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Clinical Radiological and Immunohistochemical Profile of Non Small Cell Lung Carcinoma

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Abstract

Objectives: *To evaluate clinical, radiological and immunohistochemichal profile of non small cell lung carcinoma (NSCLC).* ***Settings and study design****: A cross sectional study conducted among all diagnosed cases of primary lung malignancy in the Department of Respiratory Medicine MES Medical College Perinthalmanna .* ***Materials and methods:*** *The 41 biopsy proven cases of NSCLC was studied during a period of 1 ½ years, A detailed history, clinical evaluation and the relevant investigation is done, small biopsy specimens are collected, a histopathological evaluation was done and the markers Thyroid Transcription Factor-1(TTF1), Epidermal Growth Factor Receptor (EGFR) and p-63 status was determine by immunohistochemistry(IHC)* ***Results:*** *Of the 41 cases, Squamous cell carcinoma was the predominant histological type with a male predominance and a peak incidence in 61-70 yrs of age. (58.53%) cases showed EGFR positivity. TTF1 positivity was predominant with adenocarcinoma and p63 positivity with squamous cell carcinoma. Smoking status and EGFR in adenocarcinoma shown that there is a significant number of EGFR positivity associated with non-smokers and all were females. The study could attain a 85.71% sensitivity and 92.59% specificity for ttf1 in adenocarcinoma and 88% sensitivity and 100% specificity for p63 in squamous cell carcinoma.* ***Conclusion:*** *IHC can be used as a rapid and effective tool for diagnosing the histologic type of NSCLC because of its high sensitivity and specificity. In adenocarcinoma, there is a significant number of EGFR positivity associated with non-smokers females.*

Introduction

Lung cancer was considered as a rare disease in the beginning of the century. But it is now the leading cause of cancer related mortality in developed countries and is rising in alarming rates in developing countries. In India, lung cancer constitutes 6.9% of all new cancer cases and 9.3% of cancer related deaths in both sexes. In men, lung cancer is the leading cause of cancer related deaths and 2nd leading cause of cancer related death in women. Kerala is one of the most affected state in India with lung cancer. Cigarette smoking is the most important modifiable risk factor for lung cancer accounting for about 80% of lung cancer death in men and 50% in women. Our understanding about the disease biology has improved over the past years and the classification is expanding from histological to molecular level.

Molecular targets and driver mutation play a major role in pathogenesis and has lead to the development of targeted therapy. Diagnostic markers and prognostic markers for evaluating the survival of patients in lung carcinomas are the latest developments in this field in which studies are in progress. Mutation of the EGFR gene in lung carcinomas makes the disease more responsive to treatment with Tyrosine Kinase inhibitor (TKI). TTF-1 is a nuclear protein expressed mainly in thyroid and lung (type II pneumocytes). It is a master regulatory transcription factor for tissue specific gene. It is a highly specific marker for primary lung adenocarcinoma and is associated with better survival in NSCLC. P-63 is considered as the single best marker to separate squamous cell carcinoma and adenocarcinoma. These proteins

can be identified with the help of specific Immunohistochemichal markers in small biopsy specimens.

Studies on the EGFR mutation and other molecular markers are available in the literature. But no much studies in this aspect is available from India especially Kerala where lung cancer is a major killer disease.

Materials and methods

All biopsy proven case of NSCLC coming to the Respiratory Medicine Department of MES Medical College from 1st January 2014 to 31st September 2015 were included in the study. The study had a cross sectional study design and the sample size was calculated as minimum 30 cases.

A detailed history, clinical evaluation and the relevant investigations are done, small biopsy specimens are collected, a histopathological evaluation was done and the markers TTF1, EGFR and p-63 status was determine by IHC (avidin-biotin complex).

