BJMHR
British Journal of Medical and Health Research
Journal home page: www.bjmhr.com

Immunohistochemistry In the Diagnosis and Typing of Lung Carcinomas

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ABSTRACT

To study the clinical and radiological presentation of lung cancer and diagnose the type of lung cancers using cytohistopathology and immunohistochemistry where-ever required. The study was carried out on 200 patients of lung cancer. Cytological investigations included sputum cytology, pleural fluid examination, percutaneous needle aspiration, trans-bronchial needle aspiration and FNAC lymph-node. Histopathology of paraffin embedded sections of lung biopsies was done. Immunohistochemical staining was done where ever required. Out of 200 cases, cytology was helpful in typing malignancy in 165 cases (82.5%).In the remaining 35 cases, 21 cases (10.5%) were suggestive of malignancy and 14 cases (7.0%) were inconclusive. On histological evaluation of 64 cases, typing was possible in 48 cases (71.8%) and 18 cases (28%) were reported as poorly differentiated carcinoma. After applying immunohistochemistry in 18 cases of poorly differentiated malignancies, 13 cases(72.2%) were positive only for CK5/6 as squamous cell carcinoma, 4 cases were positive for TTF1, out of which 3 were positive for CK7 making it as 4 cases(22.2%) of adenocarcinoma. One case (5.5% was positive for CK5/6, CK7 and TTF1 and was diagnosed as adeno-squamous carcinoma. Combination of various cytological techniques following additional use of histological evaluation of lung biopsies where ever required has raised the sensitivity of diagnosing lung cancer from 82.5% to 91.0% and immunohistochemistry clinched the diagnosis in poorly differentiated lung cancers.

Keywords: Lung cancer, Cytohistopathology, Immunohistochemistry

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Received 19 June 2017, Accepted 30 June 2017

Please cite this article as: Akhtar K *et al.*, Immunohistochemistry In the Diagnosis and Typing of Lung Carcinomas. British Journal of Medical and Health Research 2017.

INTRODUCTION

Lung cancer is the most frequently diagnosed cancer and also the leading cause of all cancer associated deaths in the world.[1] Previously bronchogenic carcinoma was considered to be infrequent in India, but in the recent past a trend of increase in its incidence has been noticed.[2] Lung cancer has been estimated to be the most frequent among all the new cases of cancers in male in this country.[3]

Unfortunately, early lung cancer has non-specific symptoms or signs and by the time patient is aware of the disease, it has progressed too far. Despite advancement in imaging techniques such as spiral computed tomography of the chest and molecular diagnostics, cytology continues to play the prime role in the diagnosis and management of lung tumors. The oldest and most fundamental method is based on sputum cytology, which depends on the spontaneous exfoliation of malignant cells.

Bronchial biopsy has also been used as the gold standard diagnostic test to assess the efficacy of other cytological techniques.[4] In general the concordance between cytology and histopathology ranges from 70% to 90% and the bronchial biopsy is confirmatory for most of the cytological findings.[3]

The current World Health Organization (WHO) classification of lung tumors states that histological typing is largely based on hematoxylin and eosin (H&E) staining, and specific histotypes can be adequately assessed only when surgical samples are available.[5] Therefore, there is an increasing need for additional diagnostic techniques such as immunohistochemistry (IHC).[3] Several different panels of IHC markers may be useful to define a specific cell lineage—namely, distinguishing squamous cell carcinoma from adenocarcinoma —not only on surgical material, but also on cytology or biopsy samples.[6,7]

The present study has been conducted with the aim to diagnose and identify the type of lung cancer based on clinicopathological investigations, using immunohistochemistry where ever required.

MATERIALS AND METHOD

This study was conducted on 200 patients attending the out-patient and in-patient departments of TB and Respiratory diseases and Pathology of JN Medical College Hospital, Aligarh. Patients were selected on the basis of complaints of cough, expectoration, hemoptysis, chest pain, fever, dyspnea and weight loss and radiological findings of lung mass and effusion on Chest X ray or CT scan. Detailed clinical history and examination was carried out along with routine investigations. Special investigations done were sputum cytology, pleural fluid (PF) examination, percutaneous needle aspiration (PCNA), transbronchial needle aspiration (TBNA) and FNAC of lymph node.

