

Cytological Analysis of Sputum: The Simplest and Preliminary Method of Lung Cancer Diagnosis - A Retrospective Analysis of 8690 Samples of Symptomatic Patients

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Abstract- Lung cancer is one of the leading cause of cancer related deaths for people of both sexes worldwide and sputum cytology has been accepted as one of the best non-invasive method for lung cancer diagnosis, but this method is not accepted as a screening technique because of its lower sensitivity.

Aim: To correlate the cytologic findings of sputum with clinical and histopathological findings and evaluate the specific reason for the lower sensitivity of sputum cytology for the laboratory diagnosis of lung cancer and its precursors.

Materials and Methods: The cytological findings of 8690 sputum samples processed for a period of ten years were analysed. Three consecutive early morning sputum samples were processed by Pick and Smear method and stained with classical Pap staining technique. Clinical and radiological findings and histopathological results were collected and cytology results were correlated with it. Statistical analysis was done using SPSS software to see the significance of each parameter.

Results: The age distribution of patients ranges from 25-91 years with the highest incidence of malignancy in patients above the age of 60 years. Adenocarcinoma was the predominant type of malignancy. 792 (9.1%) samples was found to have atypical cells. Chest pain was found as the significant symptom on chi-square analysis.

Conclusion: The low sensitivity of sputum cytology is due to the degenerative changes caused to cells which can be significantly improved by developing more sensitive techniques.

Index Terms- Sputum cytology, Lung cancer, Clinical and cytological correlation

I. INTRODUCTION

Lung cancer is one of the leading causes of cancer related deaths for people of both sexes and accounts for 1.3 million deaths annually. In India also as per the report of the population based cancer registry data (New Delhi), it is the first leading cancer in males (10.5%) and 7th in females (2.5%).^{1, 2} The high

mortality rate of lung cancer is due to the fact that these cancers are detected in the advanced stage only, when prognosis is very poor. The selection of the diagnostic method for a suspected lung cancer is based on the location of tumour in the lung, the presence of potential metastatic spread and treatment plan. Sputum cytology has been accepted as the first test in centrally located lesions because it lowers the medical care costs and the mortality risk along with chest X-ray.^{4,5}

In the present study we have retrospectively analyzed the sputum cytology reports of 10 years and correlated the cytopathology findings with histological, clinical and radiological findings to see how far sputum cytology helped in the diagnosis of lung cancer in clinically suspicious patients.

II. MATERIALS AND METHODS

A total of 8690 sputum samples received during the period from 2000 to 2009 were selected for the study. These patients were referred for sputum cytology from Sanatorium for Chest disease and Medical College hospital with Chronic Obstructive Pulmonary Disease (COPD). Three to five consecutive early morning sputum samples were obtained and processed using the conventional pick and smear method⁶ and were stained with classical Pap stain technique. The slides were initially screened by a staff cytotechnologist and all positive samples were reported by a senior cytologist. Ten percentages of the negative samples have been re-screened by a senior cytotechnologist. The clinical and radiological findings recorded by the clinician on the request form were collected. Histopathological results of subjects with positive sputum cytology were obtained from the case files. Samples which were reported to contain atypical cells in three consecutive specimens but could not give a definite diagnosis of malignancy due to inadequacy of cells and poor cellular morphology or degenerative changes and unsatisfactory due to the absence of macrophages were repeated for two more days. Samples with atypical cells in five consecutive days were advised further investigations. This work has been approved by the Institutional Review Board and Ethical committee.

Statistical analysis was done using SPSS software to correlate the cytological findings with clinical, histopathological and radiological findings. Cytological diagnosis were categorised mainly in five groups NMC (Negative for malignancy),

squamous cell carcinoma, adenocarcinoma, non small cell carcinoma(samples which could not be categorised as adeno/squamous),small cell carcinoma, atypical/suspicious(a definite diagnosis of malignancy could not be given due to poor morphology preservation or due to lack of adequate number of cells). The clinical findings were correlated with cytological findings. Significance of each of the feature was assessed using Pearson chi-square test.