EGFR scoring is done by H-Score method. Here a score of 0-300 is assigned to each patient. This is obtained by multiplying percentage of cells stained and intensity of staining. Intensity of staining is divided into absent, mild, moderate and severe staining. Absent staining is given 0 staining, mild staining is given 1 score, moderate staining is given 2 score and strong staining is given 3 score. H-Score </= 200 is considered as negative and >200 is taken as positive. The data is accumulated in a preformed proforma and statistical analysis done.

Results

A total of 41 patients were included in the study that had biopsy proven diagnosis of NSCLC. Squamous cell carcinoma was the predominant histological type identified from my study (table:1).

Table 1

total	adenocarcnoma	Squamous cell carcinoma
41	14	27

Mean age at diagnosis was calculated as 62.66 years. Among the total cases studied, 80.49%were males and 19.51% cases were males. Squamous cell carcinoma was the predominant histological type among males and adenocarcinoma among female (fig: 1) s. Squamous cell carcinoma was more among smokers and adenocarcinoma among non-smokers (fig: 2). All females were non smokers but gave a history of passive smoking.

Most of the patients had dyspnoea as the presenting symptom and cough was the next commonest symptom. Chest pain and haemoptysis were more common among squamous cell carcinoma. Clubbing was more associated with squamous cell carcinoma (73%). The most common radiological presentation was mass lesion (54%) both in adenocarcinoma and squamous cell carcinoma. The next commonest presentation among squamous cell carcinoma was collapse (22%) and in adenocarcinoma, it was pleural effusion (28%) (Fig: 3). Most of the cases presented at a late stage of the disease, 56% of cases presented as Stage 4 disease (fig: 4).

Small biopsy specimens were collected for histopathlogical diagnosis. The commonest procedure used was CT guided biopsy (table: 2). Squamous cell carcinoma were more centrally placed whereas adenocarcinomas were more peripheral in distribution.

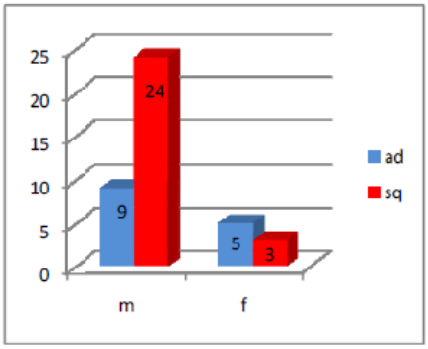

Figure 1. male female distribution.

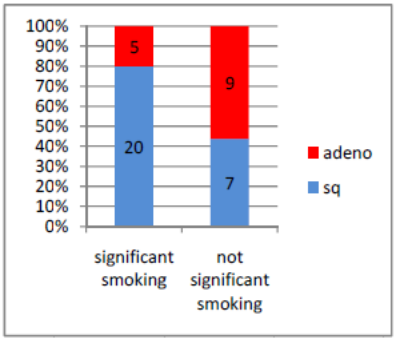

Figure 2. smoking status in the histological types.

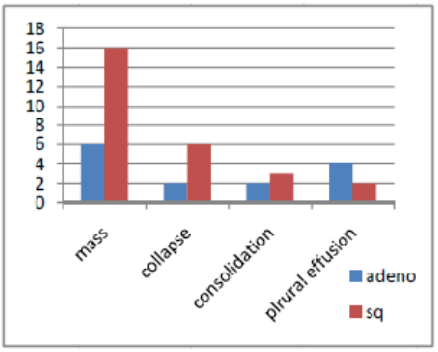

Figure 3. Radiological presentation of NSCLC

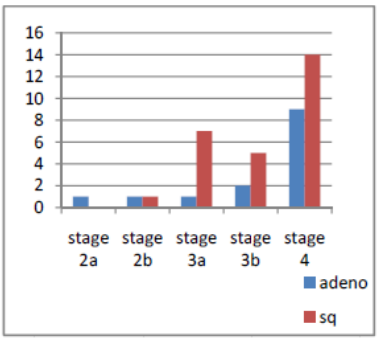

Figure 4. Stage at presentation.

