ABSTRACT

Introduction: Pulmonary adenocarcinoma harbors various molecular abnormalities that include epidermal growth factor mutation, anaplastic lymphoma kinase gene re-arrangement, K-RAS mutations. The availability of targeted therapy against these molecular markers has revolutionized personalized medicine. Accurate cytological diagnosis of pulmonary adenocarcinoma will aid in utilizing the cytology smears for molecular testing.

Objective: The objective of this retrospective study was to evaluate the diagnostic efficacy of various cytology samples in the diagnosis of pulmonary adenocarcinoma.

Materials and Methods: The study included a retrospective case series of 50 patients with biopsy proven non small cell lung carcinoma of adenocarcinoma subtype. The corresponding cytology reports of all the 50 cases were analyzed for different samples including broncho-alveolar lavage (BAL), bronchial washings, bronchial brush smears, pleural fluid, sputum and guided fine needle aspiration cytology (FNAC) of lung and metastatic lymph nodes. The overall cyto-diagnosis efficacy as well that of various cytological samples were analyzed.

Results: Multiple cytology samples were received in 14 of 50 cases. The overall diagnostic efficacy of the various cytology samples in the diagnosis of malignancy was 78% and 66.6% cases were accurately typed as non small cell lung carcinoma-adenocarcinoma. The best cytological sample for the detection of pulmonary adenocarcinoma was bronchial brush smears which had a detection rate of 70%. In fine needle aspiration cytology samples and bronchial washings the detection rate was 65.5% and 25% respectively.

Discussion and Conclusion: In cases where the cytological diagnosis is certain the tissue biopsies can be simultaneously tested for EGRF and ALK gene mutations. Repeat biopsies are often required due to small amount of tumor tissue or necrotic biopsies. Alternate use of cytological specimen for molecular testing can be done when a diagnosis of pulmonary adenocarcinoma is established.

Key words: Adenocarcinoma; cytology; pulmonary

Introduction

Lung cancer is a complex neoplasm. Lung cancer is responsible for more deaths each year than colon cancer, breast cancer, and prostate cancer combined (12.6% of all new cancers and 17.8% of cancer deaths).[1] In India, approximately 63,000 new lung cancer cases are reported each year. Lung cancer is relatively more important in the developed than developing countries as it accounts for 22% versus 14.6% of cancer deaths, respectively.

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This dismal mortality makes early diagnosis and treatment essential for improvement in the morbidity and mortality.[2,3]

Cytology is being increasingly used for the evaluation and diagnosis of pulmonary malignancies. Various sampling techniques are available to procure samples for cytologic evaluation in lung malignancies. These include exfoliative cytology samples such as induced sputum, abrasive cytology samples [bronchial brushing, bronchial washing, bronchioalveolar lavage (BAL)] and fine-needle aspiration cytology (FNAC), which can be endobronchial ultrasound-guided, transesophageal, computed tomography (CT)-guided percutaneous or transthoracic.[4,5]

Non-small cell lung carcinoma (NSCLC), adenocarcinoma subtype harbors various molecular abnormalities that include epidermal growth factor mutation, anaplastic lymphoma kinase gene rearrangement, and KRAS mutations. The availability of targeted therapy against these molecular markers has revolutionized personalized medicine.[2] Accurate cytological diagnosis of pulmonary adenocarcinoma will aid in utilizing the cytological smears and biopsy tissue for molecular testing. The objective of this retrospective study was to evaluate the diagnostic efficacy of various cytology samples in the diagnosis of pulmonary adenocarcinoma.

Materials and Methods

This was a retrospective case series conducted in the department of pathology of a tertiary care hospital. The study included 50 cases of NSCLC-adenocarcinoma subtype, which was diagnosed on histology with or without the aid of immunohistochemistry. Immunohistochemistry was performed on formalin fixed paraffin-embedded tissue sections using the standard protocol for cytokeratin 7, cytokeratin 5 out of 6, or p63 and thyroid transcription factor 1 (TTF-1). The corresponding cytology reports of all the 50 cases were retrieved from the records. A comparative analysis of the cytological and histological diagnosis was done. The various cytology samples submitted for evaluation included BAL, bronchial washings, bronchial brush smears, pleural fluid, sputum, FNAC of primary lung lesions, and metastatic lymph nodes. The cytological samples were processed using the standard protocol and both wet-fixed and air-dried slides were prepared. The slides were stained with May-Grünwald-Giemsa (MGG), Papanicolaou (Pap), and hematoxylin and eosin (H and E) stains. The overall cytodiagnostic efficacy as well as that of various cytological samples were analyzed.

Results

This retrospective case series included 50 biopsy proven cases of NSCLC. In 14 cases, more than one cytological sample was submitted for evaluation and 11 out of 14 cases (78.57%) cases were reported as malignant or suspicious of malignancy. In cases where a single sample was submitted, 28 out of 36 (77.5%) cases were reported as malignant or suspicious of malignancy.