III. RESULTS

Analysis of age distribution of patients with a positive diagnosis in sputum cytology and which were confirmed in histopathology revealed that the incidence of lung cancer was higher in older age group of above 60 years and males dominate over females in all histological types of lung cancer [Figure1]. A total of 681 samples were diagnosed as positive for malignancy, out of this 85.4% of patients were males.

The major clinical symptoms associated with lung cancer were cough, fever, breathlessness, dyspnoea, haemoptysis, chest pain, weight loss, loss of appetite etc. Cough was found to be a frequent symptom in patients with malignancy (31.9%).However Chi-square analysis failed to get any significance for it. Among clinical symptoms, chest pain was found to have significance on Chi-square test ($p=0.025$) [Table1]. Cough, haemoptysis and hoarseness of voice were the frequent complaints for patients with adeno carcinoma, but it was chest pain for squamous cell carcinoma.

Among x-ray findings, mediastinal mass, consolidation, cavitation, and hilar adenopathy were the usual findings observed for patients referred for sputum cytology and mediastinal mass was the frequent one associated with most of the positive samples (9.27%).However none of these findings were found to have significance in statistical analysis.

The incidence of malignancy was higher in males than in females and adenocarcinoma was the predominant type of malignancy both in males (18.4%) as well as in females(4.6%) and small cell carcinoma had the lowest incidence (5.68%).Squamous cell carcinoma was the second leading type 107(7.7%) .

A total of 90 cases (6.47%) were reported as non small cell carcinoma as this could not be sub-classified into adeno or squamous cell carcinoma on sputum cytology. 792(9.1%) samples were found to have atypical cells of which 73% were found positive for malignancy on further evaluation. When abnormal cells present, a definite diagnosis of malignancy was given in 784 cases (56.3%) with three consecutive samples and 420(30.2%) cases were repeated for 2 more days to give a definite diagnosis of malignancy.

Cytology results were correlated with histopathology reports. Among 792 atypical samples, 515cases were diagnosed as adenocarcinoma, 229 squamous, 30 non small cell carcinoma, 5 small cell carcinoma and 13 cases of other types of malignancy. In the case of adenocarcinoma reported by sputum cytology 7 samples turned out as non-small cell carcinoma in histopathology [Table 2].Five cases of adenocarcinoma were turned out as metastatic deposits on further evaluation. A review of the cytology slides of these samples revealed that the morphological distortion of cells caused by reactive or

degenerative changes and presence of large amount of mucus and inflammatory cells masking the cell morphology were the misleading factors on the microscopic evaluation.

IV. DISCUSSION

The high mortality rate of lung cancer is mainly due to the lack of reliable techniques for its detection at an early stage. Sputum cytology is one of the cost effective, non traumatic and simple techniques which can be used for the detection of lung cancer in symptomatic patients. Hartveit *et al.* have reported that the first positive morphological diagnosis of lung cancer was obtained from sputum cytology in the cases in which there had been a delay in diagnosis.⁷ So sputum examination for malignant cells remained as the sheet anchor of diagnosis for over 60 years and it continues to be used because of the ease of sample acquisition.⁸ However, this technique is not being used in many of the centres because of its lower sensitivity. The present analysis confirms the concept of sputum cytology as a diagnostic method in symptomatic patients, for the first morphologic diagnosis. As expected, the incidence of lung cancer was found to be more prevalent among males than females, which may be due to personal habits like cigarette smoking and occupation. Also the incidence of lung cancer in both sexes, were higher in the age group above sixty years. This is in line with the NCI statistics where the age adjusted incidence is 62.5 per 100,000 men and women per year The Hospital Based Cancer Registry data (2008) also observed the lung cancer incidence in male and female in the ratio 5:1.

