Original Research Article

Epidermal growth factor receptor protein expression in lung cancer and survival analysis

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ABSTRACT

Background: Epidermal Growth Factor Receptor (EGFR) is one of the important molecules involved in lung cancer initiation and progression. Studies on over expression of EGFR and its survival in relation with Non-small cell lung cancer (NSCLC) patients have yielded controversial results. Prevalence of EGFR expression in NSCLC patients and 6-month survival in south Indian population is unknown.

Methods: We carried out a prospective study in tertiary hospital. Diagnosed patients with NSCLC were included in the study and were interviewed with questionnaire containing demography and investigations like Chest X-ray, CT thorax, Bronchoscopy were recorded. EGFR expression analysis was done for all patients and were followed up monthly for 6 months and details of survival and treatment were collected. Cox regression analysis was used to assess their survival.

Results: 50 patients with NSCLC were included. Forty-four (88%) were men, median age of study group was 65 years. Twenty-seven patients (54%) had Adenocarcinoma, 14 patients (28%) had Squamous cell carcinoma, 7 patients (14%) had poorly differentiated carcinoma and 2 patients (4%) had large cell carcinoma. Thirty-four (68%) samples were positive for EGFR expression. On multivariate analysis we found patients who took chemotherapy and with good performance status (Karnofsky score >65 and Eastern Cooperative Oncology Group >2.5) had better survival at 6 months.

Conclusions: Patients with EGFR positivity had better survival with chemotherapy but worse with radiotherapy. Patients who took chemotherapy and had good performance status had better survival on multivariate analysis. We didn't find any correlation between EGFR positivity and poor survival.

Keywords: Chemotherapy, EGFR, Lung cancer, NSCLC, Survival

INTRODUCTION

Lung carcinoma is one of the most common cancers in the world causing significant morbidity and mortality. It is one of the most common cause of death from cancer worldwide, responsible for nearly one in five (1.69 million deaths, 19.4% of the total).[1] Recent systematic analysis for global burden of disease found lung carcinoma to be leading cause of death and DALYs in men (1.2 million death and 25.9 million DALYs).[2] Epidermal Growth Factor Receptor (EGFR) is a trans membrane protein that is expressed on the surface of

many human epidermal and mesenchymal cells. EGFR is one of the key molecules in lung cancer initiation and progression, and EGFR is expressed or overexpressed in a wide variety of malignancies including lung carcinoma, especially Non-Small Cell Lung Cancer (NSCLC) and breast, gastric, colo - rectal and bladder cancer. Various studies have suggested that over expression of EGFR is associated with advanced disease and a poor survival in solid tumors.[3-5] Over expression of EGFR and its decreased survival in relation with NSCLC patients have been evaluated by a number of studies, but have yielded controversial results.[3,5-9] Prevalence of EGFR expression in NSCLC patients in the South Indian population is not known. EGFR and 6 months survival in South Indian population is not studied. Hence, we took up this study to evaluate the expression of EGFR in lung cancer, intensity of EGFR expression (H Score) and to correlate it with survival.

METHODS

Study design

We carried out a prospective observational study in an 1800 bedded tertiary care teaching hospital between October 2012 and September 2014. Patients with non-small cell lung cancer (NSCLC) diagnosed at our hospital by FNAC/Biopsy and Histopathological and cytological examination were included in the study after taking written informed consent. Patients with histologically/cytologically unconfirmed cases, unwilling to undergo Fine Needle Aspiration Cytology /Biopsy and patients refusing to consent were excluded from the study and patients included in the study were followed up every month for 6 months.

Data collection

Patients were interviewed with questionnaire containing demographic details, general physical examination including pulse rate, respiratory rate and blood pressure were recorded. Details of investigations like Chest X-ray, CT thorax, Bronchoscopy were recorded. Lung tissue of patients included in the study were subjected to immunohistochemistry for EGFR expression. Immunohistochemistry was done on the paraffin embedded histopathological tissue or cell blocks using Rabbit monoclonal IgG primary antibody (EGFR-EP22) which is a synthetic phosphopeptide corresponding to residues Tyr1068 of human EGFR isoform 1 (PathnSitu, Epitomics Inc.,). Tissue sections were deparaffinised in 3 changes of xylene and slides are hydrated in a series of graded alcohols to water. Tris-EDTA buffer solution was used as antigen retrieval solution.

Antigen retrieval was done by Heat Retrieval Method: sections were retrieved under steam pressure for 15 minutes using Multi Epitope Retrieval System. The sections were then allowed to cool for 10 minutes and

transferred to distilled water and standard staining protocol was followed.