Table 2 common procedures to get histological specimen.

PROCEDURE	total	adenocarcinoma	Squamous cell carcinoma
CT guided biopsy	22	9	13
Lymphnode biopsy	4	2	2
Bronchoscopic biopsy	11	2	9
Pleural biopsy	4	1	3

The immunohistchemichal markers which were considered in this study were EGFR, TT1, p63. Of the total, 24 cases (58.53%) cases showed EGFR positivity (fig: 5). EGFR protein expression by IHC was associated with squamous cell carcinoma (79.16%) (Fig: 6). TTF1 positivity was predominant with adenocarcinoma (fig: 7). the study could attain an 85.71% sensitivity and 92.59% specificity for ttf1 in adenocarcinoma. Majority of the squamous cell carcinoma showed P63 positivity (fig 8) and none of the adenocarcinoma showed P63 positivity. 88% sensitivity and 100% specificity for p63 in squamous cell carcinoma was attained from this study (Table 3).

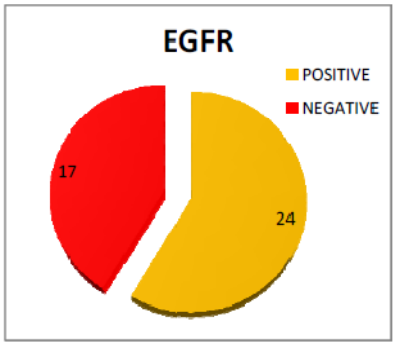

Figure 5. EGFR positivity in total

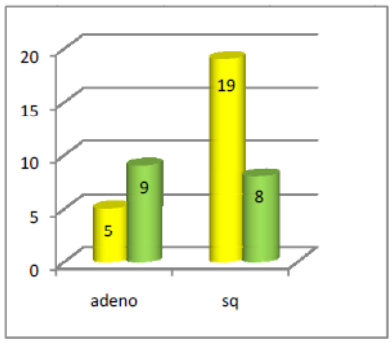

Figure 6. EGFR protein expression by IHC

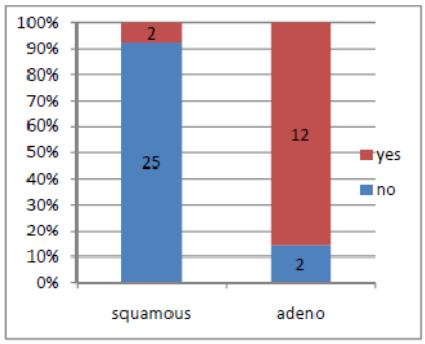

Figure 7. TTF1 Positivity was predominant with adenocarcinoma.

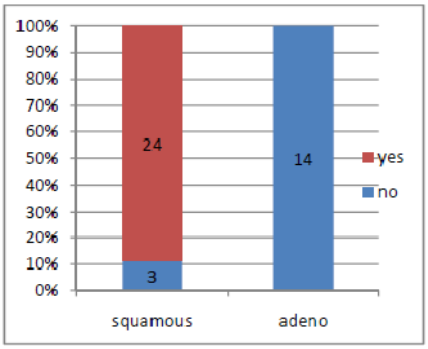

Figure 8. p63 positivity was more with squamous cell carcinoma.

Table 3. Adenocarcinoma

sensitivity	85.71%
specificity	92.59%

Squamous cell carcinoma

sensitivity	88%
specificity	100%

Smoking status and EGFR in adenocarcinoma shown that there is a significant number of EGFR positivity associated with non-smokers (fig: 9) and all were females (fig: 10). There was no relation between stage at diagnosis and EGFR status.

