

Research Article

To evaluate the role of sputum in the diagnosis of lung cancer in south Indian population

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ABSTRACT

Background: The main advantage of sputum cytology is its simplicity, non-invasiveness and minimal discomfort to the patient. Though, the sputum is evaluated in the diagnosis of lung cancer, the report on the same in the South Indian population was lacking. Therefore, the present study has been undertaken to evaluate the role of sputum in the diagnosis of lung cancer in South Indian population.

Methods: The material consisted of sputum samples from 133 patients and was collected in clean wide mouthed disposable plastic containers. Patients were asked to collect sputum the next morning after washing the mouth properly. The sputum was immediately brought to the laboratory and poured into a watch glass. Four smears were prepared from each sample, out of which two smears were immediately fixed in methanol and the other two were air-dried. The methanol fixed smears were stained with Papanicolaou stain. Out of the two air dried smears, one was stained with May Grunwald Giemsa and the other with Gabbot's method for AFB. The smears were screened for malignant cells and a cytological diagnosis was made. The cytological diagnosis was correlated with the histopathological diagnosis. The data obtained were represented as mean percentages.

Results: The observation of sputum smears showed numerous pleomorphic keratinized squamous cells, keratinized squamous cell with hyper chromatic nucleus in well differentiated squamous cell carcinoma, pleomorphic cells having vacuolated cytoplasm and vesicular nucleus with prominent nucleoli as in adenocarcinoma of the lung, cells arranged in small clusters and having scanty cytoplasm in small cell carcinoma and cells are slightly larger than lymphocyte with scanty cytoplasm and hyper chromatic, grooved nuclei in small cell carcinoma.

Conclusion: Cytology of sputum is extremely useful and highly sensitive. The diagnostic accuracy is directly proportional to the number of samples. Sputum cytology is highly sensitive for the centrally located squamous cell carcinoma rather than the peripherally located adenocarcinoma. Properly collected, simple sputum examination alone can give results similar to other highly expensive methods like bronchoscopic material for the diagnosis of lung cancer.

Keywords: Sputum cytology, Histopathological correlation, Papanicolaou stain, May Grunwald Giemsa stain

INTRODUCTION

Respiratory cytology has been widely used for the last many decades in diagnosis of both neoplastic and non-neoplastic conditions. Sputum and bronchoscopic material have been found to be very useful especially in

the detection of pulmonary malignancies. "Sputum cytology is a simple, accurate, reliable, cost-effective and noninvasive procedure for the assessment of respiratory diseases, including pre-invasive and invasive pulmonary malignancies."^{1,2}

The role of any diagnostic technique usually refers to its utility and limitations. The main advantage of sputum cytology is its simplicity, non-invasiveness and minimal discomfort to the patient. It was well reported that the presence of tissue fragments of malignant tumors in sputum, malignant cells in the sputum of a patient with cancer of pharynx. Meyer et al in 1969 reported that, the sputum cytology not only helps in the diagnosis of lung cancer but also in the early detection of cancer.³ They found malignant cells in the sputum of an asymptomatic person who was diagnosed to have lung cancer 5 years later. Similarly by Dudgeon and Wrigley in 1935 on wet film fixation of sputum smears in a mixture of ethyl alcohol, mercuric chloride and acetic acid for the identification of malignant cells.⁴

Erozan and Frost in 1970 in their study on pulmonary specimens concluded that careful and experienced cytological examination of cellular specimens from the respiratory tract had great accuracy in diagnosing pulmonary malignancies.⁵ In 1972, Dahlgren and Lind compared the diagnostic results obtained by transthoracic needle biopsy and sputum cytology.⁶ In a series of 125 patients they studied 93% were diagnosed by aspiration biopsy and 64% were diagnosed by sputum cytology. In 1974, Chopra et al performed fiberoptic bronchoscopy (brushing, washings and biopsies) and pre and post-bronchoscopic sputum were also collected in 70 patients with histopathological proven lung cancer.⁷ Of all the various methods of obtaining specimens, they observed that bronchial brushing and bronchial biopsy gave high percentage yield.

It was found that the sputum cytology as a first test conducted by Raab et al. in 1997 in case of suspected lung cancer was likely to be cost saving without adversely affecting the patients outcome.⁸ Nakhosteon et al. in 1998 concluded that automated sputum cytometry had a great advantage in the detection of lung cancer.⁹ They also reported that it had a high sensitivity and specificity in the detection of early lung cancer. Though, the sputum material is evaluated in the diagnosis of lung cancer, the report on the same in the South Indian population was lacking. Therefore, the present study has been undertaken to evaluate the role of sputum in the diagnosis of lung cancer in South Indian population.

