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DIAGNOSTIC ROLE OF SPUTUM CYTOLOGY IN PULMONARY DISEASES: A PROSPECTIVE STUDY IN A TERTIARY CARE CENTRE

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Abstract:

Background: Respiratory cytology has been widely used for the last many decades in diagnosis of both neoplastic and non-neoplastic conditions. Sputum is a pathologic material expectorated from lungs and bronchi. Sputum cytology is an example of exfoliative cytology, which is based on spontaneous shedding of cells derived from the lining of an organ into the cavity.

Objectives: The present prospective study was conducted to evaluate the role of cytological examination of sputum in the diagnosis of various pulmonary diseases and to correlate the cytological diagnosis with the clinical diagnosis.

Material and Method: 200 sputum samples were collected from patients attending Chest and TB hospital, who presented with respiratory complaints and abnormal chest x-rays along with relevant clinical investigations. Smears were made after gross examination of the sample, stained with May Grunwald Giemsa, Papanicoloau, Ziehl Neelson stain and examined microscopically.

Results: Of total 200 samples of sputum, 96 cases were diagnosed as pulmonary tuberculosis, 54 cases as pneumonia and 15 cases as lung carcinoma. Out of remaining cases, 7 were of chronic bronchitis, 4 were of lung abscess and 2 were of bronchial asthma and fungal infection each. Significant p value i.e. 0.015 calculated after correlation of clinically suspected cases with cytological diagnosis proves that sputum examination is an effective diagnostic tool for various benign and malignant pulmonary diseases

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Conclusion-Sputum cytology is an extremely useful, cheap, non-invasive and highly sensitive tool. It may be utilized as screening procedures for patients who are unfit for bronchoscopy and in patients for their routine evaluation of underlying infection.

Keywords- Sputum, cytology, screening, pulmonary diseases.

Introduction:

Respiratory cytology has been widely used for the last many decades in diagnosis of both neoplastic and non-neoplastic conditions. Sputum examination plays an important role in the diagnostic cytopathology. Sputum cytology is an example of exfoliative cytology, which is based on spontaneous shedding of cells derived from the lining of an organ into the cavity. It is a simple, accurate, reliable, cost effective and non-invasive procedure for the assessment of respiratory diseases including pre-invasive and invasive malignancies. [1-3]

Sputum is a pathologic material expectorated from lungs and bronchi. It consists of saliva and secretions from nasal, laryngeal and mouth cavities in different proportions, when evacuated from mouth. [3-6]

The main advantage of sputum cytology is its simplicity, non-invasiveness and minimal discomfort to the patient. Hence, this study was carried out to evaluate the role of cytological examination of sputum in the diagnosis of various pulmonary diseases including lung malignancies and to correlate the cytological diagnosis with clinical diagnosis. As the production of sputum in quantities sufficient to be expectorated does not occur in the absence of respiratory disease, the examination of such specimens generally demands a greater challenge.

Sputum can be collected by one of the following methods:

1. Early morning spontaneously produced sputum. [6-8]
2. Aerosol produced sputum.[2]

Examination of fresh sputum is recommended, in which a fresh, early morning specimen before meal produced by deep cough is collected and brought to the laboratory without any fixative. However, if it is not possible, then the sputum sample can be collected in 70% ethyl alcohol solution for fixation.[3] If a patient is unable to produce sputum, then expectoration can be

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induced by inhalation of steam from saline water. Consecutive three morning samples are recommended for better yield of cells and to increase diagnostic sensitivity.

A sputum sample is considered adequate if alveolar macrophages or bronchial epithelial cells are present because this shows that the sample originates from deep within the lung and just not the salivary secretions. There should be at least 10 alveolar macrophages to label it as adequate. [9-12]

Material and methods:

This prospective type of study was conducted in the department of Pathology, Government Medical College of North India. A total of 200 patients were taken into consideration over a period of 2 years. Ethical approval was taken from institutional ethical committee and written consents were obtained from all the patients in the vernacular language. Patients having respiratory complaints and abnormal chest x-rays attending the Chest and TB Hospital, were included. Unsatisfactory samples (sputum samples containing only squamous cells, ciliated cells, bacteria) were excluded. Clinical data and other relevant investigations were obtained from the case files of the patients. After receiving sample in the laboratory, it was examined grossly for its volume, color, presence of blood, pus, tissue fragments, consistency etc. In each case, 3 smears were prepared from the representative material and stained with MGG (May-Grunwald Giemsa), Papanicolaou and ZN (Ziehl Neelson) stain. PAS (Periodic Acid Schiff) stain was also done in suspected cases of fungus.