IHC evaluation and H-score

Cytoplasmic staining intensity was the basis of EGFR assessment and was scored as 0 (negative, < 10% of cells stained), 1+ (weak, 10-25% of cells stained), 2+ (moderate, 25-50% of cells stained), and 3+ (strong, > 50% of cells stained). Tumors with 1+ to 3+ staining were considered as positive for EGFR expression. EGFR expression was further scored using H-scoring method. Visual assessment was used to determine the percentage of cells with varied staining intensities and with the score calculated using the formula $1 \times$ (% of 1 + cells) + $2 \times$ (% of 2 + cells) + $3 \times$ (% of 3 + cells).

Final score gives more relative weight to higher-intensity membrane staining (3+ > 2+ > 1+). The outcome-based discriminatory threshold IHC H-score as per the FLEX analysis was set at 200 and scored according to the above method. All patients included in the study were followed up for every month for a period of six months. At each follow up visit, details of survival and treatment taken (Surgery / Chemotherapy/Radiotherapy/no treatment) were collected. Patients who did not come for follow up visits were contacted through telephone and follow up data was collected.

The Institutional Ethics Committee approved the study with IEC number JSSMC/PG/2666/2017-18. Informed consent from patient/legal representative was taken prior to inclusion in the study.

Analysis

Descriptive data are expressed as frequencies (percentages) for discrete variables and as means (SDs) or medians for continuous variables. Mann-Whitney U test was used for comparisons between two groups and when appropriate, two-sample t-test was used. Chi square test was used to evaluate categorical factors. Cox proportional hazard analysis was used to assess survival in all patients and EGFR positive subgroup.

Receiver operating characteristic (ROC) curves were constructed to obtain the value with highest sensitivity and specificity for continuous variables. All the statistical tests were 2-tailed, and variables were considered statistically significant for $p < 0.05$. IBM SPSS version 22, New York, USA and CDC Epi Info version 7, Atlanta, USA was used for analysis.

RESULTS

The flow of patients into the study is presented in Figure 1. In this prospective study, a total of 50 patients with diagnosed NSCLC were included. Among them, 44 (88%) were male and 6 (12%) were female patients with

a median age of 65 years (IQR 42-90). Loss of appetite was the most common symptom (Table 1).

Thirty-four (68%) samples were positive for EGFR and 16(32%) were negative for EGFR expression. Eleven (22%) patients had IHC- H score > 200 and rest 39 (78%) were either EGFR negative or EGFR positive with H score < 200.

Among them, both Adenocarcinoma and squamous cell carcinoma showed highest rate of EGFR positivity followed by poorly differentiated carcinoma and none of the large call carcinoma showed EGFR H score >200. Seventy percent of the patients had tumour size of more than 7 cm with stage 4 (50%), with stage 3(20%) and rest in stage 2b with tumour size 3cm-7cm.

We found on Univariate cox regression analysis, patients who have taken treatment, have taken chemotherapy, having good performance score (Karnofsky score >65 and ECOG >2.5) and EGFR H score >200 had better survival (Table 2).

Table 1: Demographic data of patients with Non-small cell carcinoma patients.

| Variables	Number=50
Age mean (SD) in years	65±12.25
Gender, male n (%)	44(88)
Shortness of breath	35(70)
Fever	14(28)
Chest pain	29(56)
Loss of appetite	45(90)
Weight loss	43(86)
Hemoptysis	9(18)
Hoarseness of voice	12(24)
Adenocarcinoma	27(54)
Squamous cell carcinoma	14(28)
Poorly differentiated carcinoma	7(14)
Large cell carcinoma	2(4)
Treatment taken	26(52)
Chemotherapy	20(40)
Radiotherapy	11(24)
EGFR positive	34(68)
Survived at 6 months	21(42)

Table 2: Univariate Cox regression analysis for factors influencing survival in all patients with Non-small cell Lung carcinoma.

Variables, n (%)	Total (50)	Survivors (21)	Non-survivors (29)	Hazard ratio (95% CI)	p value
Age (>67 years)	12	3 (25%)	9 (75%)	2.7 (0.63-11.55)	0.20
BMI (<23.8)	43	16 (37.2%)	27(62.7%)	2.99 (0.71-12.6)	0.13
Symptom duration (>1.5 months)	31	16 (51.61%)	15(48.38%)	1.72 (0.83-3.58)	0.14
Comorbidities	22	8 (36.36%)	14 (63.6%)	0.8 (0.38-1.66)	0.55
Smoking Index (>632)	36	13(36.1%)	23(63.9%)	0.59 (0.24-1.47)	0.26
Size of tumor (>67.5mm)	30	9(30%)	21(70%)	0.54 (0.23-1.22)	0.14
Peripheral lymph node involvement	16	4 (25%)	12 (75%)	0.53 (0.25-1.12)	0.10
Mediastinal lymph node involvement	41	15 (35.7%)	27 (64.3%)	0.30 (0.07-1.26)	0.10
EGFR positivity	34	15(44.1)	19 (55.9)	1.13 (0.52-2.43)	0.75
EGFR H score (≥200)	11	2 (18.2)	9 (81.8)	0.40 (0.16-1.01)	0.04
Treatment taken	26	15 (57.6)	11 (42.3)	2.44 (1.14-5.22)	0.02
Chemotherapy	20	14 (70%)	6 (30%)	3.69 (1.48-9.14)	0.004
Radiotherapy	10	1 (10%)	9 (90%)	0.47 (0.21-1.03)	0.06
Karnofsky performance score (>65)	14	11 (78.6)	3 (21.4)	5.02 (1.51-16.6)	0.008
ECOG score (>2.5)	33	9 (27.3)	24 (72.7)	0.26 (0.09-0.69)	0.006