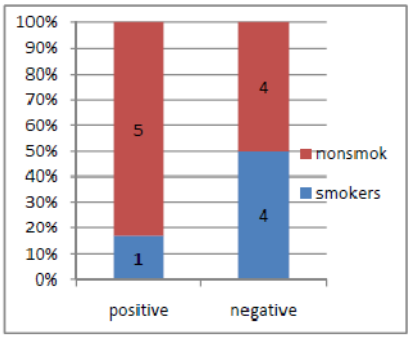

Figure 9. EGFR positivity was associated with Non-smokers

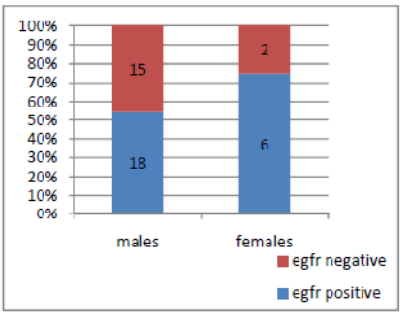

Figure 10. EGFR status and gender

Discussion

The world wide statistics shows that lung cancer is the most commonly diagnosed cancers (1.61 million, 12.7% of the total) and the most common causes of cancer deaths (1.38 million, 18.2% of the total) . The incidence of lung carcinoma in India is on the rise, Non-small-cell

lung cancer constitutes 75 - 80% of lung cancers with a male predominance (M: F ratio: 1.7:1)[1] and Kerala is one of the most affected state in India[2, 3]. More than 70 % of them are in Stages III and IV thus curative surgery is not an option for treatment.

When age group affected was considered, this study results showed that the maximum number of cases were diagnosed in 61-70 year age group, which were consistent with the World wide Globcon statistics and studies done in India by D.Behra[4.5]. The male female ratio was calculated to be 4.1:1 and the datas from various studies over years shows that even though lung cancer is seen more in males, there is an increasing trend in occurrence of lung cancer in females[4].

Smoking remains the most important preventable risk factor associated with lung cancer[6,7,8]. It is being well proved that Passive smoking or second hand smoking can cause cancer in non-smokers. The main mechanism of tobacco smoking induced lung cancer is the damage to the DNA, including the genes that protect against cancer.

Lung cancer is broadly classified as Small cell and NSCLC. NSCLC is further classified into squamous cell, adenocarcinoma and large cell carcinoma[9]. The statistical data from India has shown that the most prevalent lung cancer is NSCLC and Squamous cell carcinoma accounts for 44.73% and Adenocarcinoma 30.26%. With newer advanced techniques, our understanding about the disease biology has improved. This has helped us to expand the diagnostic modalities from histological to molecular level. Until recently, a non confirmatory diagnosis of non-small cell lung carcinoma—not otherwise specified (NSCLC-NOS) was accepted because of the inability of morphology to distinguish some poorly differentiated tumors usually because of inadequacy of specimen[10]. Now with the advancement in the therapeutic strategy, accurate histological diagnosis of non-small cell carcinoma and its sub typing in small specimens is important[11,12] and evidence suggest that IHC is a highly effective supporting tools for distinguishing adenocarcinoma and squamous cell carcinoma and its subtype[13]. The ESMO consensus conference that predictive IHC can reduce the NSCLC-NOS rate to <10%, typically 5%–6%[14] and so, IHC can be used in the routine diagnosis of lung cancer, in order to identify biological markers (diagnostic and prognostic). The role of IHC is to recognize antigens and, thus to identify and classify specific cells within a cell population whose morphology is heterogenous or apparently homogenous. Common markers used for non-small cell carcinoma subtyping include TTF-1 for adenocarcinoma whereas p63 and high-molecular weight keratins (CK5/6 and 34bE12/CK903) for squamous cell carcinoma[15]. TTF1 is a transcription factor that regulates the expression of multiple genes involved in lung development. It is predominantly expressed in lung adenocarcinomas and is been evaluated as a potential prognostic parameter in patients with lung cancer[16,17]. TTF1 is most commonly used to distinguish primary lung adenocarcinoma and metastatic lung cancer and also for differentiating primary lung adenocarcinoma from pleural mesothelioma[18,19]. P63 is a P53 homologous nuclear protein, which is expressed in basal cells of stratified squamous and glandular epithelia. In the lung, the highest expression is being consistently noted in squamous cell carcinomas.