Statiscal analysis: The observations were recorded and data was analysed statistically. Sensitivity, specificity, positive predictive value, negative predictive value and p value were calculated using IBM SPSS version 22.

Results:

In the current study, maximum number of cases (54) was observed in the age group of 61-70 years with a mean of 54.70±14.46. Majority of the samples were from male patients (138 cases, 69%) with male: female ratio being 2.2:1. After microscopic examination of 200 cases, a definitive diagnosis was reached in 180 cases while 20 smears were rendered inconclusive because of presence of only squamous cells or bacteria. The factors responsible for inconclusive results were poor expectorating technique, peripherally located lesions, submucosal lesions, non-cohesive and nonproductive pulmonary lesions. With regard to cytological diagnosis, maximum

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number of cases were of Pulmonary TB (96 cases – 48%) while minimum number comprised of bronchial asthma (2 cases – 1%) and fungal infection (2 cases - 1%) as depicted in **Table 1.**

Table 1: Cytological diagnosis of 200 cases

Nature of disease	Disease entity	Number	%age
Neoplastic cases	Carcinoma	12	6
	Suspicious	3	1.50
	Total	15	7.5
Non Neoplastic cases	Pulmonary TB	96	48
	Pneumonia	54	27
	Chronic Bronchitis	7	3.50
	Lung abscess	4	2
	Bronchial Asthma	2	1
	Fungus	2	1
	Total	165	82.50

Cellular constituent's frequency wise: Most common finding in sputum smears was the presence of polymorphs, which were seen in 164 cases (82%) followed by presence of lymphocytes (seen in 102 cases). Least commonly encountered was presence of eosinophils and fungus (2 cases each) as depicted in **Table 2.**

Sputum cytology in pulmonary tuberculosis cases (96 cases):

Cytology smears showed lymphocytes, neutrophils and ill formed giant cells. The sputum smears of all 200 cases were examined with ZN staining. All the sputum samples of pulmonary tuberculosis (96 cases) were positive for AFB with Ziehl-Neelson stain and rest cases showed negative results for AFB.

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Table 2: Findings of sputum smear cytology (MGG and PAP staining)

Sputum smear cytology findings		Number	%age
Polymorphs	Positive	164	82
	Negative	36	18
Lymphocytes	Positive	102	51
	Negative	98	49
Macrophages	Positive	70	35
	Negative	130	65
Any other findings	Malignant Cells	12	6
	Giant Cells	9	4.50
	Necrotic debris	4	2
	Suspicious Cells	3	1.50
	Fungus	2	1
	Eosinophils	2	1

Sputum cytology in pneumonia (54 cases):

The cytological diagnosis was made after clinical correlation. Patients presented with complaints of high-grade fever and cough with expectoration. The cytology smears showed mainly inflammatory cells comprising of neutrophils and macrophages.

Sputum cytology in chronic bronchitis (7 cases):

All the patients were chronic smokers with history of chronic cough and dyspnea. The cytology smears showed acute and chronic inflammatory cells comprising of neutrophils, lymphocytes and macrophages.

Cytology findings in cases of bronchial asthma (2 cases):

The sputum samples were mucinous and contained numerous eosinophils along with some lymphocytes and few neutrophils.

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Cytology findings in cases of lung abscess (4 cases):

The patients had fever, cough and x – ray findings suggestive of lung abscess. Cytology smears showed mainly necrotic debris, neutrophils and macrophages.

Sputum cytology in lung carcinoma (15 cases):

Clinical and radiological correlation was taken into account. The smears showed malignant epithelial cells lying in loose clusters exhibiting nuclear pleomorphism, hyperchromasia, irregular nuclear contours and nucleomegaly. Definitive diagnosis was made in 12 cases while 3 cases were reported as suspicious for malignancy.

Cytology findings in cases of fungal infection (2 cases):

Aspergillus (1 case) was seen as thick septate hyphae with acute angle branching. Some of the hyphae had expanded ends (Conidiophores) leading to production of further hyphae. Another case of candidiasis showed elongated pseudopyphae and spores. Periodic acid Schiff (PAS) stain was used for better visualization of fungus **Figure 1**.

The findings in **Table 3** depict that sputum cytology has high sensitivity and moderate specificity for diagnosing infectious conditions like pulmonary tuberculosis, pneumonia, chronic bronchitis and lung abscess. The sensitivity and specificity for lung carcinoma was 93.75% and 75% respectively. The high sensitivity (100%) and high specificity (100%) noted in cases of bronchial asthma and fungal infection could be attributed to less number of cases.

