Original Article

Clinicopathological and immunohistochemical profile of non-small cell lung carcinoma in a tertiary care medical centre in South India

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ABSTRACT

Background: Lung cancer is a highly aggressive malignancy causing high morbidity and mortality. An increasing incidence of lung cancer has been observed in India. Currently, the classification of lung carcinoma has gone beyond small cell lung carcinoma and non-small cell lung carcinoma (NSCLC). Precise subtyping of poorly differentiated NSCLC into adenocarcinoma and squamous cell carcinoma has a direct impact on patient management and prognosis. With this background, many molecules are under study for developing targeted therapies. Epidermal growth factor receptor (EGFR) is one such biomarker considered to be useful in targeted therapy for adenocarcinoma. **Objective:** The aim of this study was to subtype poorly differentiated NSCLC based on the expression of thyroid transcription factor-1 (TTF-1) and p-63 and to evaluate EGFR expression in adenocarcinomas. **Materials and Methods:** A retrospective analysis of 84 cases of poorly differentiated carcinomas of the lung was performed. Paraffin sections were immunostained with TTF-1 and p-63 and the tumors were subtyped. EGFR expression was assessed in adenocarcinomas by immunohistochemistry. **Results:** Fifty-five percent of the NSCLC were adenocarcinoma, with a peak incidence between 61 and 70 years of age and a male predominance. EGFR was expressed in 89% of the adenocarcinomas. **Conclusions:** Poorly differentiated non-small cell carcinoma can be subtyped by immunohistochemical markers and hence has a direct impact on the current therapeutic strategies.

KEY WORDS: Epidermal growth factor receptor, lung carcinomas, p-63, TTF-1

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INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide. It is estimated that about 1 million people die of cancer every year.[1,2] Non-small cell lung carcinoma (NSCLC) accounts for 80-85% of all lung carcinomas, and adenocarcinoma is the predominant histologic type. The prognosis of these patients remains poor, with an overall 5-year survival rate of less than 15% despite the advanced therapeutic options available.[3] Recent studies suggest the existence of two distinct molecular pathways in the carcinogenesis of lung adenocarcinomas, one associated with smoking and activation of the k-ras oncogene and the other not associated with smoking and activation of epidermal growth factor receptor (EGFR). Introduction of targeted therapy with epidermal growth factor receptor–tyrosine kinase inhibitors (EGFR-TKI) has revolutionized the treatment of adenocarcinoma. Patients with these tumors survive significantly longer with EGFR-TKI therapy than with conventional chemotherapy.[3-5] The aim of this study was to subtype poorly differentiated NSCLC based on the expression of thyroid transcription factor-1 (TTF-1) and p-63 and to evaluate EGFR expression in adenocarcinoma. Somatic mutations within the tyrosine kinase catalytic domain of EGFR lead to conformational changes that promote permanent active status and are found in approximately 20% of lung adenocarcinomas. They are considered to be the most reliable predictors of response to EGFR-TKIs.[6-8] A large number of studies on EGFR expression status in lung carcinoma are available in the Western literature. Studies on EGFR mutation status in Indian NSCLC patients are limited.

MATERIALS AND METHODS

Paraffin blocks of all the cases of poorly differentiated NSCLC reported between 2006 and 2011 were retrieved from the archives of the Pathology Department of Sri Ramachandra Medical College and were included in the study. Five-micron-thick paraffin sections were cut and immunostained for TTF-1 and p-63 and the tumors were subtyped.[9] Immunohistochemical staining for TTF-1 was performed using the biogenex monoclonal TTF-1 mouse antibody; clone (BGX-397A) diluted in phosphate-buffered saline (PBS). Staining for p-63 was performed using biogenex monoclonal p-63 mouse antibody; clone (4A4) diluted in PBS. Subtyping of NSCLC into adenocarcinoma and squamous cell carcinoma was based on the morphology and immunohistochemistry (IHC). Subtyping of NSCLC was based on the algorithm followed by the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IATC/ATS/ERS) International Multidisciplinary Team.[10,11]