Smears were fixed in 95% ethanol and stained by Papnicolaou (PAP) and hematoxylin & eosin (H&E) stains. Histopathology of paraffin embedded sections of lung biopsies was done using Hematoxylin & Eosin. Periodic Schiff (PAS) and Van-Gieson (VG) staining was done wherever required. The cytological and histological diagnoses were compared. Immunohistochemical staining with immunomarkers CK5/6, CK7, CK20, CDX2, Synaptophysin, Chromogranin and TTF1was performed on paraffin sections for typing of malignancy according to the instructions on the kit supplied. (Bio Genex, CA 94583,USA)

RESULTS AND DISCUSSION

Majority of the cases (83.0%) were males with male- female ratio of 4.8:1. The mean age of the sample was 55 years with a range of 19-90 years. Duration of illness ranged from 1/2 to 24 months with a mean of 5 months. The most common clinical feature was chest pain, which was present in 70.0% cases.

Out of 200 cases, 155 cases (78.0%) were smokers and 45 cases (22.0%) were non-smokers. Mean pack year was found to be 19.2 and ranged from 2.5-55 pack years.

Among the various techniques applied in cytological diagnosis, percutaneous needle aspiration (PCNA) was done in maximum number of cases i.e. 137 out of 200 cases and it gave a positivity of 77.0%. Twenty four of the 200 cases were subjected to transbronchial needle aspiration (TBNA), which showed 83.0% positive result. Six cases of secondaries in cervical lymph node yielded 100.0% positive result on FNAC. Sputum cytology showed positivity only in 35.0% cases of squamous cell carcinoma. Pleural fluid cytology showed 100% positivity for mesothelioma and sarcoma followed by 83.0% in adenocarcinoma.

Out of 200 cases reported in cytology, typing of malignancy was possible in 82.5% cases. Eight percent were highly suggestive of malignancy, 2.0% were suggestive of adenocarcinoma and 7.0% were inconclusive and were advised biopsy for further confirmation and typing. In 16 out of 35 cases which were suggestive of malignancy on cytology, 7 cases turned out to be squamous cell carcinoma on histology, 4 cases as adenocarcinoma and 5 cases as Small cell carcinoma. All 5 cases which were suggestive of adenocarcinoma on cytology were diagnosed as squamous cell carcinoma on histology. Fourteen cases reported as inconclusive on cytology were diagnosed as 4 cases of squamous cell carcinoma, 3 cases as bronchiolo-alveolar carcinoma, 2 cases as small cell carcinoma and 5 cases as poorly differentiated carcinoma on histology.

Immunohistochemistry was performed in 18 cases of poorly differentiated malignancies. Thirteen cases were diagnosed as squamous cell carcinoma with CK5/6 positivity. Four cases were positive for TTF1, with malignant cell nuclei lining the glands showing strong positivity (Figure 1), and CK 7and diagnosed as adenocarcinoma. One case was positive for both

CK5/6, CK7 and TTF1 and was diagnosed as adeno-squamous carcinoma, with cytoplasmic Cytokeratin 5/6 positivity in the malignant squamous component. (Figure 2) Squamous cell carcinoma was found to be the commonest lung cancer in our study (46.0%), followed by 40.0% cases of adenocarcinoma along with bronchiolo-alveolar carcinoma and 10.0% of small cell carcinoma, with cytoplasmic positivity of synaptophysin in the small pleomorphic tumor cells with hyperchromatic moulded nuclei. (Figure 3)