Bronchial brushing smears were received in 12 out of 50 cases (24%) and 10 out of 12 cases (83.3%) were reported as either malignant or suspicious for malignancy. In 70% of the cases, the cytodiagnosis was accurately reported as pulmonary adenocarcinoma [Figure 1].

Figure 1: Bronchial brush smear cytology: (a-d) A case reported as non-small-cell lung carcinoma: Sheets and clusters of atypical cells with high nucleocytoplasmic ratio, moderate amount of cytoplasm, and pleomorphic vesicular nuclei on a background of hemorrhage and reactive bronchial epithelial cells (red arrow) (e-h) A case reported as pulmonary adenocarcinoma: brush smears with high cellularity, sheets, clusters, and aggregates of malignant glandular cells with marked nuclear enlargement and vesicular nuclear chromatin. [H&E = Hematoxylin and eosin, Pap = Papanicolaou]
FNAC of the lung was done in 35 out of 50 cases (70%) and 29 out of 35 (82.8%) were reported as either malignant or suspicious of malignancy. Based on the cytomorphology, nine cases were reported as NSCLC while accurate subtyping of NSCLC-adenocarcinoma subtype was performed in 19 cases (65.5%) [Figure 2]. One case was reported as suspicious of malignancy due to low cellularity.

Bronchial washings were received in nine out of 50 cases (18%) and 44% cases (four out of nine) were reported as either malignant or suspicious for malignancy. NSCLC-adenocarcinoma subtype was accurately diagnosed in one case [Figure 3] while two cases were reported as suspicious for malignancy. In one case, the cytodiagnosis rendered was poorly differentiated carcinoma.

BAL specimens were received in five out of 50 cases and all five cases were reported as negative/inadequate.

Post-bronchoscopy sputum samples were received in eight cases and in one (12.5%) case, the cytomorphology was suspicious of malignancy.

Pleural fluid was received for cytopathological evaluation in four out of 50 cases and one case (25%) was reported as positive for malignant cells [Figure 4].

FNAC from suspicious axillary lymph nodes was performed in two out of 50 cases and in one case (50%), the node revealed metastatic deposits from adenocarcinoma.

The overall diagnostic efficacy of the various cytology samples in the diagnosis of malignancy was 78% and 66.6% of the cases were accurately typed as NSCLC-adenocarcinoma.

**Discussion**

The global trend of rise in adenocarcinoma is paralleled in India.[6] Pulmonary adenocarcinoma usually arises in a peripheral location and has many variants including acinar, papillary, mixed acinar papillary, and solid. Bronchoscopic-aided cytological sampling is useful in the evaluation of pulmonary adenocarcinoma. Cytological sampling is usually done as a daycare procedure; hence, it contributes as an additional benefit as hospitalization is avoided.[4]
Bronchoscopy and guided techniques play a definitive role in the diagnosis of endobronchial lesions. Bronchoscopy provides direct visualization of the airways and permits focused sampling of the lesion with high yield of cells/tissue. Bronchial washings are obtained by instilling normal saline into the bronchus and withdrawing the fluid by suction to collect washings from a large area. Bronchial brushings are obtained by a brush sample from the surface of the tumor under direct visualization. BAL samples the cellular exudates in the peripheral airways and alveolar spaces by instillation and aspiration of aliquots of normal saline into a bronchoscope trap. Leyden in 1883 and Menbriel in 1986 introduced pulmonary FNAC as a diagnostic tool for pulmonary malignancies and infections. FNAC for pulmonary lesions can be endobronchial ultrasound-guided, transesophageal, or CT-guided percutaneous or transthoracic.[4,5,7,8]

The diagnostic efficacy of multiple cytological samples is generally higher than a single sample. In our study, multiple samples were received in 14 cases and 78.57% were reported as malignant or suspicious of malignancy as compared to single samples where the diagnostic efficacy was 77.5%. This finding was similar to the results obtained by Gaur et al.[5]

Karahalli et al.[7] stated that a combination of washings and brushings, along with forceps biopsy increase the sensitivity of diagnosis of endobronchial lesions from 83.17% to 85.64% and 90.65%, respectively.

