INTRODUCTION

Today, lung cancer is the most common cause of cancer-related mortality in both sexes in the world. In India, it was considered infrequent, but in the recent past, a trend of increase in its incidence has been noticed. Its incidence is increasing at an alarming rate of 0.5% per year, and now, it has been estimated to be the most frequent among all the new cases of cancers in male in this country, especially across all urban registries. The increasing incidence could be due to increase in smoking habit, change in lifestyles of the people, increased environmental pollution, and at the same time,

ABSTRACT

Background: Bronchogenic cancer is the most common cause of cancer-related mortality in both sexes all over the world, especially across urban population. However, early diagnosis and timely intervention may minimize the mortality rate to a certain extent and increase the 5-year survival rate to 70–80%. Among the diagnostic modalities available, bronchoscopic biopsy, though gold standard, is less feasible in peripheral centers where we depend more on cytological techniques such as bronchoalveolar lavage (BAL) fluid and sputum cytology for quick diagnosis. Aims and Objectives: This study was conducted at a tertiary care center for studying usefulness of post-bronchoscopic sputum cytology and also to correlate sputum cytology with brushing and washing cytology taking biopsy as the gold standard in the diagnosis of lung cancer. Materials and Methods: A cross-sectional observational study was done in Nil Ratan Sircar Medical College, Kolkata, over a period of 18 months in clinically and radiologically suspected patients of bronchogenic carcinoma. Bronchoscopic samples using a fiber-optic bronchoscope were collected from 50 such patients. In every case, following sequence of events was performed: Pre-biopsy washing (BAL fluid), brushing, biopsy, post-biopsy washing (BAL), and post-bronchoscopy sputum. Cytological examination done and compared with bronchoscopic biopsy specimens of the same. Results: All the available information were meticulously documented in tables and charts along with other variables such as age, sex, morphological types, and cytological and histological diagnosis. SPSS software was used to calculate the efficacy and statistical significance, if any, of these different diagnostic tools and its correlation with the final histological diagnosis. Conclusion: We found that BAL cytology had higher sensitivity and positive predictive value in early and accurate diagnosis of lung malignancy. However, post-bronchoscopy sputum cytology had no additional benefit with respect to BAL and brush cytology (in addition to bronchial biopsy) in diagnosing the same.

Key words: Bronchoalveolar lavage fluid; Bronchoscopic biopsy; Lung cancer; Sputum
due to availability of different modern diagnostic modalities
to detect lung cancer. To treat the disease successfully, it
should be diagnosed at the earliest possible stage. Several
studies have demonstrated that early detection, localization,
and aggressive treatment of lung cancer result in the 5-year
survival rate of 70–80%. Recent developments in molecular
study of lung cancer along with subsequent targeted
therapeutic approaches have given a new ray of hope.
Nowadays, bronchoscopy is an invaluable tool for diagnosis
of lung cancer and various diagnostic tools have been
developed using flexible fiber-optic bronchoscopy (FOB).
Among them, histopathological examination of bronchial
biopsy specimen remains the confirmatory or the gold
standard test in these situations. However, bronchial biopsies
cannot be satisfactorily performed in more peripheral sites
or in narrow bronchial lumen. Hence, alternative methods
including cytological methods for diagnosis are required.
Sputum is the collection of mucoid material that contains
cells from the buccal cavity, the pharynx, the larynx, the
trachea, the bronchial tree and the pulmonary alveoli, as well
as inflammatory cells and microorganisms. Sputum cytology
is an easy and often the earliest cytological method for the
detection of malignant lung tumor. Cytological assessment
of specimens obtained through FOB-guided washing and
brushing specimens of the respiratory tract offer excellent
and accurate information about the lesion as well as the
site of the lesion. In this context, cytological assessment
of specimens obtained through sputum, bronchoscopic
washing, and brushing of the respiratory tract is important,
and often, the initial diagnostic technique carried out in a
patient with suspected malignant lung lesion. The utilities
of cytology are extensive, and sometimes, they help in planning
the treatment without the requirement for an open biopsy.
This cross-sectional, observational study was conducted at
a tertiary care center over the period of 18 months with
the aim to study usefulness of sputum cytology and also
to correlate sputum cytology with brushing and washing
cytology taking biopsy as the gold standard in the diagnosis
of lung cancer.

**Aims and objectives**

This study was conducted at a tertiary care center of eastern
India for studying usefulness of post-bronchoscopic sputum
cytology in diagnosing lung malignancy and also
to correlate sputum cytology with brushing and washing
cytology taking biopsy as the gold standard by using fiber
optic bronchoscopy in the suspected cases of lung cancer.