Significant p value i.e. 0.015 calculated after correlation of clinically suspected cases with cytological diagnosis proves that sputum examination is an effective diagnostic tool for various benign and malignant pulmonary diseases as shown in **Table 4**.

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Figure 1: Shows cytological findings of various lesion on sputum smears examination.

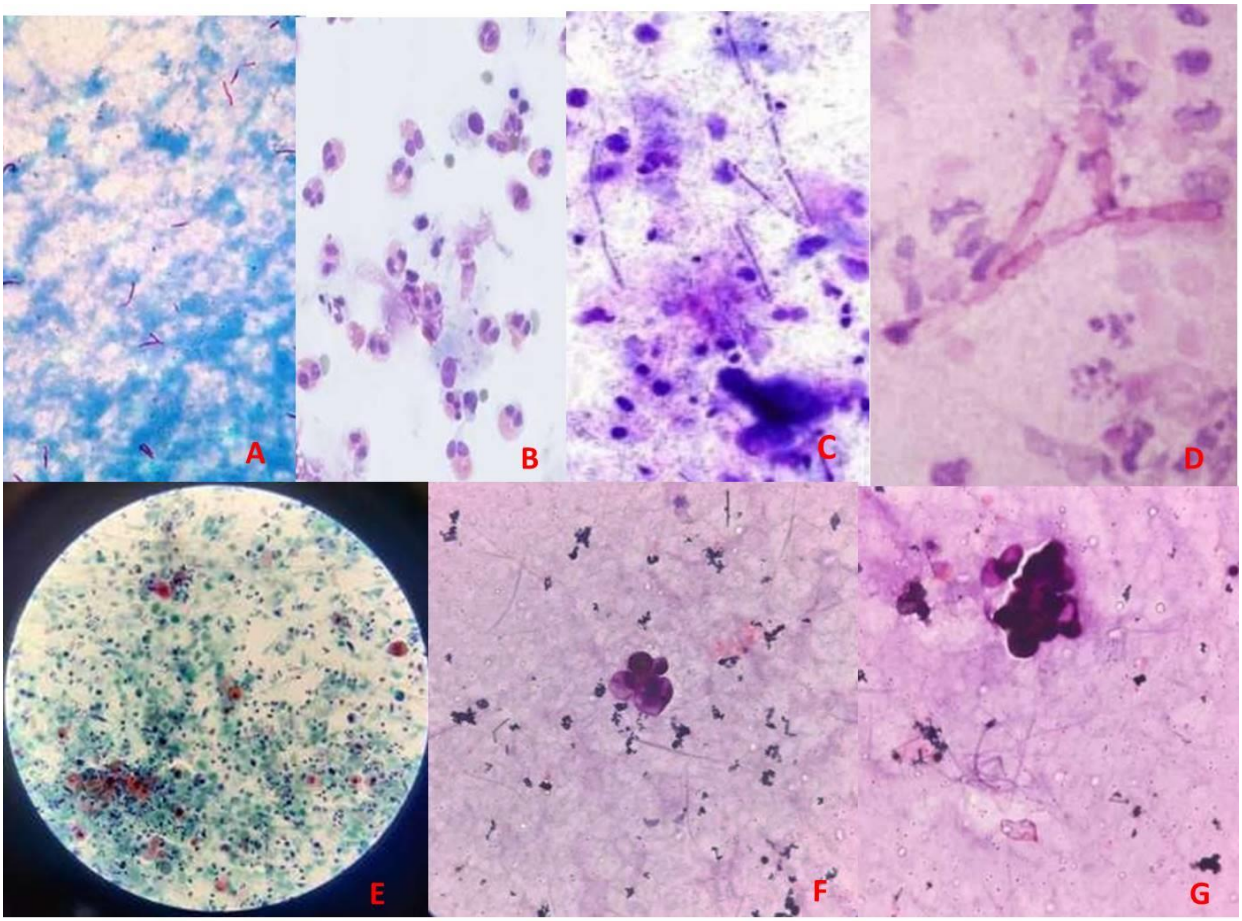

FIGURE 1. (A) Rod shaped bacilli seen in case of pulmonary TB (ZN stain, oil immersion). (B) Sputum smear showing numerous eosinophils and very few macrophages in case of bronchial asthma (MGG stain, oil immersion). (C) Candidal spores and pseudohyphae representing fungal infection (MGG stain, oil immersion). (D) Aspergillus showing acute angle branching and septations (PAS stain, oil immersion). (E) Keratinized malignant squamous epithelial cells, tadpole cells and inflammatory infiltrate seen in a case of Squamous Cell Carcinoma of lung (PAP stain, 400x). (F) Sputum smear showing malignant epithelial cells with eccentrically placed hyperchromatic nuclei and cytoplasmic vacuolations indicating Adenocarcinoma (MGG stain, 400x). (G) Small cluster of suspicious looking cells exhibiting hyperchromatic nuclei (MGG stain, 400x)

