Abstract

Background: Cytological evaluation of specimens from respiratory tract is the initial investigation in patients suspected to have pulmonary diseases. The various cytological specimens submitted for analysis include bronchoalveolar lavage, bronchial wash, brush smears, transbronchial needle aspiration, guided fine needle aspiration cytology (FNAC) smears and pleural fluid. Present study was undertaken to study the spectrum of lesions diagnosed by cytomorphological analysis of various cytological specimens. Materials and Methods: Centrifuged and direct smears from received samples were stained with MGG and PAP stain. Special stains (ZN and PAS) were used wherever required. Cytohistological correlation was done wherever biopsy was available. Results: This study included 671 samples from 583 patients suspected clinicoradiologically of having a respiratory pathology. A male preponderance (65.87%) was noted with 73.59% of patients in age group 40–80 years. Cytological diagnoses were classified as non-diagnostic (14.90%), negative for malignancy (59.76%), specific benign lesions (5.22%), positive for atypical cells (3.87%) and malignant (16.25%). Tuberculosis (TB) was found in 17 cases. Adenocarcinoma (both primary and metastatic) was the commonest malignancy of the 99 cases positive for malignancy. Incidence of primary lung adenocarcinoma and squamous cell carcinoma were found to be equal. Conclusion: Lung carcinoma is presently the leading cause of cancer deaths while TB is still a common cause of death in developing countries. Cytopathology has a definite role in diagnosis of malignant lesions and also contributes in diagnosis of unsuspected chronic infections. Cytological diagnosis is fairly accurate if the specimen obtained is adequate and representative.

Keywords: Cytology, lung carcinoma, pulmonary, tuberculosis

Introduction

Exposure to various environmental factors like airborne microorganisms, natural allergens, automobile exhaust fumes and smoking causes a vast array of pulmonary diseases ranging from infective to neoplastic which account for majority of mortality and morbidity worldwide. Accurate and early diagnosis is required to treat them successfully. Lung carcinoma is the leading cause of cancer-related deaths and tuberculosis (TB) is still a common cause of death in developing countries.[1] Cytology is non/minimally invasive procedure for evaluation of pulmonary lesions and plays a significant role in cases where biopsy cannot be attempted due to high risk of hemorrhage.[2]

We evaluated the spectrum of pulmonary lesions which were diagnosed by cytomorphological analysis of bronchoalveolar lavage (BAL), bronchial wash, transbronchial needle aspiration (TBNA), guided-fine needle aspirate (FNA) and pleural fluid. Cytohistological correlation was done where biopsies were available.

Materials and Methods

This prospective study was done in the Department of Pathology of a tertiary care teaching hospital after approval by the institutional ethical committee. The study group included 671 samples from 583 patients attending the pulmonary medicine OPD from August 2015 to March 2017. The samples were collected by pulmonologist or radiologist. Flexible fiberoptic bronchoscope was used to obtain bronchoalveolar lavage, bronchial washing, bronchial brush smears and
transbronchial needle aspirate. Pleural fluid was obtained by thoracocentesis. Fine needle aspirations were performed under USG or CT guidance.

The samples were processed within 2 h of receipt. Two smears from each sample were air-dried and stained with MGG stain while rest of the smears were fixed in 100% methanol followed by PAP stain. Special stains (ZN and PAS stain) were used wherever indicated.

Samples were deemed adequate/satisfactory for inclusion in the study based on the following criteria:
(I) Adequate quantity (minimum 50 ml) for pleural fluid
(II) No visible clot
(III) No obscuration of cytomorphology by hemorrhage
(IV) Sample from representative site.

Samples were examined and categorized cytologically as malignant, atypical, specific benign lesions, negative for malignancy and non-diagnostic. Cytohistological correlation was done in 99 cases.

RESULTS

The age of the patients in the study group ranged between 13 and 96 years with male preponderance (M:F ratio is 1.9:1). Majority of patients were in the age group of 40–80 years [Table 1].