All the adenocarcinoma cases were immunostained with EGFR antibody and the results were analyzed. Immunohistochemical staining for total EGFR protein was performed using the biogenex monoclonal EGFR rabbit antibody; clone (Ep38Y) pre-diluted in PBS on both control and test sections according to the manufacturer's instructions. Slides were scored based on the cytoplasmic and/or membrane staining intensity as follows: 0 - no staining or faint staining intensity in < 10% of tumor cells; 1+ = faint staining in > 10% of tumor cells; 2+ = moderate staining; 3+ = strong staining.[6]

RESULTS

Ninety cases of carcinoma lung were diagnosed during the 5-year period from 2006 to 2011. Of these, non-small cell carcinoma accounts to 93% (84 cases), followed by small cell carcinoma (3%), carcinoid tumor (1%), mucoepidermoid carcinoma (1%) and pleomorphic carcinoma (1%)[Table 1] [Chart 1]. Of the 84 cases of NSCLC, 53 cases (63%) were males and 31 cases (37%) were females. Fifty-five percent of the NSCLC were adenocarcinoma[Figure 1] with a peak incidence between 61 and 70 years of age [Charts 2 and 3]. Of the 84 cases of NSCLC, 46 cases (55%) were immunohistochemically proven primary adenocarcinoma of the lung. This was followed by squamous cell carcinoma (24%), adenosquamous carcinoma (4%) and non-small cell carcinoma-not otherwise specified (NSCLC-NOS)(18%)[Table 2]. Subtyping of NSCLC was based on the algorithm followed by the IATC/ATS/ERS International Multidisciplinary

Team [Table 3]. TTF-1 was expressed in 55% of the adenocarcinoma cases[Figure 2] and p-63 was positive in 24% of squamous cell carcinoma[Figures 4 and 5]; both markers were positive in different tumor cells in 4% of the cases and were considered adenosquamous carcinoma. Eighteen percent of the tumors were negative for both markers and were hence considered as NSCLC-NOS with a possibility of metastasis [Chart 4]. Semiquantitative scoring of adenocarcinoma for EGFR positivity showed 3 + positivity in 85% of the cases[Figure 3], 2 + positivity in 10% of the cases and 1 + positivity in 5% of the cases. EGFR was positive in 89% of adenocarcinoma and negative in 11% of the cases [Charts 5 and 6].

DISCUSSION

The incidence of lung carcinoma in India is on the rise. NSCLC accounts for 80-85% of all lung carcinomas, and adenocarcinoma is the predominant histologic type with a male predominance (M: F ratio: 1.7:1). Tobacco smoking continues to be the leading cause of lung cancer worldwide. However, an increase in the incidence of adenocarcinoma among non-smokers and women is noted in North America and Europe. Globally, the overall lifetime risk of lung cancer is about 1 in 13 for men and 1 in 16 for women. The risk is significantly higher for smokers and lower for non-smokers. Unfortunately, despite the therapeutic advances, the prognosis of patients with lung cancer (5-year overall survival rate of 15%) has not changed dramatically in the past 30 years.[12-14] Currently, diagnostic and treatment approaches to lung carcinoma, mainly adenocarcinoma, are undergoing a revolution. The classification of lung

Table 1: Distribution of all types of lung carcinoma

Tumor types	Number of cases (90)	%
Adenocarcinoma	46	51
Squamous cell carcinoma	23	26
Small cell carcinoma	3	3
Carcinoid	1	1
Mucoepidermoid carcinoma	1	1
Pleomorphic carcinoma	1	1
NSCLC-NOS	15	17

NSCLC/NOS: Non-small cell carcinoma-not otherwise specified

Table 2: Subtyping of non-small cell lung carcinoma based on immunohistochemistry

TTF	P-63	Subtypes	%	Number of cases (84)
+	−	ADC	55	46
−	+	SqCC	24	20
+	+	Adenosquamous	3	3
-	-	NSCLC-NOS	18	15