METHODS

The experiment was conducted after the institutional and ethical clearance. One hundred and eighty patients with the history, clinical features and radiologically suspected lung cancers were included in the current study. The material consisted of sputum samples from 133 patients. The material was collected from the patients attending the district government hospital and the university medical centre after the consent from all the patients. Sputum was collected in clean wide mouthed disposable plastic containers which were provided to the patients on the previous day. They were asked to collect sputum the next

morning after washing the mouth properly. Patients were also instructed not to eat anything before the collection of sputum. In a similar manner 3-5 early morning samples were also collected on 3-5 consecutive days.

The sputum was immediately brought to the laboratory and poured into a watch glass. A careful naked eye examination was done for the presence of any tissue particles, blood streaks and foreign bodies. Four smears were prepared from each sample, out of which two smears were immediately fixed in methanol and the other two were air-dried. The methanol fixed smears were stained with Papanicolaou stain. Out of the two air dried smears, one was stained with May Grunwald Giemsa and the other with Gabbot's method for AFB. The smears were screened for malignant cells and a cytological diagnosis was made. The cytological diagnosis was correlated with the histopathological diagnosis. The data obtained were represented as mean percentages.

RESULTS

The present study pertains to the cytological study of materials obtained from patients suspected to have lung cancer based on the clinical history and radiological findings. The cytological material included sputum from 133 patients. Cytological features of squamous cell carcinoma are shown in Table 1. Nature of cytological specimens is shown in Table 2. Incidence of primary malignancy is shown in Table 3. The observation of sputum smears showed numerous dust laden macrophages (Figure 1), metaplastic squamous cells in the background of polymorphs (Figure 2), numerous pleiomorphic keratinized squamous cells (Figure 3), keratinized squamous cell with hyper chromatic nucleus in well differentiated squamous cell carcinoma (Figure 4), pleiomorphic cells having vacuolated cytoplasm and vesicular nucleus with prominent nucleoli as in adenocarcinoma of the lung (Figure 5), cells arranged in small clusters and having scant cytoplasm as in small cell carcinoma (Figure 6) and cells are slightly larger than lymphocyte with scant cytoplasm and hyper chromatic, grooved nuclei (Figure 7) in small cell carcinoma. A tiny cluster of cells with abundant vacuolated cytoplasm and hyper chromatic nuclei with inconspicuous nucleoli was seen in bronchioloalveolar carcinoma (Figure 8).

Table 1: Cytologic features of squamous cell carcinoma. N=133 patients.

	Cytologic presentation	Sputum
1.	Cytoplasmic keratinisation	Marked
2.	Abnormal nucleocytoplasmic ratio	Often present
3.	Nuclear pyknosis	Marked
4.	Single cancer cells	Very frequent
5.	Cancer cells in sheets	Infrequent
6.	Details of nuclear structure	Rarely observed
7.	Large nucleoli	Very rare

Table 2: Nature of cytological specimens. N=133 patients.

Nature of sputum material	Number of cases
1. Early morning sample	105
2. Post nebulized sputum	15
3. Immediate post bronchoscopy sputum	04
4. 72 hour post bronchoscopy sputum	09
Total	133

Table 3: Incidence of primary malignancy. N=133 patients.

Type of cancer	Number of cases
1. Squamous cell carcinoma	33
2. Poorly differentiated squamous cell carcinoma	20
3. Adenocarcinoma	24
4. Bronchioloalveolar carcinoma	14
5. Small cell carcinoma	23
6. Adenosquamous carcinoma	19
Total	133

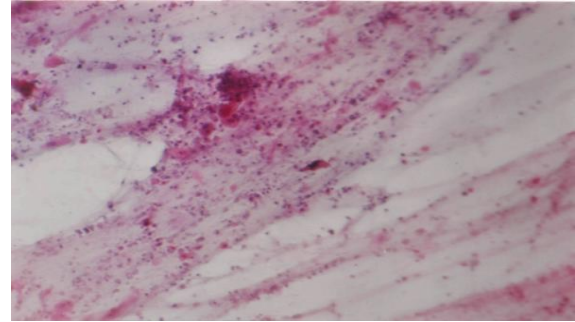


Figure 3: Sputum showing numerous keratinized squamous cells which are pleomorphic. N=133 patients. Pap x120).