The overall diagnostic efficacy of the various cytology samples in the diagnosis of malignancy was 78% and 66.6% cases were accurately typed as NSCLC-adenocarcinoma. The sensitivity and specificity of pulmonary cytology in the diagnosis of both benign and malignant lesions are variable.[8] This discrepancy can be attributed to a number of factors, which include the site and size of the lesion, the stage of the disease and the technical skill employed to obtain the sample. The cellular yield of pulmonary cytology samples and cytomorphology is greatly affected by procedural factors. The presence of inflammation, necrotic debris, and crushing artefact has an impact on the diagnostic efficacy of cytology samples. Rapid and adequate fixation of cytology samples are also essential as excessive air drying artefact leads to misdiagnosis.[6,9]

Radiotherapy- or chemotherapy-induced cellular changes may induce significant cellular atypia.[9] Hence, it is essential for a cytopathologist to obtain proper clinical history to avoid overdiagnosis of malignancy. In a study conducted by Thivolet-Bejui,[10] the rate of false positive results in pulmonary lesions was about 1%. It is also essential to be aware of the mimics of pulmonary adenocarcinoma, which include reactive bronchial cells, reactive pneumocytes, bronchial cell hyperplasia, and goblet cell hyperplasia.[4,10]

In this study, the best cytological sample for the detection of malignancy as well as pulmonary adenocarcinoma was bronchial brush smears, which had a detection rate of 83.3% and 70%, respectively. This finding was in concordance with the results of the studies conducted by Chaudhary et al., Gaur et al. and Rawat et al.[5,8,11] The cellular yield of the bronchial brush cytology samples was high and the cytological characteristics were well-preserved.

The diagnostic efficacy of FNA samples for the detection of malignancy was 82.8% while 65.5% cases were accurately reported as pulmonary adenocarcinoma. Mondal et al.[12] stated that FNA is a simple, rapid, and reliable technique for the diagnosis of pulmonary mass lesion with a diagnostic efficacy of 91.93% for the detection of malignancy and 52.63% for the diagnosis of adenocarcinoma. In the FNA samples, the cellular yield was high and cytomorphology was well-preserved.

The cellular yield was low in bronchial washings and the detection rate was 25% for NSCLC-adenocarcinoma. In the study conducted by Rao et al.,[9] the detection rate for malignancy was 32.9%. The authors attributed low cellular yield-crushing artefacts and secondary inflammation as the factors responsible for low accuracy.

In this study, all the five BAL samples were reported as negative. This finding was contradictory to the results of Ahmed et al.[13] who stated that BAL cytology is a highly sensitive and specific test for diagnosis of carcinoma in the lung.
However, in the study conducted by Binesh et al.,[14] the detection rate for malignancy was 39% and the sensitivity for the detection of lung carcinoma was low.

Post-bronchoscopy sputum was positive for malignant cells in one case in this study. Sputum cytology is a noninvasive and relatively inexpensive technique for the detection of malignancy. However, three to five samples are necessary to achieve a good sensitivity and specificity.[4]

In this study, pleural fluid cytology was positive in one case and axillary lymph nodal metastasis was detected in one case. In such cases, the detection of the primary lesion is largely dependent on clinic-radiological correlation. To improve the diagnostic efficacy, cell block preparations can be prepared and immunohistochemical subtyping can be done to locate the primary tumor.[5,6]

The categorization of NSCLC into squamous cell type and adenocarcinoma is essential as the treatment protocol of both these tumors is different. In this study, an attempt was made to classify the lesions based on cytomorphology. In this study, the overall diagnostic efficacy for malignancy was 78% while 66.67% of the cases were accurately subcategorized as adenocarcinoma. Specific subtyping can be achieved in a high proportion of lung FNAs with high accuracy.[13]

In a retrospective study conducted by Yildiz-Aktas et al.[16] 54.6% of the tumors were accurately subcategorized as adenocarcinoma based on cytomorphology alone. This subcategorization was statistically significant suggesting that pathologists are cognizant of the impact that more specific cytomorphologic interpretations have on directing molecular triage and therapy.

The diagnostic difficulties in accurate cytological subtyping are due to nonrepresentative sampling with poor cell yield, secondary inflammation, or necrotic debris. Poor fixation and cell morphology preservation also play a major role. Subcategorization is also difficult in high grade tumors due to lack of acinar pattern and keratinization or in lesions with mixed cytomorphology [Figure 5].

In such cases, it is recommended that cell blocks be prepared and a primary panel of immunohistochemistry markers that include CK7, TTF-1, and p63 be done to accurately subcategorize the tumor.

Molecular testing in cytology samples is increasing widely. Various cytological preparations including FNAC, exfoliative samples, and liquid-based cytology preparations are being utilized for the testing of both prognostic and predictive markers in pulmonary adenocarcinoma. The preliminary results of the studies conducted by Schuurbiers et al.[17] and Smouse et al.[18] state that the detection rate of molecular markers in cytology samples is comparable to the histology/resections samples.[17,18]

**Conclusion**

Cytology is a useful tool in the diagnosis of pulmonary adenocarcinoma. In cases where the cytological diagnosis is certain, the tissue biopsies can be simultaneously tested for epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) gene mutations. Repeat biopsies are often required due to the small amount of tumor tissue or necrotic biopsies. Alternate use of cytological specimen for molecular testing can be done when a diagnosis of pulmonary adenocarcinoma is established.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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