Pleural fluid formed the bulk of samples evaluated (53.05%) followed by BAL (23.70%), bronchial washing (09.09%), guided FNA (6.86%), TBNA (5.81%) and brush smears (1.49%). About 15.0% of the samples were found to be non-diagnostic in our study [Table 2].

A cytological categorization of atypical cells was offered when cytomorphology lacked definite evidence of malignancy, quantitatively or qualitatively. 3.87% of cases showed cells with atypia falling short of a definite malignant diagnosis, but more than what could be assigned to reactive changes [Table 2].

Smears of adequate samples which did not show a specific pathology and had no cells exhibiting atypia, comprising 59.76% of cases, were considered negative for malignancy [Table 2]. A definite diagnosis of malignancy was offered in 109 of the 671 samples, evaluated [Table 2]. Of the 5.22% of samples where specific non-neoplastic pathology was detected on cytology, TB formed 48.57% of diagnoses.

Of the total 99 cases diagnosed as malignant by cytology, 30 (30.30%) cases could not be typed and additional ancillary investigations were recommended. Adenocarcinoma (primary and metastatic) was found to be the commonest type (28.28%) of malignancy followed by squamous cell carcinoma (26.26%) and small cell carcinoma (11.11%). There were 02 cases of Non-hodgkin’s lymphoma and one case each of malignant mesothelioma and adenosquamous carcinoma.

Adequate biopsy was available in 111 cases. The cytology specimens were inadequate for opinion in 12 cases (10.81%), hence cytohistological correlation could be performed in 99 cases only. Cytohistological concordance was found in 85 cases (85.85%) [Table 3]. The overall accuracy, sensitivity and specificity of cytology of adequate samples from pulmonary lesions were found to be 86.87%, 81.48% and 93.33%, respectively. The positive predictive value and negative predictive value of cytology were 93.62% and 80.77%, respectively.

DISCUSSION

In the present study patients of all age group were included ranging from 13 to 96 years, similar to studies by Ahmad et al.[2] and Pavani et al.[3] Correlating well with observations

| Table 1: Distribution of cases according to age in the study group (n=583) |
| Age          | n (%) |
|--------------|-------|
| 0-20         | 15 (2.57) |
| 21-40        | 112 (19.21) |
| 41-60        | 224 (38.43) |
| 61-80        | 205 (35.16) |
| 81-100       | 27 (4.63) |

| Table 2: Broad categorization of the lung lesions based on the cytological examination of various samples (n=671) |
| Categories                              | BAL | Bronchial wash | Brush | TBNA | Guided-FNA | Pleural fluid | Total | Percentage |
|-----------------------------------------|-----|----------------|-------|------|------------|---------------|-------|------------|
| Malignancy                              | 7   | 27             | 1     | 11   | 27         | 36             | 109   | 16.25      |
| Atypical                                | -   | 7              | -     | 3    | 1          | 15             | 26    | 3.87       |
| Specific benign lesions                 |     |                |       |      |            |                |       |            |
| Tuberculosis                            | 14  | 1              | 0     | 1    | 1          | 0              | 17    | 2.53       |
| Nocardia                                | 1   | -              | -     | -    | 0          | 1              | 1     | 0.15       |
| Fungal                                  | 12  | -              | -     | -    | 0          | 0              | 12    | 1.80       |
| Parasitic                               | 1   | -              | -     | -    | -          | -              | 1     | 0.15       |
| Non-caseating granulomatous lesion      | -   | -              | -     | 2    | 2          | 0              | 4     | 0.59       |
| Negative for malignancy                | 104 | 21             | 5     | 12   | 8          | 251            | 401   | 59.76      |
| Non-diagnostic                          | 21  | 6              | 3     | 10   | 6          | 54             | 100   | 14.90      |
| Total                                   | 159 | 61             | 10    | 39   | 46         | 356            | 671   | 100        |

BAL: Bronchoalveolar lavage, TBNA: Transbronchial needle aspiration, FNA: Fine needle aspirate
made by other study groups, we found that all malignant cases were >40 years of age.[4,5] A male predominance was observed as was reported in other studies.[1–3]