Other markers associated with squamous cell carcinomas are, p40 and cytokeratin CK 5/6, while TTF1, Napsin A and CK7, as well as mucin stains, are associated with adenocarcinomas. The problem is that none are individually entirely tumor-type sensitive and specific. But the least IHC panel for subtyping NSCLC was TTF1 for adenocarcinoma and P63 for squamous cell carcinoma. The IATC/ATS/ERS has subtyped NSCLC based on the IHC status (table 4)

Table 4. Subtyping of NSCLC based on IHC markers

Markers	Adenocarcinoma	Squamous cell carcinoma	Adenosquamous carcinoma	NSCLC-NOS/ metastasis
TTF-1	+	-	+	-
p-63	-	+	+	-

EGFR is a trans membrane glycoprotein with an extra cellular ligand -binding domain and an intracellular domain possessing intrinsic tyrosine kinase(TK) activity[20]. In-frame deletions in exon 19 and the exon 21 L858R substitutions are the most common *EGFR* mutations[21] and, combined, represent approximately 90% of all mutants[22]. The cause of EGFR mutation is unknown, but is not related to tobacco carcinogenesis. Eventhough not exclusive, these mutations are therefore more common in never smokers and distant ex-smokers, and females of younger age[23,24]. EGFR gene copy number determined by FISH, protein expression determined by IHC and EGFR tyrosine kinase mutations are all potential markers to be used as selection criteria in EGFR-targeted therapy of which IHC seems to be a simple, rapid, sensitive and reliable method to identify the commonest EGFR mutation in NSCLC and can be used as a rapid screening method. Phase III trials involving Asian, European and North American patients with metastatic disease whose tumours have activating EGFR mutations have demonstrated high response rates (70%) and significantly longer progression-free survival (PFS) in patients treated with EGFR TKIs, (Gefitinib, Erlotinib, Afatinib) as initial treatment when compared with those receiving chemotherapy[25,26]. The identification of active mutation in tyrosine kinase domain of EGFR and their response to novel tyrosine kinase inhibitors was one of the greatest leap in the management of NSCLC in selected cases. From multiple studies, it was identified that the phenotypes that respond to EGFR-TKI (Gefitinib, Erlotinib) were adenocarcinoma, non-smoker females-East Asian descent which was due to the presence of mutation in EGFR domain. Gefitinib is approved by the Food and Drug Administration (FDA) to treat locally advanced or metastatic non-small cell lung cancer (NSCLC) that has not gotten better after treatment with other chemotherapy[27].

NSCLC corresponds to 80%-85% of lung cancer and, although there is progression in the development of new chemotherapeutics, NSCLC prognosis remains unsatisfactory with a 5-year overall survival of less than 15%. Preliminary results of randomized clinical trials conducted with these TKIs have shown that their use in patients with advanced disease is effective and significantly increasing the survival of these patients, especially if they harbour mutations in the EGFR which are more frequently found in a subgroup of non-smoking, female patients, of Asian ethnicity and with adenocarcinoma histological sub-type[28].

Conclusion

Evaluating the profile of lung cancer was helpful in demonstrating the general pattern of distribution of NSCLC in the defined study group. IHC can be used as a rapid and effective tool for diagnosing the histologic type of NSCLC because of its high sensitivity and specificity. In adenocarcinoma, there is a significant number of EGFR positivity associated with non-smokers females.

Since there are supporting and contradicting reports on the relationship between EGFR protein expression by IHC and mutation status, the response to EGFR-TKI based on IHC status need to be evaluated further and correlated to mutation status for EGFR-TKI response. For this a large multicentered long term study is needed.

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