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Table 3: Sensitivity and specificity of sputum cytology in each case.

	Sensitivity	**Specificity**	**Positive Predictive Value (PPV)**	**Negative Predictive Value (NPV)**	**Predictive Value**	**X^2**	**p value**
Pulmonary TB	98.96%	75%	98.96%	75%	98%	37.13	0.001
Pneumonia	98.04%	84.62%	96.15%	91.67%	95.31%	41.19	0.001
Bronchogenic carcinoma	93.75%	75%	93.75%	75%	90%	8.98	0.003
Chronic bronchitis	87.50%	50%	87.50%	50%	80%	11.25	0.008
Lung abscess	80%	50%	80%	50%	71.43%	5.41	0.020
Bronchial asthma	100%	100%	100%	100%	100%	4.00	0.045
Fungal infection	100%	100%	100%	100%	100%	4.00	0.045

Table 4: Comparison of clinically suspected cases and cytologically proven cases.

S.No	**Disease**	**Number of clinically suspected cases**	**Number of cytologically proven cases**	**X^2 (Chi square test)**	**p value**
1.	Pulmonary TB	100	96	5.96	0.015
2.	Pneumonia	64	54		

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3.	Lung carcinoma	20	15		
4.	Chronic bronchitis	8	7		
5.	Lung abscess	5	4		
6.	Bronchial asthma	2	2		
7.	Fungal infection	1	2		

Hence in the current study, we found good statiscal correlation between clinical and cytological diagnosis with a p value of 0.015. Further we examined lung biopsies to confirm the cytological diagnosis.

DISCUSSION

Sputum cytology of smears is an extremely useful and important diagnostic tool. Proper collection of material, adequate fixation, prompt processing and methodical staining is essential for perfect cytological study. [13-15] The diagnostic accuracy is directly proportional to the number of samples. A lot of work has been done in the field of exfoliative cytology of lower respiratory tract stressing the diagnostic value of cytology in various respiratory diseases especially in the diagnosis of lung cancer. For this purpose, sputum and bronchial secretions have been used for cytological diagnosis. Use of sputum has been reported to be superior to bronchial secretions in the cytological diagnosis. Much more time is required for sputum examination than bronchial secretions but sputum is preferred because it is much easier to obtain. [16-19]

The most important aspect of sputum cytopathology is in the diagnosis of malignancy of the lung. In general, screening of sputum for lung cancer may detect about 80% of the cases. Unlike the bacterial and viral infections, fungi can be identified with precision in respiratory samples because of their characteristic morphology. Pulmonary Aspergillosis, Candiasis, Histoplasmosis, Blastomycosis and Crytococcosis have been frequently reported. [19-21]

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The most important value of sputum cytology is in the diagnosis of obscure cases. In some instances, where bronchoscopy and aspiration cytology are negative and X-ray examination inconclusive, a positive sputum cytology may reveal the true nature of the lesion. [21-25]

The present study comprised of a total of 200 cases of sputum samples of various respiratory disorders. The study was conducted to evaluate the role of sputum cytology in the diagnosis of malignant and non-malignant conditions of lung. Sputum cytological diagnosis was carried out using the "fresh pick and smear" method, which employs examination of sputum for blood tinged, discolored or solid particles and preparation of thin and even smears from these selected portions. This method enables effective concentration of diagnostic material by selection of suspicious areas. [26]

In the present study, out of 200 cases, 96 cases were of pulmonary tuberculosis, which stained positive for acid fast bacilli with ZN stain. Smears showed mainly lymphocytes, neutrophils and ill – formed giant cells in few cases. This result is in conformity with that of Kumar R et al, 2018. [21] Sensitivity of sputum cytology for diagnosing pulmonary tuberculosis was 98.96% and specificity was 75%. These findings suggest that sputum cytological examination combined with ZN staining is a sensitive and specific tool for pulmonary TB.

