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EPIDERMAL GROWTH FACTOR RECEPTOR OVEREXPRESSION IN IMMUNOHISTOCHEMISTRY HAS NO SIGNIFICANCE IN THE SURVIVAL OF PATIENTS WITH NONSMALL CELL LUNG CANCER

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ABSTRACT

Background: Lung cancer is the most common cause for the cancer related mortality worldwide. It is important to define the prognostic markers for this deadliest disease so that we can predict the survival of the patient and treatment outcome.

Objectives: *T*o estimate the prevalence of EGFR overexpression in NSCLC with immunohistochemistry and to determine its prognostic significance.

Materials & methods: 45 cases with histological diagnosis of NSCLC diagnosed in a tertiary care hospital, north Kerala were selected and immunohistochemistry for was performed on their bronchoscopic specimens and they were followed up and their survival at 6th month was noted.

Results: EGFR positivity was noted in 28.89% of nonsmall cell lung carcinoma. In this study We didn't get any significant correlation between EGFR overexpression and survival/histology/stage of the disease. A statistically significant correlation was seen between the stage of the disease and survival (p values – 0.003). Patients with stage III disease showed higher proportion of EGFR positivity but it was not statistically significant.

Conclusion: The prognostic significance of EGFR remains to be defined. IHC assesses the EGFR as total cellular level rather than its activated form, which is probably the only form affecting prognosis and IHC relies more on the subjective judgements. So the priority should be given to the standardisation of the techniques used to assess the overexpression of EGFR. But EGFR overexpression can be used as a predictive factor.

Keywords: EGFR – Epidermal Growth Factor Receptor; NSCLC – Nonsmall Cell Lung Carcinoma; IHC - Immunohistochemistry

INTRODUCTION

Lung cancer is the most common cancer in the world today and it accounts for about 12.6% of all new cancers and 17.8% of cancer deaths[(1)]. 20% and 41.5% of the current 5 million deaths in world, and 2.41 million in developing countries respectively is contributed by India and this

figure is estimated to reach 1.5 million by 2020[2]. Over the past fifty years there is only meagre increase in the five year survival rate of patients with lung carcinoma from 5 to 14% . The factors that independently determine the prognosis of nonsmall cell carcinoma lung include stage of the disease, performance status of the patient and histological subtype. EGFR , HER 2 , p16INK4 , cyclin E etc., are the known biomarkers that are involved in the development of carcinogenesis [3,4,5,6,7].

The EGFR and its ligands are essential for the normal and neoplastic epithelial cell growth. The epidermal growth factor receptor (EGFR) belongs to ErbB family of signalling receptors and it is a receptor with tyrosine kinase activity, and it is encoded by the gene located on chromosome 7p12. EGFR is one of the pathway that is essential for regulation of proliferation, cell survival, angiogenesis, and metastasis of the tumour cells , making the EGFR an attractive therapeutic target[8]. The EGFR has been implicated as a progression or prognostic factor in NSCLC, in which its overexpression is often detected. When the literature was reviewed there was heterogeneity of reports in relation between EGFR and prognosis of NSCLC. In our study we aimed at determining the prevalence of EGFR overexpression in NSCLC and to find out its significance in the survival of the patient.

MATERIALS & METHODS

The study was a Prospective study from January 2012 to September 2013. 45 patients diagnosed as non small cell carcinoma lung in bronchial biopsy specimens in a tertiary care hospital, North Kerala, India . Stage of the disease was assessed using 7th edition of the TNM staging for lung cancer. Samples of bronchoscopic biopsy obtained were fixed in 10% formalin and processed in automatic tissue processor (Leica Biosystems) and their paraffin embedded blocks were prepared. Thin sections were cut from the tissue blocks. One section was stained with Hematoxylin & Eosin (H&E) and one section stained by immunohistochemical marker for EGFR(Biogenex). EGFR immunohistochemistry staining was done on bronchial biopsies based on standard protocols and the intensity was analysed visually. Each patient was followed up for a minimum period of 6 months and survival at the 6th month was noted. We lost the follow up of one patient , so for the prognostic analysis only 44 patients were considered.