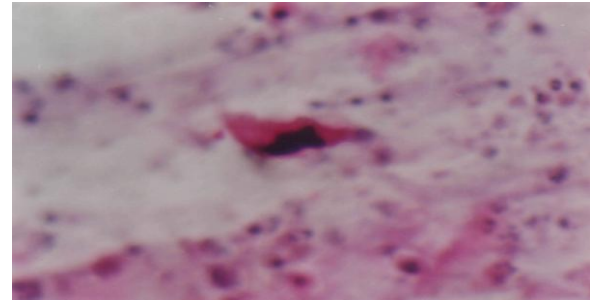


Figure 4: Sputum smears showing a keratinizing squamous cell with hyperchromatic nucleus in well differentiated squamous cell carcinoma. N=133 patients. (Pap x500).

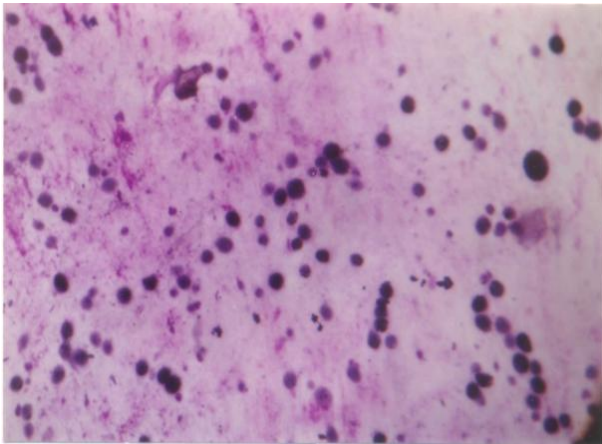


Figure 1: Sputum smears showing numerous dust laden macrophages. N=133 patients. (Pap x500).

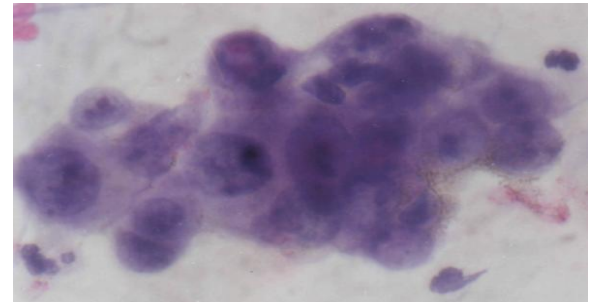


Figure 5: Adenocarcinoma of the lung. The cells are pleomorphic having vacuolated cytoplasm and vesicular nucleus with prominent nucleoli. N=133 patients. (Pap x1250).

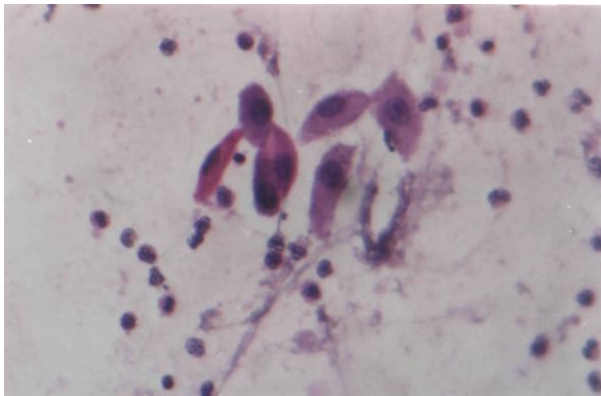


Figure 2: Sputum smears showing metaplastic squamous cells in the background of polymorphs. N=133 patients. (Pap x500).

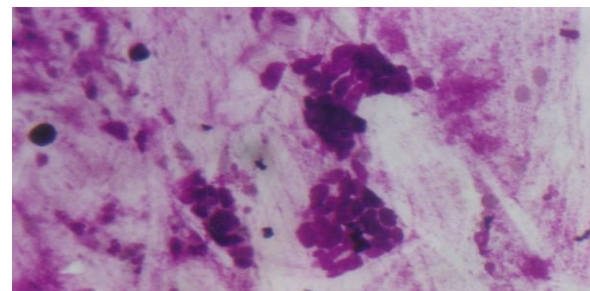


Figure 6: Sputum showing small cell carcinoma. The cells are arranged in small clusters and having scant cytoplasm. N=133 patients. (MGG x400).