Among clinical symptoms associated with malignancy, persistent cough was found in majority of cases but it is difficult to distinguish cough associated with lung cancer from that of chronic lung diseases like COPD that has resulted from smoking. The other clinical symptoms which were found associated with malignancy in our study were similar to the findings of other workers like Beckles *et al.* especially chest pain, which was found significant on chi square analysis, and the association of cough, haemoptysis and hoarseness of voice with adenocarcinoma was also noticed in previous studies.⁹ In our study, the most prevalent clinical symptoms were dyspnoea, breathlessness, haemoptysis and hoarseness of voice in adenocarcinoma patients whereas it was dyspnoea, haemoptysis and chest pain in small cell carcinoma. In squamous cell carcinoma breathlessness, cough, haemoptysis, and loss of appetite and chest pain were the frequent complaints. Fever, cough, chest pain and hoarseness of voice were usually associated with patients having atypical cells in their sputum samples. So it appears reasonable to consider these clinical findings favourable for arriving at a positive diagnosis in samples with atypical cells. But it was also observed that, there was not a single clinical symptom specific for a particular histologic type of malignancy and the specific signs and symptoms depends on the location of the tumour, its loco-regional spread and the presence of metastatic disease.

It was found in many studies that chest X-ray alone cannot contribute in lung cancer diagnosis and it can be used as a supportive measure by the physician in symptomatic group. Abnormal X-rays are main trigger for referral to respiratory physician to rule out lung cancer.^{10, 11, 12, 13.} In adenocarcinoma

and squamous cell carcinoma, mediastinal mass and mediastinal adenopathy were the main X-ray findings. The cytologically atypical samples which were turned out as malignancy on histopathology were also found to have abnormal X-ray findings mainly mediastinal mass in majority of cases. So abnormal x-ray findings, especially mediastinal mass can be considered as factor favouring malignancy if presented along with significant clinical findings.

The increase in incidence of adenocarcinoma in our study coincides with the world scenario where the incidence of squamous cell carcinoma was found to be decreasing and there is a steep rise in adenocarcinoma particularly in older age group.¹⁴ The Hospital Based Cancer Registry(HBCR) data also observed higher incidence of adeno carcinoma (19.56%) compared to squamous cell carcinoma(10%).The changing trends in the incidence of different histological types of lung cancer can be attributed to change in cigarette smoking pattern and changing life style of the people.¹⁵ More over malignant transformation and progression are characterised by genomic instability due to many key step events.¹⁶

An important drawback of sputum cytology is its failure to sub classifies a fair number of malignancies as either adeno carcinoma or squamous cell carcinoma. The sub- classification of non-small cell carcinoma is significant in selecting treatment modalities. The present analysis observed a total of 90 samples reported as non small cell carcinoma.After histopathologic confirmation, 12 cases remained as non small cell carcinoma which could not be distinguished into adeno or squamous cell carcinoma. The Hospital Based Cancer Registry (HBCR) data (2008) also reported 19.67% total lung cancer cases having a diagnosis of non small cell carcinoma. The ambiguity in distinguishing non small cell carcinoma into adeno or squamous was mainly due to unsatisfactory sample, inadequate number of cells and poor morphology preservation due to drawbacks in sputum processing techniques.

Cytologic atypia of exfoliated cells in sputum has been shown to be associated with both prevalent and incident lung cancer.^{17,18,19,20} In previous studies, 14% of the patients with sputum cytologic atypia graded as moderate or worse later progressed to lung cancer as compared with only 3% without atypia.^{21,22} Cytologic atypia could be associated with increased lung cancer risk due to morphological changes in bronchial epithelial cells that correlate with broad changes in the bronchial epithelium and this association is considerably stronger for samples collected within few months of the diagnosis of lung cancer, and perhaps lung cancer itself also exfoliate abnormal cells into the sputum. So further investigations of patients with atypical /suspicious diagnoses are prime importance in patient management and to distinguish between prevalent and incident lung cancer and there by selecting treatment protocol.