Table 3: Multivariate Cox regression analysis for factors influencing survival in all patients with Non-small cell Lung carcinoma

Variables	Hazard ratio (95%CI)	p value	Multinomial Hazard ratio (95%CI)	p value
Chemotherapy	3.69(1.48-9.14)	0.004	2.25(1.75-5.25)	0.032
Karnofsky performance score (>65)	5.02(1.51-16.6)	0.008	3.04(1.75-8.92)	0.02
ECOG score (>2.5)	0.26(0.09-0.69)	0.006	0.12(0.04-0.19)	0.01
EGFR H score (≥200)	0.40(0.16-1.01)	0.04	0.32(0.11-1.59)	0.06

On multivariate analysis we found patients who took treatment, chemotherapy and with good performance status (Karnofsky score >65 and ECOG >2.5) had better survival at 6 months (Table 3). Among EGFR positive subgroup of patients, symptom duration >1.5 months, peripheral lymph node involvement, EGFR- H score >200 and patients who had taken radiotherapy (HR-0.30, p=0.012) were significantly associated with poor survival. Patients who had taken chemotherapy (Paclitaxel with cisplatin in combination with EGFR inhibitor Geftinib) and Karnofsky performance score >65 and ECOG >2.5 had better survival. Kaplan meier survival graph for patients who took chemotherapy are depicted in Figure 1.
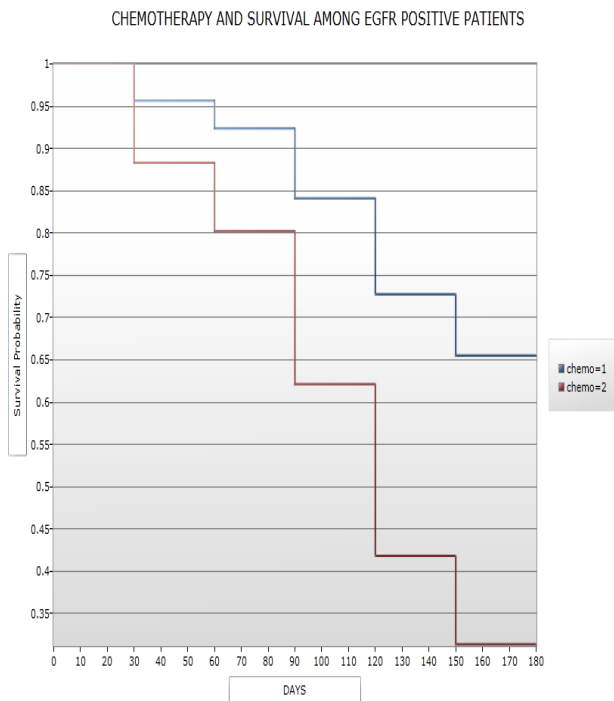
Hazard Ratio	95% Confidence Interval		p-value
	Lower limit	Upper limit	
2.7370	1.0329	7.2528	0.0429

	EGFR – Positive	Chemotherapy taken
	EGFR – Positive	Chemotherapy not taken

Figure 1: Survival among EGFR positive patients who have received chemotherapy vs patients who have not received chemotherapy.

During follow up of 6 months, 21 (42%) patients survived and 29 (58%) patients succumbed. Twenty-four patients took treatment (Radiotherapy, chemotherapy or both) and of them 15 patients survived to 6-month follow up. Twenty patients were on chemotherapy with Paclitaxel, cisplatin and EGFR inhibitor-Geftinib and 14 patients survived at 6 months. Twelve patients took radiotherapy only (6 adenocarcinoma and 6 squamous

cell carcinoma) and only 1 patient with squamous cell carcinoma (SCC) survived through follow up period.