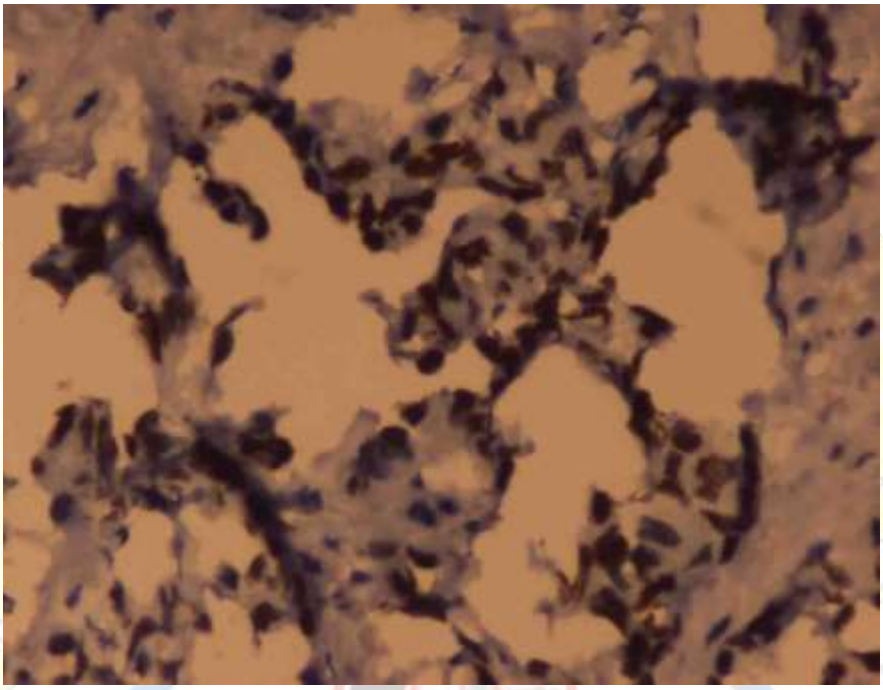

Figure 1: Adenocarcinoma: Malignant cell nuclei lining the glands showing positivity for TTF1. TTF1 x 500

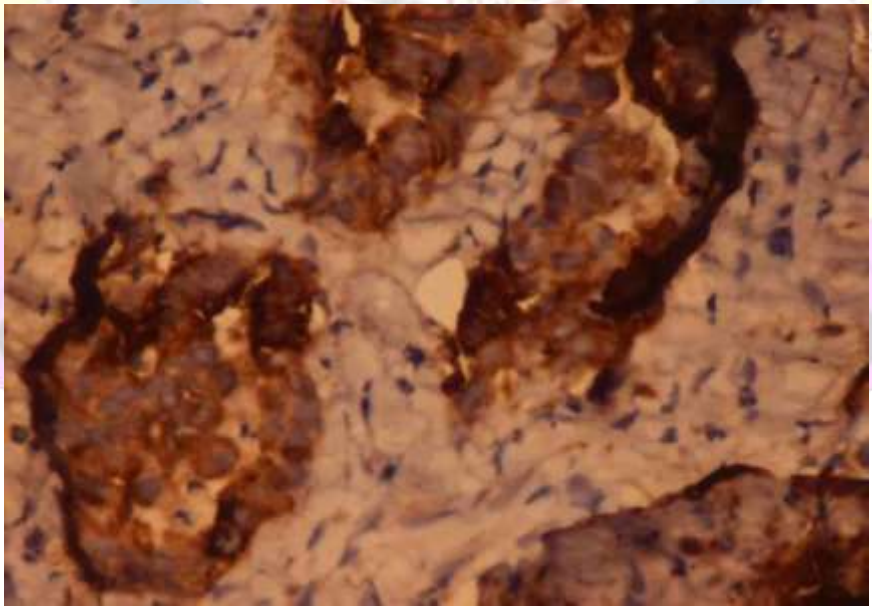

Figure 2: Adeno-squamous carcinoma: Groups of malignant cells with dense cytoplasm showing cytoplasmic Cytokeratin 5/6 positivity in the squamous component. CK5/6 x500

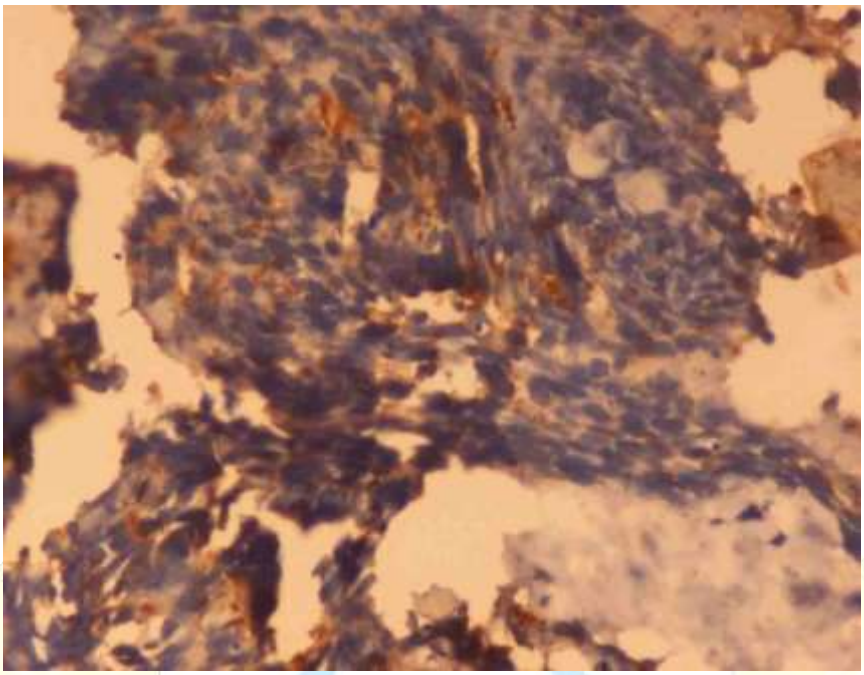

Figure 3: Small cell carcinoma: Small pleomorphic tumor cells with hyperchromatic moulded nuclei with cytoplasmic positivity of synaptophysin. Synaptophysin x500

DISCUSSION

The present study was conducted with the objectives to correlate the findings of bronchoscopy and biopsy for diagnosis of lung cancer and diagnose the type of lung cancers using routine stains and immunohistochemistry.