The ambiguity existing in the diagnosis of lung cancer by sputum is attributed to lack of improved techniques in the laboratory processing of samples.^{19, 25, 26} Due to low sensitivity of sputum examination, it cannot be considered as a very reliable test ,especially in the modern times of bronchial brush biopsy and CT guided Fine Needle Aspiration Cytology. Another fact is that a negative sputum cytology report may not represent a truly benign lesion; it is due to the failure to get representative samples. If we get adequate well preserved cells in sputum samples, morphological evaluation can be supplemented by molecular tests, especially those with clinical symptoms and abnormal X -ray findings in low resource settings.^{23, 24}

The present analysis suggests sputum cytology is of great diagnostic value in this modern era of more sophisticated techniques, as it is a reliable and non invasive method for patients in low resource settings .If some molecular biology techniques are combined along with sputum cytological examination, it will be promising for the early detection, evaluation and staging of lung cancer.

Table 1. Correlation of Cytological findings with clinical symptoms

Clinical Symptoms (chi-square test value)	Cytology Report					Total
	Adeno Ca (%)	Sq. □ Ca. (%)	N S□ Ca. (%)	Small cell Ca (%)	Atypical/ Suspicious (%)	
Cough (0.405)	23.9	8.7	7.6	6.1	53.6	444
Fever (0.806)	19.1	8.2	5.4	5.4	61.8	110
Breathlessness (0.397)	26.3	8.8	8.8	4.7	51.5	171
Dyspnoea (0.061)	35.6	4.1	4.1	8.2	47.9	73
Haemoptysis (0.197)	25.1	8.8	7.5	7.9	50.6	239
Chest pain (0.025)	15.8	10.8	10.1	8.6	54.6	139
Hoarseness of voice (0.904)	27.5	3.4	6.9	6.9	55.2	29
Weight loss (0.792)	25	8.3	0	0	66.7	12
Loss of Appetite (0.188)	38	14.3	0	0	47.6	21

Table 2. Correlation of cytology with histopathology

Cytology Report	Adeno Ca.	Sq□ Ca.	N.S□ Ca.	Small cell ca.	Atypical /Sus□	Total
	323	107	90	79	792	1391
Corresponding Histopathology						
Adeno ca	257	8	53	1	515	834
Sq.Ca□	31	89	22	0	229	371
NS Ca. □	30	7	12	1	30	80
Small cell ca.	2	0	0	66	5	73
PD Ca	3	3	3	11	13	33
Total positive cases	323	107	90	79	792	1391

Sq.Ca□-squamous cell carcinoma.

NS Ca. □-Non small cell carcinoma.

Sus. □-Suspicious.

PDCa NOS□-Poorly differentiated carcinoma not otherwise specified.