Assessment of EGFR immunohistochemistry:

EGFR positivity was graded as 0, 1+, 2+ & 3+

EGFR immunohistochemistry score = 1*(% of cells staining weakly[1+]) + 2*(% of cells staining moderately [2+]) + 3*(% of cells staining strongly[3+]) [9].

3+ - strong; visible at low levels of magnification, 5X objective lens;
2+ - moderate; staining visible at intermediate levels of magnification, 10X objective lenses
1+ - weak; only reliably confirmable at high magnification, 40X objective lens
0 - no staining visible at high magnification

Data was analyzed using standard analytical techniques with SPSS version 16.0 software. The associations between study variables were analyzed using Chi-square test and p values <0.05 were considered significant.

RESULTS

Most of the patients in this study were between the ages of 51- 60 with mean of 59.3. fig.1 and most of them were males with a male female ratio of 14:3 (males – 42; females – 3). Out of 45 patients 35 had squamous cell carcinoma and 10 had adenocarcinoma (Table – 1). About 80% of the patients presented with an advanced disease. As far as EGFR staining was considered 13 patients (28.89%) showed positivity [squamous cell carcinoma – 10(28.6%) & adenocarcinoma – 3 (30%) ; p value – 0.930] (Table – 2). Higher incidence of EGFR positivity was seen in stage III disease (Table – 3). As the stage advanced survival of the patient decreased and there was a statistically significant p value of 0.03 (Table – 4) and there was no statistically significant correlation between EGFR positivity and survival of the patients (p value- 0.205)(Table – 5).

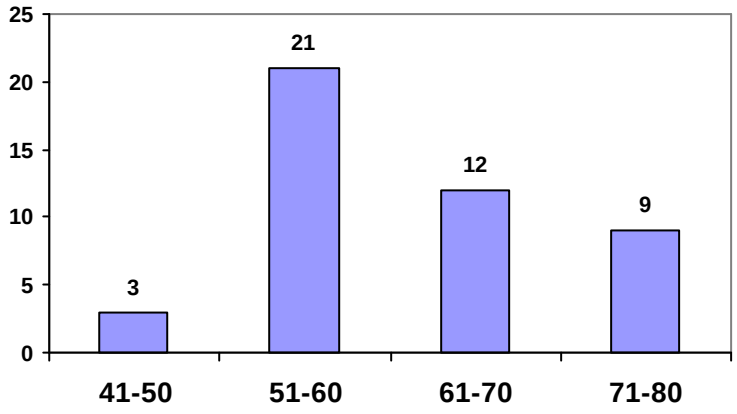

Figure 1 – Age distribution of the patients

Table- 1 : Diagnosis

Diagnosis		Frequency	Percent
Squamous Cell Carcinoma (N= 35)	Well differentiated	3	6.7
	Moderately differentiated	21	46.7
	Poorly differentiated	11	24.4
Adenocarcinoma (N=10)	Well differentiated	7	15.6
	Moderately differentiated	1	2.2
	Poorly differentiated	2	4.4
Total		45	100

Table – 2 : EGFR immunostaining

Diagnosis	Ihc score		Total
	Positive(%)	Negative(%)	
Squamous cell carcinoma	10(28.6)	25(71.4)	35(100)
Adenocarcinoma	3(30.0)	7(70.0)	10(100)
Total	13(28.9)	32(71.1)	45(100)
Pearson chi-square value- 0.008	P value – 0.930		

Table – 3: Stage and EGFR immunostaining

Stage	Frequency (%)	EGFR STAINING	
		Positive N (%)	Negative N (%)
IIA	7(15.6)	3(25.0)	4(12.1)
IIB	8(17.8)	2(16.7)	6(18.2)
IIIA	8(17.8)	3(25.0)	5(15.1)
IIIB	10(22.2)	4(33.3)	6(18.2)
IV	12(26.7)	0	12(36.4)
TOTAL	45(100)	12(100)	33
Pearson Chi-Square value- 6.718		P value – 0.152	

Table – 4: Stage of the disease & survival of the patients

Stage	Survival		Total(%)
	<=6 months(%)	>6 months(%)	
IIA	0 (0)	7 (100)	7 (100)
IIB	1 (12.5)	7 (87.5)	8 (100)
IIIA	4 (50)	4 (50)	8 (100)
IIIB	6 (60)	4 (40)	10 (100)
IV	9 (81.8)	2 (19.2)	11 (100)
TOTAL	20 (45.5)	24 (54.5)	44 (100)
Pearson Chi-Square value- 16.124		P value – 0.003	