NSCLC/NOS: Non-small cell carcinoma-not otherwise specified,
ADC: Adenocarcinoma

Table 3: Subtyping of NSCLC based on IHC markers

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

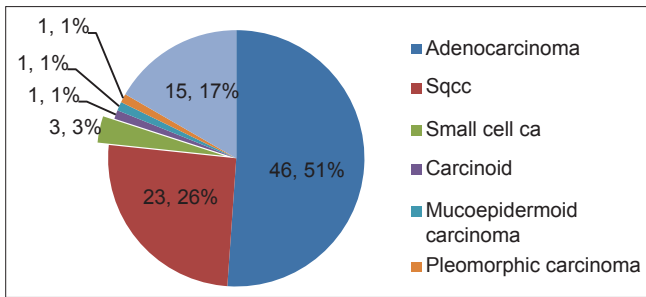SCC: Squamous cell carcinoma, ADC: Adenocarcinoma, NSCLC/NOS: Non-small cell carcinoma-not otherwise specified
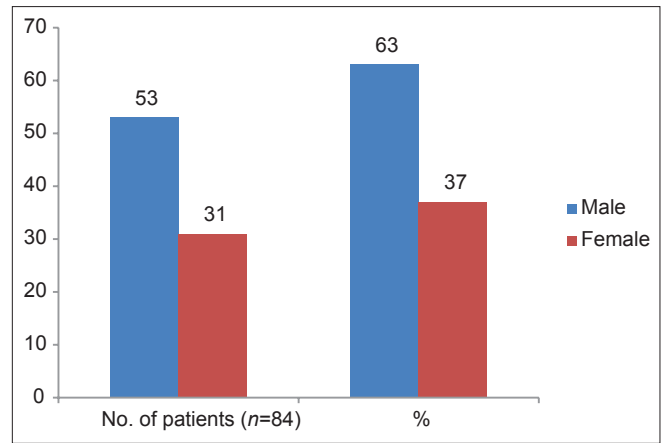
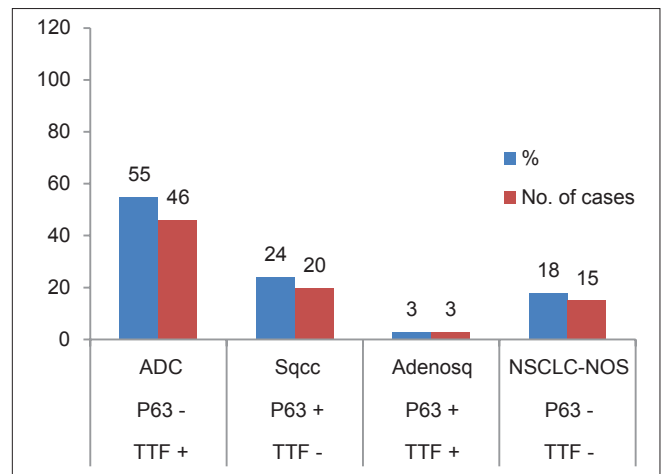**Chart 1:** Prevalence of lung carcinoma

Chart 3: Age-wise distribution of non-small cell lung carcinoma

Chart 5: Immunohistochemical scoring of epidermal growth factor receptor in adenocarcinoma

Chart 2: Demographic profile based on gender distribution

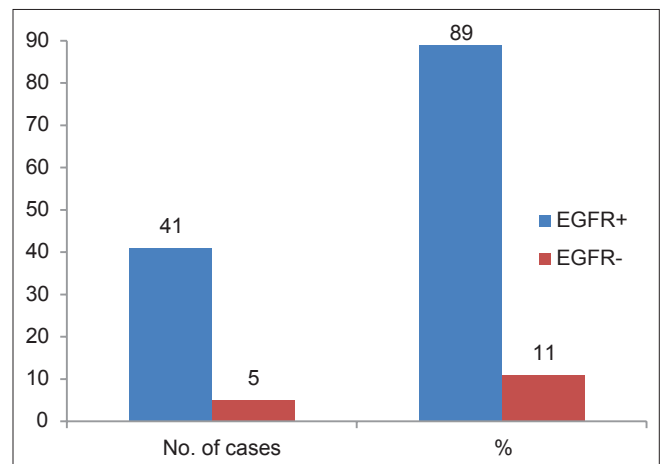**Chart 4:** Subtyping of non-small cell lung carcinoma