We examined PAP-stained and MGG-stained smears and broadly categorized them into five groups, positive for malignancy (16.25%), atypical (3.87%), specific benign lesions (5.22%), negative for malignancy (59.76%) and non-diagnostic (14.90%). Chandra et al.[6] who evaluated the role of rapid onsite evaluation with cytohistological correlation in lung lesions, broadly categorized the lesions as malignant (52.09%), negative for malignancy (18.13%), inflammatory (11.16%), inadequate for diagnosis (8.37%), atypical cells suggestive/suspicious of malignancy (5.58%) and atypical cells reactive (4.65%). The number of malignant cases in this study was considerably higher (52.09% vs 16.25%), as they only included patients suspected clinically or radiologically of malignancy. Since rapid on-site evaluation was not done in our study the number of non-diagnostic samples was observed to be higher (14.90% vs 8.37%).

Thirty five specific benign lesions in our study included, 17 (48.57%) cases of TB [Figure 1a], 7 (20%) of candidiasis, 4 (11.43%) of aspergillosis, 4 (11.42%) of non-caseating granulomatous lesions and 1 (2.86%) case each of mucormycosis [Figure 1b], nocardia and parasitic infestation [Figure 1c]. A study conducted by Rohtagi et al.[7] found TB as the commonest specific benign lesion in concordance with our observation. Frequency of TB in patients being evaluated for pulmonary mass lesions ranges from 2 to 12% in various studies.[8] We found TB in 2.5% of our study population which is similar to findings of other authors.

A changing trend in incidence of subtype of lung cancer is noted worldwide and adenocarcinoma has replaced squamous cell carcinoma as the commonest malignancy. We found adenocarcinoma (primary and metastatic) (28.28%) as the commonest type of malignancy with an equal incidence of primary lung adenocarcinoma and squamous cell carcinoma of 26.26% each [Figure 2a and b]. Results obtained by other researchers like Piplani et al.,[5] found adenocarcinoma in 44.5% of cases followed by squamous cell carcinoma (37%), small cell carcinoma (7.4%) and undifferentiated carcinoma (4%). Pavani et al.[3] also reported adenocarcinoma (46.60%) as the commonest subtype. However, Razia et al.,[1] Ahmad et al.[3] and Chandra et al.,[6] reported squamous cell carcinoma to be the commonest type of lung malignancy.

In the present study, cytohistopathological correlation could be performed in 99 cases. In 85.85% of cases a good

![Figure 1: (a) Tuberculosis – AFB++. BAL (Ziehl–Neelsen stain, ×1000). (b) Mucormycosis – Broad non-septate fungal hyphae. BAL (Periodic acid–Schiff, ×1000). (c) Parasite – Strongyloides stercoralis. BAL (May–Grünwald–Giemsa, ×1000)](image-url)
cytohistopathological correlation was achieved while 14.5% cases showed discordance. Overall sensitivity and specificity of cytology in evaluation of lung diseases were found to be 81.48% and 93.33%, respectively. Cytological accuracy achieved in our study was 86.87%. The positive predictive and negative predictive values were 93.62% and 80.77%, respectively. Our findings were similar to a study by Razia et al.[9] who evaluated efficacy of bronchial wash and brush cytology and found the sensitivity, specificity and accuracy of bronchial cytology to be 80.5%, 92.85% and 80.5%. A study in 2015 on efficacy and utility of bronchial cytology achieved a sensitivity of 93.75% and specificity of 83.33%.[9] The overall sensitivity and specificity of bronchoscopic techniques ranges from 60 to 96% in different studies.[9] The results of the present study have been compared with the other similar studies in a tabulated form [Table 4].

