increased attention has been given in the development and application of drugs that target specific molecules which expressed on NSCLC cells and great success has been reported in NSCLC patient study groups. These methods include targeted therapy against signal transduction molecules such as the epidermal growth factor receptor (EGFR) and EML4-ALK fusion gene (4). Lung cancers that harbor EGFR activating mutations accounts for a large number of NSCLC among Asian, young, female, non-smoker patients (5).The two most common mutations - the point mutation at exon 21 codon 858(L858R) and in-frame deletions in exon 19 account for > 90% of the EGFR positive cases(5).Although inferior to molecular genetic analysis of the EGFR gene, mutant specific EGFR IHC has good specificity and sensitivity for identifying targeted activating EGFR mutations. The antibodies are likely to be of clinical value in cases where limited tumor material is available, or in situations where molecular genetic analysis is not readily available (6).

In the year 2007, a fusion gene EML4-ALK has been identified in a subset of NSCLC, strongly associated with adenocarcinoma histology and mostly in young and light-smokers or non-smokers patients.5 These patients typically present in late stages, which is not amenable for surgical resection. Therefore, the molecular target regimens that target the EML4-ALK fusion protein would be an effective, novel therapeutic method for those patients.

Patient with EGFR and ALK expression demonstrate unique clinico-pathological characteristics and show a dramatic response and longer progression-free survival after TKI-based targeted therapy than those without these protein expressions.[7]

Despite a large number of studies performed on lung cancers showing EGFR and EML4-ALK expression, detailed clinico-pathological profiles remain unclear and only few studies have been performed on EFGR and ALK expression in NSCLC in India till date.

So, to study the prevalence and to correlate the expression of EGFR and ALK with the histological types of NSCLC cases, and also with their age at presentation, sex, smoking habit, tumor stage and to guide the personalized targeted therapy for the patients who are positive for EGFR and ALK expression, we intended to perform the present study.

MATERIALS AND METHODS

An institutional based cross sectional observational study was conducted in a tertiary care center of West Bengal from February 2017 to July 2018 in the Department of Pathology in collaboration with Department of Pulmonary Medicine, NRS Medical College and Hospital. Specimens obtained by bronchoscopic biopsies, guided trucut biopsies, cell block preparation from broncho-alveolar lavage fluid, and lobectomy specimens were included in the study with the exclusion of specimens with a history of chemotherapy. A total 67 numbers of lung biopsies were received during the study period and examined histopathologically, of which 15 cases were diagnosed to be small cell lung carcinoma and hence excluded. Two cases of non-small cell lung carcinomas were excluded due to very scanty viable materials in subsequent sections for IHC examination. Hence a total of 50 cases could be enrolled. Census method of sampling was used. Data was collected using a pre-designed, pretested semi-structured schedule on dependent variables like EGFR and ALK expression and independent variables like clinico-pathological profile including age at presentation, sex, occupation, smoking history, histological type, stage and other relevant parameters. Data was collected by personal interview, observations, record review and laboratory techniques including histopathology and immunohistochemistry. Reporting was done by Pathology experts.

Histopathology

All tissue samples were collected in 10% buffered formalin and processed for routine histopathological examination. Five µm thick sections from formalin fixed paraffin embedded blocks were cut and stained with hematoxylin and eosin for histopathological diagnosis of tumor type and subtype.

Immunohistochemistry (IHC)

For IHC staining, 3 µm thick sections from formalin fixed paraffin embedded tissues were taken on poly L Lysine coated slides. IHC was done using EGFR antibody(clone SP125) and mouse anti-human ALK D5F3 antibody and the steps mentioned in the kit supplied were followed. The criterion for a positive immune reaction for EGFR was complete membranous staining and under a light microscope at 400X magnification. Criterion for ALK positivity was presence of granular cytoplasmic staining in tumour cells (any percentage of positive tumour cells).