Chart 6: Distribution of epidermal growth factor receptor in adenocarcinoma

carcinoma is going beyond small cell carcinoma and non-small cell carcinoma. Precise subcategorization of NSCLC into adenocarcinoma and squamous cell carcinoma has a direct impact on patient management and prognosis. Presence of EGFR mutations in adenocarcinoma is a predictor of responsiveness to EGFR tyrosine kinase inhibitors. The diagnosis of lung carcinoma is a multidisciplinary process requiring correlation with clinical, radiologic, molecular and surgical information.[10] The World Health Organization has published guidelines for the classification of lung cancer in resection specimens based on the examinations of the entire tumor; however, 70% of the lung cancers present at advanced stages and are unresectable and hence subjected

to platinum-based chemotherapy or radiation therapy. Therefore, these guidelines are often not applicable. Hence, a new classification has been proposed by the IASLC/ATS/ERS for small biopsies and cytology samples. This new classification emphasizes the use of histochemical (mucin
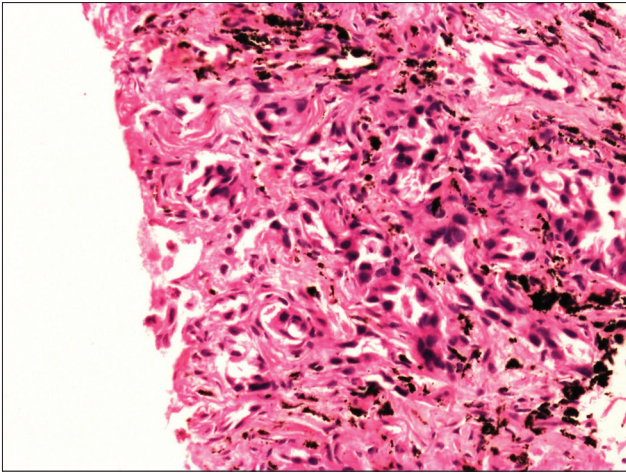
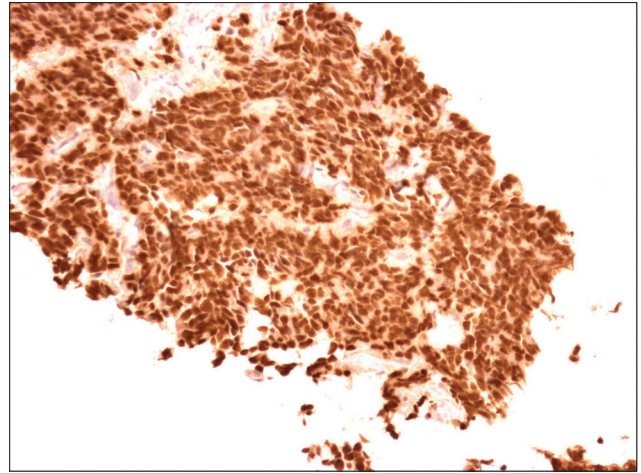**Figure 1:** Microphotograph of adenocarcinoma (hematoxylin and eosin, x200)

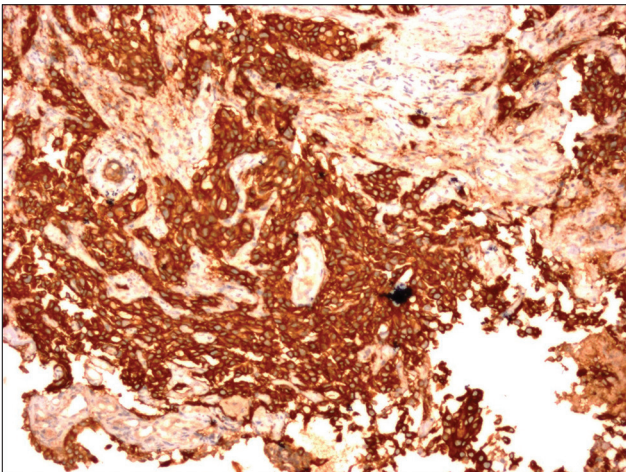**Figure 2:** Microphotograph of adenocarcinoma (thyroid transcription factor-1, x200)

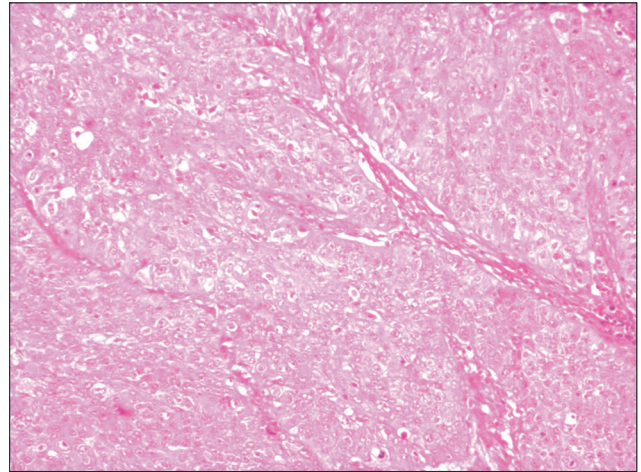**Figure 3:** Microphotograph of adenocarcinoma (epidermal growth factor receptor, x200)