**MATERIALS AND METHODS**

It was a cross-sectional observational study conducted
in the Department of Pathology in association with the
Department of Respiratory Medicine, Nil Ratan Sircar
Medical College and Hospital (NRSMCH), Kolkata.
The study was approved by the Institutional Ethics
Committee of NRSMCH. After getting the permission,
the samples for cytological and histological examination
were collected from the indoor/outdoor patients in whom
clinical findings, radiological examination suggested lung
malignancy. Chronic cough, hemoptysis, significant weight
loss, pallor, and lymphadenopathy were among the most
significant clinical findings that were considered. Among the
radiological findings, mass with or without consolidation
was the most characteristic indicator apart from pleural
effusion. Among these, suspicious patients, who were
considered for bronchoscopy, endobronchial growth, and
narrowing of bronchial lumen (due to compression from
outside), were the predominant presentations. Patients
with hemorrhagic diathesis, poor general condition,
and sputum positive for acid-fast bacilli were excluded
from the study. A total of 50 cases were studied in the
stipulated time frame of 18 months (January 2014–July
2015) which fulfilled our inclusion and exclusion criteria.
Bronchoscopic samples were obtained by Pentax flexible
FOB done by the pulmonologists following standard
protocol. Bronchial brushings were obtained by the use
of a stiff bristle disposable brush (outer diameter of
brush is 2 mm and outer diameter of sheath is 1.8 mm).
In every case we performed the following sequence of
events: Pre-biopsy washing (bronchoalveolar lavage [BAL]
fluid), brushing, biopsy, post-biopsy washing (BAL), and
post-bronchoscopy sputum. In the present study, sputum
samples, taken 30 min after bronchoscopic procedures
by deep cough, were collected in a wide-mouthed sterile
plastic container and were brought without any fixative.
Only one sputum sample of adequate quantity and quality
was taken. The delay was avoided as far as possible because
samples usually degenerate after 8–10 h of collection.
Sputum cytodiagnosis was carried out using the “fresh
pick and smear” method, which employed examination
of sputum for blood-tinged, reddish, discolored, or solid
area with the preparation of thin and even smears from
these selected portions. Cytological examination with
Leishman-Giemsa stain, hematoxylin and eosin (H and E)
stain, and Papanicolaou stain of sputum sample is done
in each case. Brushing material smeared directly onto
at least four clean glass slides. The two air-dried smears
were stained with Leishman-Giemsa stain and two slides
are fixed with ethanol-ether mixture for Pap and H and E
stain. Bronchial wash fluids (BAL fluids) taken both before
brushing and after biopsy were first centrifuged (1500 rpm
for 5 min) and then prepared into air-dried and ethanol
fixed smears (total four slides as before) and stained with
Giemsa, H and E, and Pap stain, respectively. Bronchial
biopsy specimens were fixed in 10% formalin, sectioned
cut at 3–4 µ thickness, and stained with H and E.
RESULTS

Most patients were in their fifth and sixth decade of life with age, the range of 31–80 years. Of 50 study subjects, lung cancer was confirmed in 38 (76%) cases by histopathology of bronchial biopsy. Among patients with lung cancer, 79% were male and 21% were female. Squamous cell carcinoma was found to be the most common lung cancer (47.4%) (Figure 1a and b), followed by adenocarcinoma (23.7%), small-cell carcinoma (15.8%), large cell neuroendocrine (5.2%), and large cell anaplastic carcinoma. All except two cases of bronchial biopsy could be differentiated into a specific type of non-small-cell carcinoma. The overall sensitivity of our post-bronchoscopy sputum sample was 7.9%, specificity 100%, positive predictive value 100%, and negative predictive value 25.53% (Tables 1 and 2).

BAL fluid cytology [Pre-biopsy (pre-brushing) and post-biopsy washing] showed high specificity of 92.31%, but a very low sensitivity of 32.43% and 35.14%, respectively. Sensitivity and specificity of brushing were found to be 74.36% and 81.82%, respectively. Positive predictive value of pre-biopsy washing, post-biopsy washing, and brushing is 92.31%, 93.55%, and 92.86%, respectively. Both sensitivity and accuracy of combined tests (wash, brush, and sputum taken together) increase significantly (Table 2). The diagnostic efficacy of post-bronchoscopic sputum is statistically insignificant. There was no statistical difference between pre- and post-biopsy wash cytology and if we consider brush and wash cytology together, combined diagnostic efficacy becomes statistically significant (Table 3).

DISCUSSION

Sputum cytology is an example of exfoliative cytology, which is based on spontaneous shedding of cells derived from the lining of an organ into a cavity from where they can be removed by non-invasive means. It is a simple, accurate, reliable, cost-effective, non-invasive procedure for premalignant and malignant diseases. It is sometimes called as poor man’s bronchoscopy. Sputum can be collected by one of the following two means – early morning spontaneously produced sputum and induced sputum. In this present study, we studied only post-bronchoscopy-induced sputum. However, it is suggested that the quality of spontaneously produced sputum and aerosol-induced sputum was comparable, with a better cell viability in the former. The pick and smear method was the most reliable method, and it has many advantages over other methods. In our study, sputum samples, produced after bronchoscopy procedure, were brought without any fixative. The delays were avoided as degeneration took place after 8–10 h of collection. However, specimens with high mucous content, like sputum, might be preserved for 12–24 h, if refrigerated. The other methods to induce sputum production are induction with a single dose of INS316 in patients with mild chronic bronchitis, induction with hypertonic saline solution, and injection of neostigmine. Cytological examination of Leishman-Giemsma stain, H and E stain, and Papanicolaou stain of sputum is accepted as a useful diagnostic tool in carcinoma of lung.