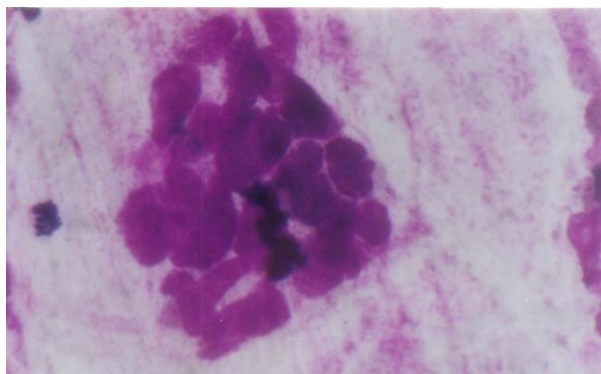


Figure 7: Small cell carcinoma. The cells are slightly larger than lymphocyte with scant cytoplasm and hyper chromatic, grooved nuclei. N=133 patients. (MGG x 1250).

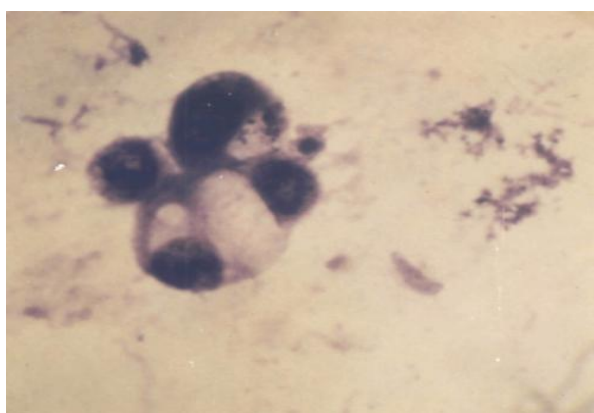


Figure 8: Sputum showing bronchioloalveolar carcinoma. A tiny cluster of cells having abundant vacuolated cytoplasm and hyper chromatic nuclei with inconspicuous nucleoli. N=133 patients. (Pap x500).

DISCUSSION

The motto of every physician should be aimed at early and accurate diagnosis of a disease utilizing simple investigations during a short hospital stay. The aim of the current study was to document the role of sputum and bronchoscope material in the diagnosis of lung cancer.

The cytological material included sputum from 133 patients. Out of 133 cases 38.8% cases were found to have malignancy in which 60% were squamous cell carcinoma, 27.14 were adenocarcinoma, 5.7% were bronchioloalveolar carcinoma, 4.2% were small cell carcinoma and one 1.4% was adenosquamous carcinoma.

Sputum

It is the most widely used diagnostic tool in the diagnosis of lung cancer. The samples of sputum studied were of different types like, early morning sputum, post nebulized sputum, immediate post-bronchoscopic sputum and 72 hour post-bronchoscopic sputum. The detection of the

malignancies also varied depending upon the type of samples.

Early morning sputum

The bulk of the current study was early morning sputum samples. A total of 296 early morning sputum samples were collected from 105 patients. Of these 46 (43.8%) showed malignant cells. Out of these 35 (76.08%) were squamous cell carcinomas, 7 (15.21%) were adenocarcinoma, 3 (6.5%) were bronchioloalveolar carcinomas and one (2.1%) was adenosquamous carcinoma.

Johnston and Frable in 1976 investigated early morning sputum samples from 420 patients who were suspected to have lung cancer clinically and radiologically and found malignancy in 253 (60.2%) patients [10]. It was also concluded that, the high diagnostic accuracy of early morning sputum was due to better concentration of malignant cells in sputum and the diagnostic accuracy could still be increased, if the sputum was collected and processed properly.

In the present study, the percentage of positivity in early morning sputum sample was 43.8%. Remaining 59 (56.2%) of the cases did not yield malignant cells. The possible reasons for the low positivity in these cases could be the following: 25 out of 59 (42.37%) cases were elderly patients (age >70). Their cough reflex was not so good and so was unable to bring out adequate sputum even after proper instructions. Radiologically in 22 out of 59 (37.28%) cases, the lesions were located peripherally. So exfoliation of malignant cells in the sputum could be minimal.