Table – 5: EGFR immunostaining & survival of the patients

		Survival		
		<= 6months	> 6 months	
IHC score	Positive(>200)	4	9	13
	Negative(<200)	16	15	31
	Total	20	24	44
Pearson Chi-Square value- 1.605		P value – 0.205		

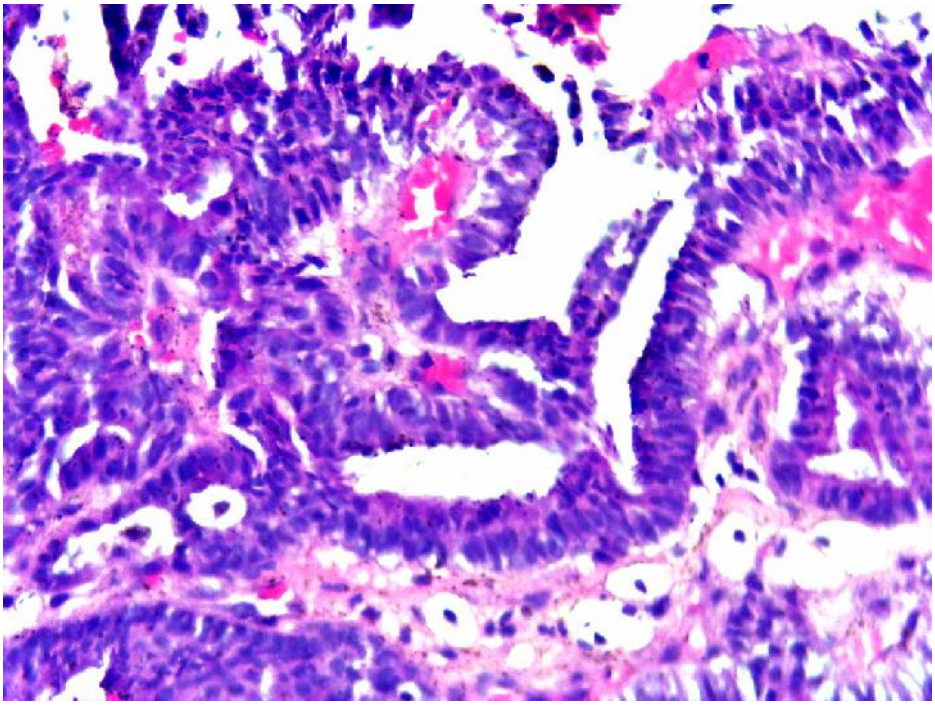

Figure 2 - Adenocarcinoma– H&E section X 400

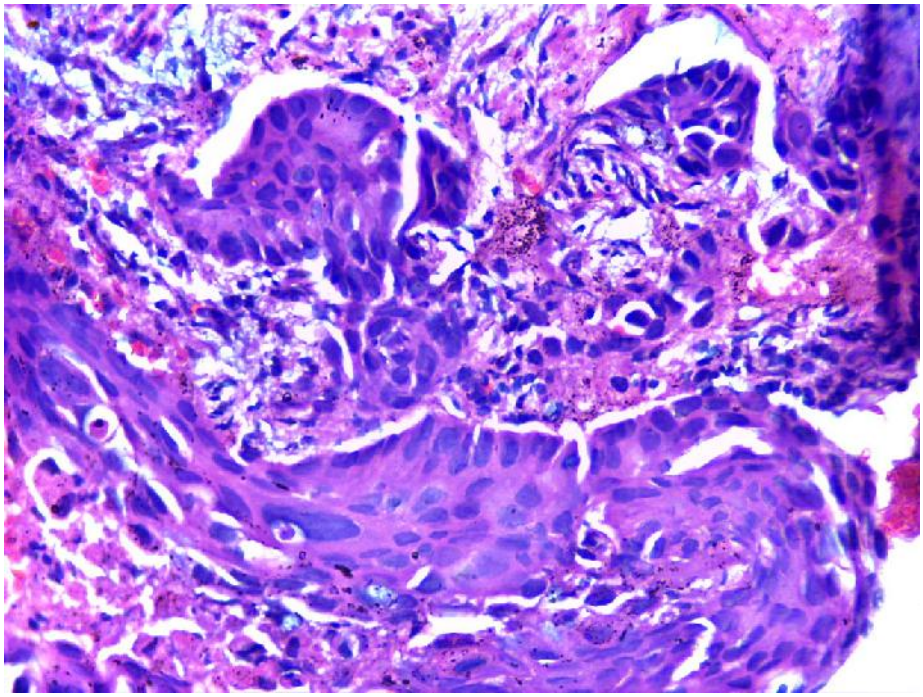

Figure 3: Squamous cell carcinoma moderately differentiated H&E section X 400

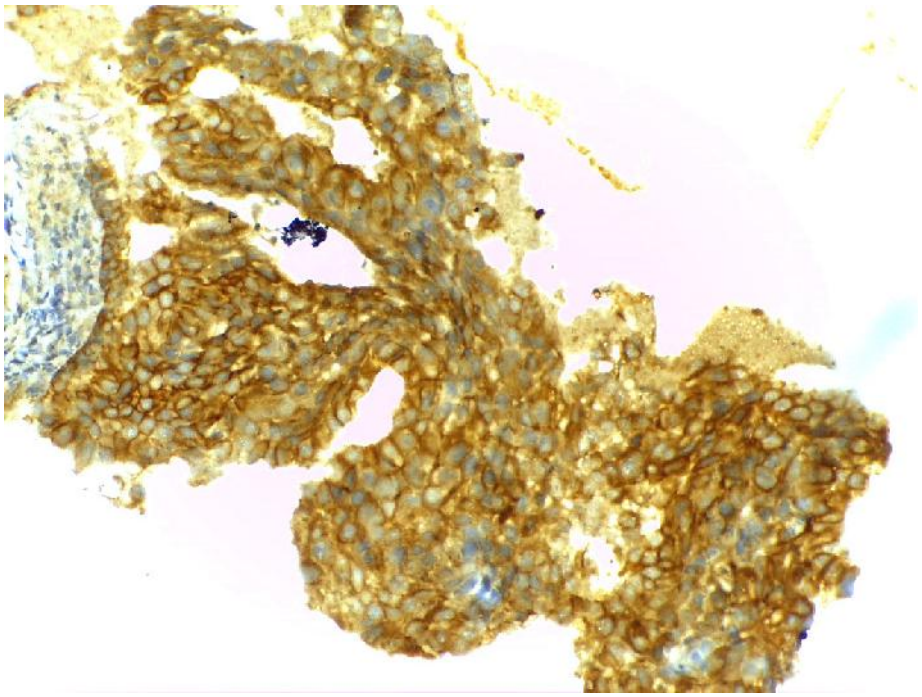

Figure 4 - Squamous cell carcinoma showing 3+ positivity for EGFR staining x 200

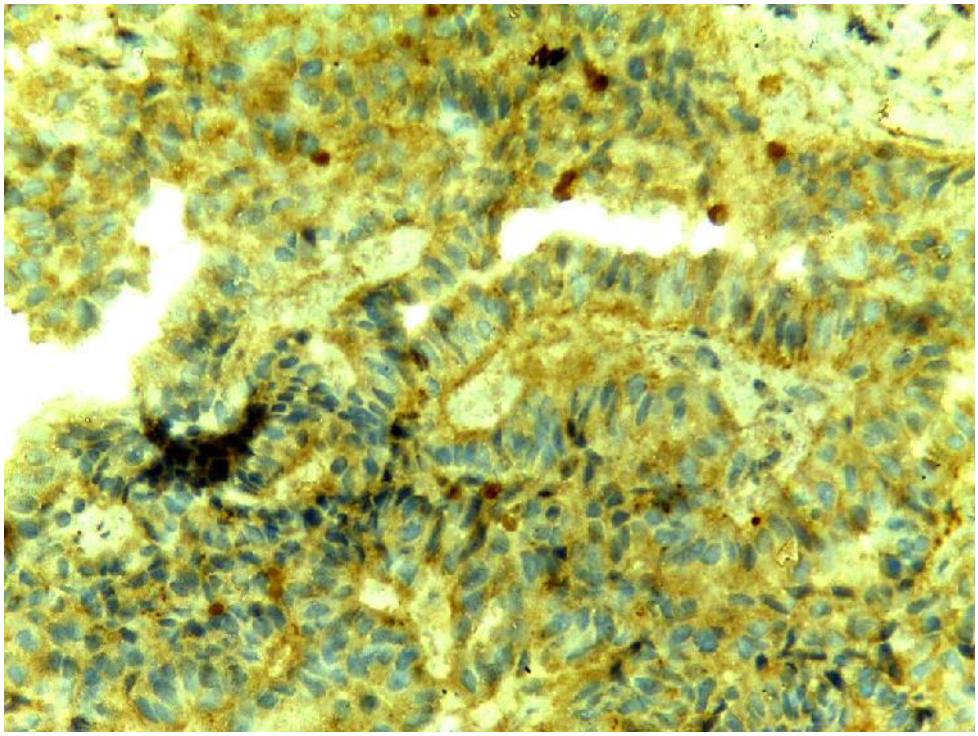

Figure 5 - Adenocarcinoma with 2+ positiivity for EGFR staining x400

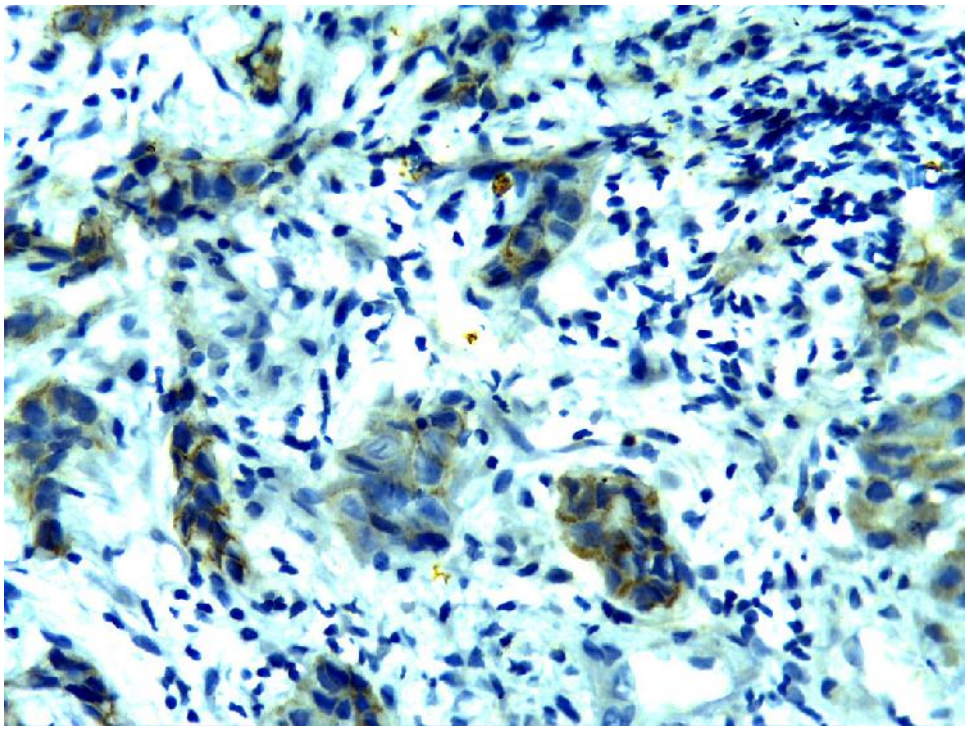

Figure 5 - Squamous cell carcinoma with 1+ positiivity for EGFR staining x 400

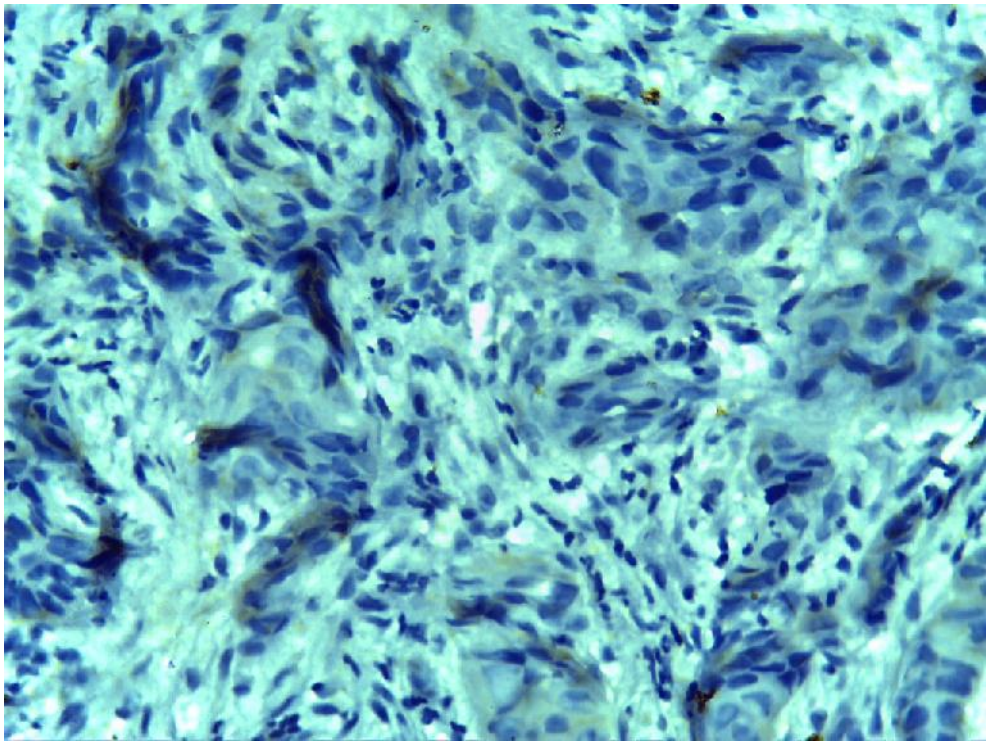

Figure 6 - Squamous cell carcinoma negative for EGFR staining x400