In the present study, the most frequent cytological technique performed was PCNA which was positive in 77% cases. Nguyen et al., found cytodiagnostic accuracy rates by PCNA of 75.5%, 72.0%, 100.0% and 50.0% for adenocarcinoma, squamous cell carcinoma, small cell carcinoma and large cell carcinoma respectively.[8] Shah et al., reported diagnostic accuracy rate of 95.0% with PCNA.[9] In a study conducted by Layfield et al.,overall sensitivity was 90.0% and specificity of 92.0%.[10] But he reported that sensitivity of PCNA varies according to size and location of the lesion, which varies between 82.0% in centrobasal portion to 100.0% in peripherally located nodule.

TBNA was positive in 83% of our cases. Castella et al., performed TBNA in 194 patients with bronchogenic carcinoma and showed that TBNA was positive in 87.0% cases of central lesion and 69.0% in peripheral lesion.[11] It shows a high yield of positive results in central lesion as compared to peripheral mass.

In our study, when histopathological evaluation was performed for inconclusive cases on cytology, the diagnostic efficacy of malignancies rose from 82.5% to 91.0%. Drăgan et al., showed cyto-histological correlation in 163 cases, out of which 66.0% cases were diagnosed as malignant on cytology and 87.0% cases on histology.[12] Gong et al., have documented the

reliability of FNA in the diagnosis of intrathoracic malignant tumors, with diagnostic accuracy of 90- 95%.[13] Tuladhar et al., showed 84.2 % diagnostic sensitivity of bronchial biopsy.[14]

In our study, one case of small cell variant of squamous cell carcinoma showed CK5/6 positivity and was synaptophysin negative. Travis et al, showed cytokeratin 5/6 was positive in 93% of the studied lung squamous cell carcinomas whereas Stojsic J et al., showed cytokeratin 5/6 immuno- expression in 100% of squamous cell carcinomas, 7.4% of adenocarcinomas and 28.6% of 7 non-small cell lung carcinomas – unclassified type.[5,15]

Out of 8 cases of adenocarcinoma in our study, 75.0% were positive for CK7 and 88.0% were positive for TTF1, which showed that adenocarcinoma did not show 100% positivity for the two markers, where the diagnosis was made on morphology. None of the cases showed positivity for either CK20 or CDX2 ruling out metastatic adenocarcinoma of the lung. Ninty four percent of adenocarcinomas have been reported to express TTF-1.[16] CK7 is particularly useful, when used in combination with CK20, in identifying colon cancer metastases to the lung, which is CK20 positive and CK7 negative.[17]

In small cell carcinoma, 2/8 cases were strongly positive and 6/8 cases were weakly positive for synaptophysin and chromogranin confirming the origin from neuroendocrine cells. Lyda and Weiss have reported that chromogranin, synaptophysin and neural cell adhesion molecule (NCAM)-CD56 are the most reliable and widely used neuroendocrine markers offering confident results with high sensitivity and specificity.[18]

Out of the 18 cases of poorly differentiated carcinoma in our study, 13cases were positive only for CK5/6 and diagnosed as squamous cell carcinoma. Four cases were positive for TTF1, out of which 3 were also positive for CK7, and were diagnosed as adenocarcinoma. One case was positive for both CK 5/6, CK7 and TTF1, and was labeled as adenosquamous carcinoma. Stojsic J et al., concluded that no one monoclonal antibody is totally specified for one histological type of tumor and its origin.[15] Combination of TTF-1, cytokeratin 7, p63, cytokeratin 5/6, CD56 and synaptophysin allows for differentiation of non- small cell lung carcinoma but Napsin-A for adenocarcinoma differentiation and chromogranin A for non-small cell carcinoma-neuro-endocrine differentiation should be added in an optimal panel.

CONCLUSION

Combination of various cytological techniques i.e. sputum, pleural fluid cytology, percutaneous needle aspiration, transbronchial needle aspiration, FNAC lymphnode with additional use of histological evaluation of lung biopsies, where ever required has raised the sensitivity of diagnosing lung cancer and Immunohistochemistry is a final tool for diagnosis.

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