Ng and Horak in 1983 investigated 438 cases of radiologically suspected lung cancer and found that the sensitivity of early morning sputum samples for the central lesions was 86.9% compared to peripheral lesions of 41.5%. They also concluded that the central lesions had a tendency to erode the bronchus and hence exfoliate cells more frequently in sputum.¹¹

The diagnostic accuracy of early morning sputum also depends on whether the sputum is fresh or not. In the present study although fresh samples were taken, in a few cases there was delay in the arrival of samples to the laboratory due to technical factors. Although sputum was labelled as early morning sample, some of the patients would have collected sputum previous night itself, instead of early morning due to lack of proper instructions to them. The diagnostic yield of early morning sputum samples also depends upon the type of cancer.

In the present study 35 out of 46 (76.08%) cases were squamous cell carcinoma. Majority of the squamous cell carcinomas had a propensity to occur in the large bronchi at the hilus of lung and hence they exfoliated cells more

frequently compared to the adenocarcinoma which were situated more peripherally. In the present study high diagnostic yield of early morning sputum samples for squamous cell carcinoma was because, 31 (88.57%) out of 35 cases were arising from the central bronchus. Out of 35 cases of squamous cell carcinoma 20 (57.14%) cases were well differentiated and the rest showed varying degrees of keratinisation. Another factor which determines the diagnostic accuracy of early morning sputum is the number of sputum samples which are submitted to the study from a particular case. In the present study, there was an absolute increase in diagnostic yield as the number of early morning sputum samples were increased in each case.

In the present study, out of 296 early morning samples collected from 105 patients, (29%) samples were inadequate and 63.9% inadequate sputum samples were of first sputum sample. The inadequacy of majority of first few samples was due to improper way of collection, reduced cough reflex and lack of knowledge. However there was an absolute increase in the adequacy of early morning samples as more number of samples was collected from a single case. Hence there was an increase in the diagnostic accuracy.

In the present study, increase in the adequacy and accuracy of early morning sputum samples were achieved by proper instruction to the patient regarding collection of sputum and proper instruction to laboratory personnel regarding processing of sputum. Erozan and Frost (1970) have compared the diagnostic accuracy in detection of lung cancer in relation to the number of samples submitted. In their series of 155 patients, they found that the diagnostic accuracy was directly proportional to the number of early morning samples submitted to the study.⁵ They also found that, most of the initial samples were unsatisfactory, because they were not early morning samples, but were collected whenever the clinicians first decided to do cytologic examination (e.g. Arrival on the ward, first clinic visits, etc.).

Post nebulized sputum

Inadequacy of material is a constant problem in sputum cytology. The inadequate material could be due to lack of cough reflex. In these circumstances cough can be induced artificially either by using 10% hypertonic saline or by 20% polyethylene glycol in a nebulizer. In the present study, out of the total 15 post nebulized sputum samples studied, only one case showed squamous cell carcinoma, with the percentage of positivity of 6.6%. The low diagnostic yield of post nebulized sputum was because, the 10 out of 15 (66.66) cases had lesions at the peripheral portions of the lung.

Histopathological correlation

Out of the total 133 cases, 54 (40.65%) were diagnosed by sputum. Histopathological correlation was available in

35 (26.3%) cases. Out of 35 histologically correlated cases, 17 (48.5%) were diagnosed by both sputum and histopathology, 8 (22.8%) were diagnosed by histopathology but missed by sputum, 10 (28.6%) were negative both by sputum and histopathology. The sensitivity of the sputum was 68% and specificity was 100% with false negative rate of 6.09% and there were no false positive cases. Sing et al (1997) in their study of 415 lung cancer patients found that the overall sensitivity of sputum technique was 40.03%.¹² They also found that the false negative results might be due to an inadequate quantity of sputum, improper fixation or inadequate selection of material for making smears.

In the present study false negative rate was minimal (6.09%) because, adequate material was collected properly and processed immediately in all the cases. Apart from histological correlation, transthoracic needle aspiration was attempted in 20 cases, out of which 9 (45%) were diagnostic of malignancy.

CONCLUSION

Cytology of sputum is extremely useful and highly sensitive. Proper collection of material, adequate fixation, prompt processing and methodical staining is essential for perfect cytological study. The diagnostic accuracy is directly proportional to the number of samples. Sputum cytology is highly sensitive for the centrally located squamous cell carcinoma rather than the peripherally located adenocarcinoma. Properly collected, simple sputum examination alone can give results similar to other highly expensive methods like bronchoscopic material for the diagnosis of lung cancer.

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Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee

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