Figure 4: Microphotograph of squamous cell carcinoma (hematoxylin and eosin, x100)
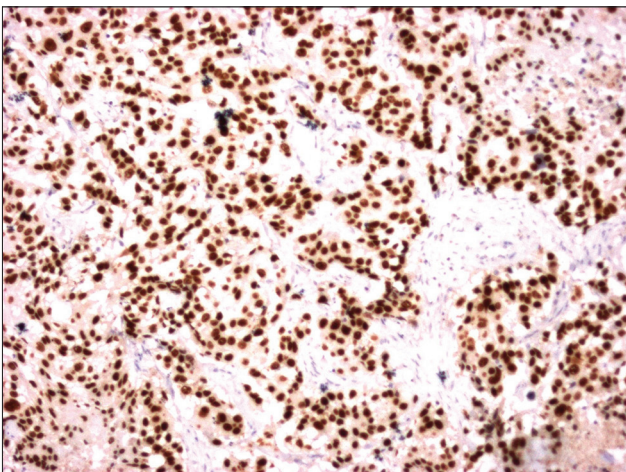

Figure 5: Microphotograph of squamous cell carcinoma (p-63, x100)

staining) and immunohistochemical stains (TTF-1, p-63) and molecular studies apart from routine histomorphology on hematoxylin and eosin-stained slides.

Morphologic diagnosis forms the basis and is further supplemented by a panel of immunohistochemical markers. Tumor cells in adenocarcinoma are positive for TTF-1, Napsin and cytokeratin-7. Squamous cell carcinomas are positive for p-63, cytokeratin-5/6 and NTRK-1 and NTRK-2. TTF-1 is a protein that regulates transcription of genes specific for thyroid, lung and developing central nervous system (diencephalon).[15,16] It is encoded by the NKX2-1 gene and is seen in chromosome 14q. It is produced by clara cells and type II pneumocytes of the peripheral bronchioalveolar unit. It is a highly specific (97-100%) but not very sensitive (54-75%) marker for adenocarcinoma.[15] Tumor protein p-63 is encoded by TP63 gene, a member of the p-53 family of transcription factors. It is mapped to chromosome 3q. It is expressed in the normal respiratory epithelium of the central air conducting system and does not carry any prognostic implications in NSCLC patients. The sensitivity of p-63 ranges from 75% to more than 95%, whereas the specificity for squamous cell carcinoma is between 70% and 100%. Napsin A is moderately sensitive (79-85%) and