Scoring of Immunohistochemisty

The scoring method followed, as was described by FLEX study.8 The staining intensity of the cell membrane was scored within a scale ranging from 0-3 and divided into 4 categories as follows: No staining, 0; weak staining, 1+ (light brown membrane staining); intermediate staining, 2+; and strong staining, 3+ (dark brown linear membrane staining). For more reliable scoring definitions, strong staining (3+) was clearly visible using a ×4 objective lens, moderate staining (2+) required a ×10 or ×20 objective lens for clear observation, and weak staining (1+) required a ×40 objective lens. Intensity scoring is not applicable in ALK positive cases. Data was entered in MS excel. For descriptive analysis, the general variables were summarized as the mean ± standard deviations, range and percentage. Spearman correlation test has been used to find the significance of study parameters on categorical scale between two or more groups, using MedCalc V 11.3.0.0. Significance level was considered at p value < 0.05.

RESULTS

A total 50 cases of non-small cell lung carcinomas were studied. The average age of the participants was estimated to be 55.08±10.78 (mean ± sd) years with a range of 48(76-28) years. There were 34(76%) male patients and 16(32%) female patients. Majority of the patients were smokers (60%).Of the 50 cases, majority (35 cases) were diagnosed to be adenocarcinoma and among the rest, 13 cases of squamous cell carcinoma, one sarcomatoid carcinoma and one large cell carcinoma were diagnosed. Baseline patient characteristics are shown in Table 1.

The results of IHC testing are shown in Table 2, figure 1,2,3. 21(42%) cases were positive for EGFR immunostaining and among the positive cases 18(86%) were adenocarcinoma, 2 squamous cell carcinoma and 1 sarcomatoid carcinoma. Out of 50 cases, only two (4%) were positive for ALK immunostaining, both were diagnosed to be adenocarcinoma and both patients were male and non-smoker. One case had solid growth pattern and the other was invasive mucinous adenocarcinoma. One of the two ALK positive cases was also positive for EGFR i.e. one (2%) out of fifty NSCLC cases was double positive for EGFR and ALK.

Table 1: Basic demographics and tumor characteristics

Characteristics	N(%)
Median age, years(range)	55.08(28-76)
Sex	
Male	34(68%)
Female	16(32%)
Smoking	
Smoker	30(60%)
Non-smoker	20(40%)
Histological types	
Adenocarcinoma	35(70%)
Squamous cell carcinoma	13(26%)
Sarcomatoid carcinoma	01(2%)
Large cell carcinoma	01(2%)

Table 2: Results of IHC with EGFR and ALK antibodies

Characteristics	N(%)
EGFR IHC	
Positive	21(42%)
Negative	29(58%)
ALK IHC	
Positive	2(4%)
Negative	48(96%)

The association of age, sex, smoking habit and stage of tumor with regardsto EGFR and ALK epression are shown in Table 3 and Table 4 respectively. While sex and smoking habits were significantly associated with EGFR expression($P<0.05$), the expression of ALK showed a significant association with age($p<0.05$).

Table 3: Distribution of EGFR in different age, sex, smoking habit and tumor stage of non-small cell carcinoma cases

Variable		EGFR		p value
		positive	negative	
Sex	Female	10	06	0.0301
	Male	11	23	
Age in years	21-30	02	00	0.3979
	31-40	01	01	
	41-50	06	05	
	51-60	06	14	
	61-70	04	06	
	71-80	02	03	
Smoking	Non smoker	12	08	0.0444
	Smoker	09	21	
Histological types	Adenocarcinoma	18	17	0.039
	Squamous cell carcinoma	02	11	
	Sarcomatoid carcinoma	01	00	
	Large cell carcinoma	00	01	
Stage	I	01	01	0.2660

	II	10	16	
	III	05	11	
	IV	05	01	

Table 4: Distribution of ALK in different age, sex, smoking habit and tumor stage of non-small cell carcinoma cases