In our study, post bronchoscopy positive sputum cytology detected only three out of 38 confirmed cases of lung cancer with sensitivity of only 7.9%, whereas BAL cytology has higher sensitivity of 31.57% and 34.13%. This finding corresponds with the study conducted by Wongsurakiat et al., where the sensitivity of post-bronchoscopy sputum was 7.7% and BAL cytology was 46.7%. In another study conducted by Yuksel et al., sensitivity of post-bronchoscopy sputum was 31.8% for visible endobronchial lesion under FOB. It increases to 42.9% when the lesion is not visible, that is, for peripheral lesion. In this study, bronchoscopic lavage fluid cytology had lower sensitivity than post-bronchoscopic sputum cytology (22.7% vs. 31.8% and 25% vs. 42.9%), whereas in our study, we got the reverse finding. Das et al. conducted similar study where he found higher sensitivity for BAL cytology (62%) in respect to post-bronchoscopic sputum cytology (14%) in endoscopically non-visible tumors. In another study conducted by Kitamura et al., the diagnostic rate with the PBS was 26.8% which is far less than overall diagnostic rate of 66.7% and no patient was able to diagnosed only with PBS.

Sensitivity of pre-biopsy washing was found to be 31.57 and that of post-biopsy washing was 34.13% in patients of
Table 2: Results of different cytological techniques compared to gold standard (bronchial biopsy)

<table>
<thead>
<tr>
<th>Cytological technique</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>7.9</td>
<td>100</td>
<td>100</td>
<td>25.53</td>
<td>30</td>
</tr>
<tr>
<td>Bronchoalveolar lavage</td>
<td>34.13</td>
<td>91.67</td>
<td>92.85</td>
<td>34.21</td>
<td>50</td>
</tr>
<tr>
<td>Brush</td>
<td>76.31</td>
<td>75</td>
<td>90.62</td>
<td>50</td>
<td>76</td>
</tr>
<tr>
<td>Combined</td>
<td>84.57</td>
<td>69</td>
<td>90</td>
<td>58.4</td>
<td>82</td>
</tr>
</tbody>
</table>

Table 3: Statistical significance of different cytological procedures

<table>
<thead>
<tr>
<th>Different procedures</th>
<th>Chi-square test</th>
<th>P</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>1.008</td>
<td>0.314</td>
<td>Not significant</td>
</tr>
<tr>
<td>Difference of pre-wash and post-wash (BAL) cytology</td>
<td>0.0985</td>
<td>0.753654</td>
<td>Not significant</td>
</tr>
<tr>
<td>BAL and brush cytology (together)</td>
<td>14.9139</td>
<td>0.000113</td>
<td>Significant</td>
</tr>
</tbody>
</table>

BAL: Bronchoalveolar lavage

Limitations of our study

Post-bronchoscopy sputum samples have low sensitivity. Early morning fresh spontaneous samples might give different result as pointed by other studies. Multiple simultaneous samples were not taken in our study which could pick up higher positive cases as shown in different studies. There are different factors contributing to the final yield such as location of the tumor (central versus peripheral) and tumor type. On bronchoscopic examination, the gross morphology of majority of these cases of adenocarcinoma was compression type lesion, that is, extrinsic compression of the bronchus by the lesion, and thus, there may be a possibility of getting less representative material by cytology techniques and bronchial biopsy in such tumors. Furthermore, in mucinous type of adenocarcinoma, bronchial biopsy specimen may contain pools of mucin, very few neoplastic cells with a relative lack of atypia that makes the diagnosis of adenocarcinoma more difficult as observed by Butnor. Majority of the previous studies that have used other techniques such as rebronchoscopy, surgery, computed tomography-guided fine-needle aspiration cytology, tumor markers, and autopsy, to prove the cases of lung cancer have shown that bronchial biopsy does not provide diagnostic yield in all cases of lung cancer. Chances of missing the diagnosis by bronchial biopsy are more in peripheral lung tumors.

CONCLUSION

Although it is difficult to derive a definite conclusion from such a small sample of subjects in a single-centered study and analysis of a larger cohort from multiple institutions including patients from different geographical regions would reflect the true pattern, we found post-bronchoscopy sputum cytology had no additional benefit with respect to BAL and brush cytology (in addition to bronchial biopsy) for an early and accurate diagnosis of lung malignancy.

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REFERENCES


Authors Contribution:

AB- Concept and design of the study, collected the data, and made the initial manuscript; MP- Interpreted the results, reviewed the literature, and corrected the manuscript; SKB- Statistical analysis and interpretation; and RMM- Concept, coordination, final manuscript preparation, and revision.

Work attributed to:

Nil Ratan Sircar Medical College, Kolkata, West Bengal, India.

Orcid ID:
Dr. Abhishek Bandyopadhyay - https://orcid.org/0000-0002-0811-6003
Dr. Mallika Pal - https://orcid.org/0000-0001-8667-6543
Dr. Saugata Kumar Bhattacharya - https://orcid.org/0000-0003-2483-3632
Dr. Ruplekha Mitra Mustaphi - https://orcid.org/0000-0002-5107-171X

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