54 cases of pneumonia were diagnosed which showed only inflammatory cells comprising of neutrophils and macrophages. However, no definite characteristic finding was noted. Tse GMK et al in 2004 [4] also documented that no characteristic finding is seen on sputum cytology except for some morphological changes in macrophages and inflammatory cells.

The present study included 7 cases of chronic bronchitis and 4 cases of lung abscess. The sputum smears on cytological examination revealed acute and chronic inflammatory cells along with necrotic debris. Nothing characteristic of chronic bronchitis and lung abscess is mentioned in the literature on sputum cytology. Two cases of bronchial asthma showed numerous eosinophils along with some lymphocytes and few neutrophils. These findings were similar to the study of Farber et al (1957). [7]

Two cases of fungal infections (Aspergillosis and Candidiasis) were encountered in sputum smears in the present study. Aspergillus was seen as thick septate hyphae with acute angle

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branching. Aspergillosis has been reported in the literature by Sandhu et al (1966), Rangaswamy et al (1972), Shome et al (1976), and Afzal (1980). [26-29]

Candida was seen as spores and elongated pseudohyphae in the sputum smears. The presence of fungus was confirmed by PAS staining. Candida has been reported in sputum cytology by Singh L et al in 2013. [14]

In cases of non-tubercular inflammatory conditions of the lungs like pneumonia, chronic bronchitis and lung abscess, inflammatory cells: both acute and chronic type, were encountered, thus revealing the inflammatory nature of these conditions. Nothing characteristic of any particular condition was found. Diagnosis was made based upon relevant clinical history and radiological findings. Thus, it is concluded that sputum cytology is of little help in diagnosis of non-tuberculosis inflammatory conditions of the lungs.

In case of bronchial asthma, eosinophils with some lymphocytes, few neutrophils and mucin were found, thus revealing the allergic nature of the condition. Hence, sputum cytology can serve as a good diagnostic tool in cases of bronchial asthma. Aspergillus, which is an opportunistic fungus, was found in sputum of 1 case in the present study. Candida was reported in another case. PAS staining was used for confirmation. Thus, sputum cytology is of great value in the diagnosis of mycotic infections of the lungs and the organisms can easily be identified on staining.

The most important role of sputum cytology is in the diagnosis of lung cancers. The screening of the sputum may detect about 80% of the cases (positive or suspicious). The cytological diagnosis of primary lung cancer is made in conformity with the World Health Organization (WHO) classification of lung tumors.[8]

Exfoliative sputum cytology is not a definitive diagnostic tool for lung cancer but it has good sensitivity and specificity for initial screening. The present study included 15 cases of lung carcinoma, diagnosed after clinical and radiological examination. Sputum smears were positive in 12 cases and suspicious cells were encountered in 3 cases.

The **table 5** compares the results of present study in view of malignant cases with various researchers. There are different studies of sputum cytology showing an average sensitivity of 65% with a wide range of 22 – 98%. This wide variability is because of the location of the

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lesion. Centrally located lesions like small cell carcinoma and squamous cell carcinoma are more likely to be detected than the peripheral lesions like large cell carcinoma and adenocarcinoma (Khalid et al, 2010). [16] Our study showed high sensitivity (93.75%) and specificity (75%) of sputum cytology for detection of malignant lesions. This is in comparison with studies done by Risse et al (1987) and A.S. Ammanagi et al (2012). [1, 30]

Table 5: Following table compares the results of present study in view of malignant cases with various researchers.

S. No	Various studies	Percentage of cytologically positive cases of primary lung cancer
1.	Farber et al (1950)[7]	55%
2.	Umiker WO (1961)[9]	65.7%
3.	Johnston and Bosson (1981)[10]	47.1%
4.	Chaudary MK et al (2010)[11]	46%
5.	Veena VS et al (2012)[12]	73%
6.	Ammanagi AS et al (2012)[1]	60%
7.	Present Study (2020)	75%

CONCLUSION

Sputum cytology is an extremely useful, cheap, non-invasive and highly sensitive tool. It may be utilized as screening procedures for patients who are unfit for bronchoscopy and in patients for their routine evaluation of underlying infection. Sputum cytology is highly sensitive for centrally located lesions like small cell carcinoma and squamous cell carcinoma are more likely to be detected than the peripheral lesions like large cell carcinoma and adenocarcinoma. Properly collected, simple sputum examination alone can give results similar to other highly expensive methods for diagnosis of lung cancer.

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Conflict of interest: None

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