		ALK		P value
		positive	Negative	
Sex	Female	00	00	0.3321
	Male	02	32	
Age in years	21-30	01	01	0.0301
	31-40	00	02	
	41-50	01	10	
	51-60	00	20	
	61-70	0	10	
	71-80	00	05	
Smoking	Non smoker	02	18	0.0799
	Smoker	00	30	
Histological types	Adenocarcinoma	02	33	0.221
	Squamous cell carcinoma	00	13	
	Sarcomatoid carcinoma	00	01	
	Large cell carcinoma	00	01	
Stage	I	00	02	0.0849
	II	00	26	
	III	01	15	
	IV	01	05	

DISCUSSION:

The last decade has been an exciting period for pulmonary oncology research, wherein targeted therapies have improved median overall survival for patients with NSCLC (14).

The uncontested benefit of EGFR TKIs for patients with EGFR activating mutations has clearly demonstrated the feasibility and power of tumor molecular profile as a guide for clinical treatment decision and has paved the way for a new era of personalized treatment for NSCLC.

- In the present study, the mean age of patients found to be 55.08± (mean±sd). This is consistent with the figure mentioned by previous studies.9

- The incidence of EGFR mutation (42%) in our study is close to reported incidence in Asian patients (47%) as stated by studies of Chougule A et al. 10 and Doval DC et al. 11

- A higher EGFR mutation rate was observed in adenocarcinomahistology (p=0.039) and in female than males (62.50% versus 32.35%, p=0.0244) and the results were statistically significant. This observation is consistent with previously published studies of Doval DC et al. 11, Sekine I et al. 12

- ALK positive patients are more likely to be men (2 out of 2), this is in line with study of Shaw et al. 13, but against the results of study of Doval DC et al. 11

- A significant correlation between young age of presentation and ALK expression could be concluded from this study (p=0.0303). This is consistent with the studies of Dover DC et al. 11 and Shaw AT et al. 13

- The result of our study demonstrate a positive correlation of EGFR expression in the non-smoker group as compared to smokers(p=0.0444) which is in line with studies of Doval DC et al.11 and Krishnamurthy A et al.14

- In this study, 2 of 50 patients classified as non-smokers were positive for ALK, whereas all 30 patients with a smoking history were ALK negative. This result suggests that ALK is associated with non-smoking history. This finding is in line with study of Shaw AT et al.13, but contrary to study of Soda M et al.15

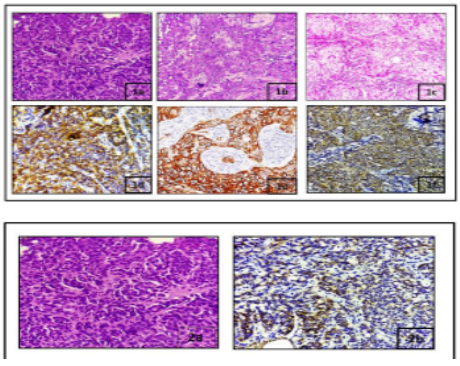
- In this study, concomitant expression of EGFR and ALK was present in one(2%) of 50 cases, which has been described as virtually impossible by several studies like Hsu SC et al. 16, Zhang X et al. 17, however, studies done by Popat S et al.18 demonstrated that the concomitant presence of EGFR and ALK is rare but not irrelevant but have a poor prognosis.

- We found two cases of squamous cell carcinoma which were positive for EGFR. This finding corroborated with studies of Miyamae et al.19

- In our study, no correlation between EGFR and ALK expression and stage of tumor could be found.

CONCLUSION:

In the present study it is found that the EGFR and ALK expressions are more prevalent in younger age group and non-smoker patients. While EGFR is more prevalent in female patients, ALK positivity is seen exclusively in male patients. As the high likelihood of response to targeted therapy with EGFR and ALK tyrosine kinase inhibitors and improvement in the overall survival of the younger patients avoiding the unnecessary side effects of conventional chemotherapy, it emphasizes the importance of detection of EGFR and ALK expression in non-small cell lung carcinoma cases.



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