DISCUSSION

Lung malignancy is associated with significant morbidity and mortality throughout the world. NSCLC contributes to around 80-85% of lung cancer and has got better prognosis than small cell carcinoma.[10] Remarkable research and development has been done in understanding and treatment of disease in the recent past few years. Targeted therapies have been recently used in the treatment of advanced cancers. Epidermal growth factor receptor (EGFR) inhibitors are used most commonly for targeted therapy. EGFR is one of the key molecules identified in lung carcinoma initiation and progression. It is overexpressed in various types of cancers, including lung cancer, especially NSCLC. Numerous studies have shown overexpression of EGFR is associated with advanced disease and very poor survival.[3,9,11] however many studies are not in agreement with it.[7,8] So, there is a need to study EGFR expression in NSCLC and its relation to survival as there is sparse data in South Indian population.

We found in present study 34/50(68%) were positive for EGFR expression which was comparatively lower than in the Italian population, which was 78% and higher compared to the Japanese population which was 34% and the Indian population (Mumbai) which had positive rate of 33%.[11,12,13] These variations can be explained by lack of uniformity in the cut-off values used for EGFR in these studies. As in our earlier study, we found adenocarcinoma more common than squamous cell carcinoma.[14] We found EGFR expression more common in adenocarcinoma subtype (58.8%) followed by squamous cell carcinoma (23.5%) which is in agreement with Korean study done by Ahn et al and study done in US by Shah et al but different from the Japanese population, Suzuki et al and in the Italian population, Ludovini et al, who found EGFR overexpression common in squamous cell carcinoma.[12,15,16,17]

EGFR overexpression and its relation to survival in patients with NSCLC has been studied for many years, but with conflicting results. We did not find any statistically significant association between EGFR status with survival (HR-1.13, p-0.75) similar to observations by Deeb et al and Lee et al who did not find association between EGFR expression and survival.[18,19] A recent meta-analysis failed to show any association between EGFR status and mortality (HR-1.14, p-0.10).[20] There are many studies which have found significant positive correlation between EGFR status and survival. Alison et al observed a 44% reduction in risk of death among EGFR positive patients.[21] Similarly studies done by Ucvet et al, Shah et al and Rusch et al found improved survival among EGFR positive patients.[16,22,23] In contrast to above studies, a meta-analysis done by Meert et al found EGFR over expression is associated with poor

survival (HR-1.13). Similarly, studies done by Selvaggi et al and Swinson et al found poor survival with EGFR over expression.[11,19,24]

We found that patients who were EGFR positive had better survival with chemotherapy [HR=3.69 (95% CI 1.48-9.14), p=0.004]. IRESSA trial done by Mok et al found that patients having EGFR mutation positive status and received Gefitinib-Paclitaxel combination chemotherapy had significantly better survival than the EGFR negative subjects who received the same combination [HR=0.48 (95% CI 0.36-0.64), p<0.001].[25] Similarly, IPASS (IRESSA Pan-Asia Study) trial done by Fukuoka et al found longer progression free survival for Geftinib-paclitaxel combination than doublet chemotherapy with carboplatin/paclitaxel in patients with EGFR overexpression positive tumors [HR=0.48 (95% CI 0.36-0.64), p<0.0001].[26] Thatcher et al and Hirsch et al also found better survival in patients receiving Geftinib who have EGFR positivity.[27,28]

Treatment with radiotherapy sequentially or concurrently with chemotherapy may provide benefit to the patients with unresectable locally advanced stage III NSCLC. We found that EGFR positive group who did not take radiotherapy had a statistically significant 30% less hazard of death compared to patients who took radiotherapy [HR=0.30 (95% CI 0.12-0.77), p=0.0012], which was in agreement with studies done previously. Animal study done by Milas et al showed that mouse which had high EGFR expression were radioresistant.[29] On multivariate analysis we found patients who took treatment, chemotherapy and with good performance status (Karnofsky score >65 and ECOG >2.5) had better survival at 6 months.

Performance status is an important prognostic factor for survival in various malignancies, including NSCLC. A population-based study done by Radzikowska et al evaluated Lung carcinoma in women (n=15657) showed that good performance status of the patients is significantly associated with better survival among lung cancer patients on multivariate analysis.[30] A study done by Mohamed et al evaluated performance status in patients with advanced NSCLC who were treated with gefitinib and found that good performance status in advanced NSCLC cases have a significant association with survival (p=0.0002).[31] Strength of present study is it is a prospective study with a six month follow up data on survival. Patients were evaluated for their EGFR status and survival, on which there was sparse data in the South Indian population. Limitations of the study were a small sample size and most subjects had advanced disease, hence our findings cannot be generalized to all subjects with lung cancer and requires further RCTs to confirm the beneficial effects of targeted therapy in EGFR positive cases.

We found in present study that patients with EGFR positivity had better survival with targeted chemotherapy but worse with radiotherapy. Patients who took chemotherapy and had good performance status had better survival on multivariate analysis. We did not find any correlation between EGFR positivity, intensity of EGFR expression (H Score) and poor survival.

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