highly specific (100%) for adenocarcinoma. Mucin stains are valuable markers, but the sensitivity and specificity for adenocarcinoma can be variable. NTRK1 and NTRK2 are highly specific for squamous cell carcinoma, but are rarely used outside research laboratories. TTF-1 and p-63 can be used as a reliable diagnostic tool in subtyping these tumors in routine practice. Difficulty arises in subtyping NSCLC in small biopsies due to sampling error and tumor heterogeneity. Loo *et al.* reported that the combination of TTF-1, Alcian blue/PAS, p-63 and CK5/6 in small biopsies is best in differentiating adenocarcinoma from squamous cell carcinoma. Nicholson *et al.* also used PAS with diastase, p-63, TTF-1 and CK5/6 to subtype NSCLC into adenocarcinoma and squamous cell carcinoma. We used a panel of two markers, TTF-1 and p-63, in subtyping 84 cases of poorly differentiated carcinomas. TTF-1 was positive in 55% of adenocarcinoma and P-63 was positive in 27% of squamous cell carcinomas. TTF-1 and p-63 were negative in 18% of the cases and favored metastatic adenocarcinoma. Terry *et al.* studied the expression of nine immunohistochemical markers on 588 lung carcinomas, of which 200 cases were adenocarcinoma and 225 were squamous cell carcinoma. The sensitivity of TTF-1 was found to be 62% and the specificity was found to be 92%. p-63 is considered as the single best marker to separate squamous cell carcinoma and adenocarcinoma, with a sensitivity of 84% and a specificity of 85%.[15] Screening for EGFR expression and mutation analysis would be of great value in designing treatment protocols in NSCLC. Patients with these tumors survive significantly longer on EGFR-TKI therapy than with conventional chemotherapy. Rosell *et al.* evaluated the EGFR mutations in 350 cases of 2105 patients (16.6%). Mutations were frequently found in women (69.7%), in those who never smoked (66.6%) and in those with adenocarcinomas (80.9%).[17] The presence of KRAS, HER2, BRAF, PI3K, LKB1 and SHP2 mutations is associated with a lack of response to EGFR-TKIs in the treatment of lung cancer. Therefore, additional mutational analysis of genes other than EGFR may be necessary to improve patient selection for EGFR-targeted therapies, and EGFR amplification and/or overexpression are also predictors of response to TKI treatment. The EGFR overexpression accounts for about 43-83% of NSCLC, being more common in squamous cell carcinoma (70%), followed by adenocarcinoma (50%) and, to a lesser extent, in large cell carcinoma.[3,18,19] The EGFR mutation status is best determined by fluorescence *in situ* hybridization (FISH) or chromogenic *in situ* hybridization (CISH) and protein expression determined by IHC with mutation-specific antibodies. EGFR is expressed in 50% of NSCLC, and its expression is correlated with poor prognosis.[3] Ruschoff *et al.* evaluated the interobserver reproducibility of the EGFR IHC scoring system based on both the tumor cell membrane staining intensity (graded 0 to 3+) and the percentage of cells staining at each intensity. This allowed a highly reproducible allocation of NSCLCs into clinically relevant high or low EGFR expression groups.[20] Our study revealed a much higher percentage of EGFR expression (89%), indicating the importance of screening our patients with adenocarcinoma lung.

CONCLUSIONS

The current therapeutic strategies for lung cancers require accurate morphological differentiation between adenocarcinoma and squamous cell carcinoma. A panel of immunohistochemical markers, TTF-1 and p-63, help in subtyping the poorly differentiated carcinomas. Adenocarcinoma was more common than squamous cell carcinoma in our study. TTF-1 and p-63 immunostain were extremely useful in making a conclusive diagnosis, especially in small biopsies with a poorly differentiated morphology. EGFR mutation is a predictive biomarker for EGFR-TKI therapy. In our study, 89% of the adenocarcinomas showed EGFR positivity and 11% were EGFR negative.

Limitation and future plan
Although the status of EGFR has been assessed by IHC, CISH and FISH, mutational analysis has been shown to be the best predictor of tumor response to EGFR-TKI, which will be assessed in future studies.

ACKNOWLEDGMENT

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The Lung India Awards

The Lung India awards for the year 2013 were given during award session of Indian Chest Society's annual general body meeting held at NAPCON conference in Chennai. A team of three experts constituted the awards committee. All the original articles and letter to editor were ranked on the basis of predefined objective parameters. The website records were analyzed for selection of the best referee and the prompt referee. Dr. Mandeep Kang of department of radiodiagnosis & imaging, at PGI, Chandigarh was selected for the best original article award. Dr. Parvaiz A. Koul of department of internal and pulmonary medicine and clinical research & geriatrics, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir, Dr. Prem Parkash Gupta of department of respiratory medicine, Postgraduate Institute of Medical Sciences, Pandit. B.D. Sharma University of Health Sciences, Rohtak, and Dr. Sajal De of department of pulmonary medicine, Bhopal Memorial Hospital and Research Centre, Bhopal were selected for the outstanding original article awards. Dr. Sunil Kumar Raina of department of community medicine, Dr. Rajendra Prasad Government Medical College, Tanda, Kangra, Himachal Pradesh was awarded for the best letter to editor. Dr. Ashutosh N. Aggarwal of department of pulmonary medicine, PGI Chandigarh received the best referee award. The prompt referees' awards were given to Dr. Navneet Singh of department of pulmonary medicine, PGI Chandigarh, Dr. Nazir A. Khan of department of radiational oncology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir, and Dr. O.D. Schoch of department of pneumology, Canton of Sankt. Gallen-Hospital, Switzerland. We congratulate them all and wish them a great success in their future carrier.