The cytomorphology has several mimics and pitfalls that can lead to false positive and false negative diagnosis. In our study two cases were cytologically diagnosed as malignant which were not corroborated by histopathology examination. One of the two biopsies was inadequate while pleural biopsy in the other case showed only mesothelial cell hyperplasia. Cytology was correlated with a single biopsy which may not have been from the representative lesion. A false positive rate of 1% was observed by Thivolet-Béjui.[10] Reactive atypia of epithelial cells or atypical histiocytes may be misinterpreted as malignant cells on cytology.[9]

Nine samples categorized as positive for atypical cells in our study were subsequently confirmed to be malignant on histopathology. Superadded inflammation and paucicellularity of the samples were the reasons for this discordance. One case

Table 4: Comparative analysis of the results of present study with similar studies

| Parameters                        | Present study | Pavani M et al. | Ahmad M et al. | Razia D et al. | Piplani S et al. | Chandra et al. |
|-----------------------------------|---------------|-----------------|----------------|----------------|------------------|---------------|
| Number of cases                   | 583           | 60              | 73             | 38             | 74               | 215           |
| Sample type                       | Pleural fluid, BAL, TBNA, bronchial brush, bronchial wash, guided-FNA | Bronchial brush, BAL, bronchial wash | Bronchial wash | Bronchial wash, bronchial wash | Guided-FNA | BAL, bronchial brush, TBNA, guided-FNA |
| Age (year)                        | 13-96         | 18-80           | 21-80          | 20-70          | 32-77            | -             |
| Male: female ratio                | 1.9:1         | 2.3:1           | 8:1            | 6:1            | 2.08:1           | -             |
| Non-diagnostic (%)                | 14.90         | -               | 2.73           | 5.3            | 4                | 8.37          |
| Negative for malignancy (%)       | 59.76         | 60              | 50.68          | 34.2           | 2.70             | 22.79         |
| Specific benign lesion (%)        | 5.22          | 06              | -              | 15.7           | 2.70             | 6.51          |
| Atypical (%)                      | 3.87          | 7               | 12.33          | 13.2           | 5.40             | 10.23         |
| Positive for malignancy (%)       | 16.25         | 27              | 34.26          | 31.6           | 85.1             | 52.09         |
| Sensitivity (%)                   | 81.48         | 93.75           | 80.5           | 80.5           | -                | -             |
| Specificity (%)                   | 93.33         | 83.33           | 96.6           | 92.85          | -                | 75.3          |
| Positive predictive value (%)     | 93.62         | 95.24           | 97             | -              | -                | -             |

BAL: Bronchoalveolar lavage, TBNA: Transbronchial needle aspiration, FNA: Fine needle aspirate

Figure 2: (a) Adenocarcinoma – Tumor cells in clusters and acinar pattern with prominent nuclei and moderate cytoplasm. Pleural fluid (May–Grünewald–Giemsə, ×400). (b) Squamous cell carcinoma – Keratinized malignant squamous cells. Bronchial wash (Papanicolaou stain, ×1000). (c) Small cell carcinoma – Atypical cells exhibiting high N/C ratio and hyperchromatic nuclei. Bronchial wash (Papanicolaou stain, ×1000). (d) Hematolymphoid malignancy – Monomorphic population of atypical lymphoid cells. Pleural fluid (May–Grünewald–Giemsə, ×400)

Figure 3: (a) Non-small cell carcinoma – Tumor cells arranged in cluster, exhibiting large nuclei, prominent nuclei and well-defined moderate cytoplasm. Bronchial wash (Papanicolaou stain, ×400). (b) NSCC-pleomorphic tumor cells. CT-guided FNAC (May–Grünewald–Giemsə, ×400)
reported as squamous cell carcinoma on endobronchial biopsy, showed no tumor cells in pleural fluid, possibly due to central location of the growth.

**CONCLUSION**

Cytology of pulmonary lesions is a reasonably accurate diagnostic tool with a sensitivity of 81.48% and specificity of 93.33% for both neoplastic and non-neoplastic lesions, if the sample obtained is appropriate, adequate and representative. Cytological evaluation must be assessed in conjunction with clinicoradiological findings. Cytology becomes more significant where biopsy is contraindicated.

With the advent of target therapy non-small cell carcinomas need to be further classified as adenocarcinoma or squamous cell carcinoma. Rapid onsite evaluation for sample adequacy and wider use of ancillary techniques like immunocytochemistry may help in increasing sensitivity and specificity of cytology of pulmonary lesions.

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**Conflicts of interest**

There are no conflicts of interest.

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