DISCUSSION

The relationship between EGFR overexpression in immunohistochemistry and survival of the patient was not statistically significant in our study. This was a prospective study with relatively small number of patients (45) and we lost follow up of one patient and in survival analysis only 44 patients were included.

EGFR positivity was observed in 26.7% of nonsmall cell carcinoma lung. Selvaggi et al [(10)] got 37% positivity but in that study both 2+ and 3+ were considered as positive. Veale et al [(11)] got 22.1% positivity in NSCLC. Out of 44 cases were followed up, 20 cases (45%) died with in first six months of diagnosis. The period of survival decreases with the advancement of the disease and we got a significant P value (0.003) for the correlation between the stage of the disease and 6 months survival. Pathologic stage was the only independent prognostic factor in a multivariate analysis for recurrence and prognosis in a study conducted by Kim [(12)] et al. In our study the higher incidence of EGFR overexpression was seen in stage III disease but significant statistical value was not there may be because of small sample size. Fontanini et al[(13)] suggested that EGFR is a better predictor of mediastinal lymphnode involvement by NSCLC.

The relationship between EGFR overexpression in immunohistochemistry and survival of the patient was not statistically significant in this study(P value – 0.205 for 6 month survival). This is in contrast to selvaggi et al [(10)] he showed that patients with EGFR overexpression had poor outcome and significantly shorter period of survival. Kim YT[(12)] et al in his study he states that previous studies that sought to determine the utility of EGFR as a prognostic factor in NSCLC have had mixed results, possibly due to confounding factors such as clinical characteristics and the small sizes of the study populations . And according to their study presence of the EGFR mutation may not be a true prognostic factor for long-term survival in NSCLC. In the review from Nicholson et al[(14)] EGFR overexpression confirmed its prognostic value in multiple tumor types, but evidence was weaker in NSCLC.

An additional bias is the lack of standardized cut-off points of normal receptor levels. The diverse results obtained by the various studies could be explained by the factors such as differences in interpreting the intensity of expression i.e., subjectivity in assessment, localization (cytoplasmic Vs membrane) of staining and by the wide range of methods in use for EGFR detection [(15)].

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