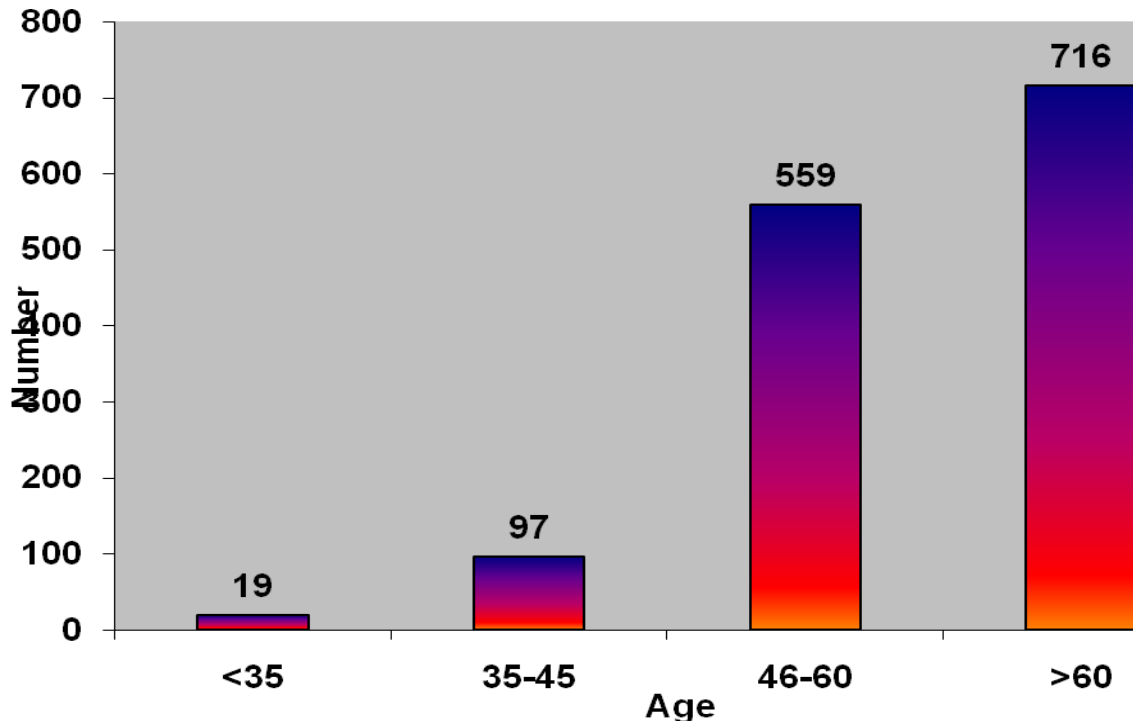


Fig1. Distribution of Age of patients who were positive in sputum cytology

REFERENCES

[1] Parker SL, Tong T, Bolden S, Wingo PA. Cancer Statistics. *Ca: Cancer J Clin* 1997; 47:5-27.

[2] Consolidated Report of Population Based Cancer Registries 2001-2004, National Cancer Registry Programme, Indian Council of Medical Research, Bangalore, India 2006;2:12-20

[3] Schreiber G, McCrory DC. Performance characteristics of different modalities for diagnosis of suspected lung cancer: Summary of published evidence. *Chest* 2003;123:115-28.

[4] Raab SS, Hornberger J, Raffin T. The importance of sputum cytology in the diagnosis of lung cancer: a cost effectiveness analysis. *Chest* 1997; 112:37-45.

[5] Melamed MR. Lung cancer screening results in the National Cancer Institute New York study. *Cancer* 2000; 89:356-62.

[6] Ellis JR, Gleeson FV. Lung cancer Screening. *The Br J Radiol* 2001; 74:478-85.

[7] Hartveit F. Time and place for sputum cytology in the diagnosis of lung cancer. *Thorax* 1981;36:299-302.

[8] Bhatia A, Singh N, Arora VK. A Perspective on cytology of lung cancer. *Indian J Chest Dis Allied Sci* 2004;46:81-3.

[9] Beckles MA, Spiro SG, Colice GL, Rudd RM. Initial evaluation of the patients with lung cancer: symptoms, signs, laboratory tests and paraneoplastic syndromes. *Chest* 2003;123:97-104.

[10] Austin JHM, Romney BM, Goldsmith LS. Missed bronchogenic carcinoma: radiographic findings in 27 patients with a potentially resectable lesion evident in retrospect. *Radiology* 1992; 182:115-22.

[11] White CS, Flukinger T, Jeudy J, Chen JJ. Use of a computer aided detection system to detect missed lung cancer at chest radiography. *Radiology* 2009;252:273-81.

[12] Barrett J, Hamilton W. Pathways to the diagnosis of lung cancer in the UK: a cohort study. *BMC Fam Pract* 2008;9:31.

[13] Allgar VL, Neal RD, Ali N, Leese B, Heywood P, Proctor G, *et al.* Urgent general practitioner referrals for suspected lung, colorectal, prostate and ovarian cancer. *Br J Gen Pract* 2006;56:355-62.

[14] Yoshimi I, Ohshima A, Ajiki W, Tsukuma H, Sobue TA. A comparison of trends in the incidence rate of lung cancer by histological type in the Osaka Cancer Registry, Japan and in the Surveillance, Epidemiology and End Results Program, USA. *Jpn J Clin Oncol* 2003; 33:98-104.

[15] Devesa SS, Bray F, Vizcaino AP, Parkin DM. International lung cancer trends by histologic type: Male:female differences diminishing and adenocarcinoma rates rising. *Int J Cancer* 2005; 117; 294-9

[16] Lantuejoul S, Salameire D, Salon C, Brambilla E. Pulmonary preneoplasia-sequential molecular carcinogenetic events. *Histopathology* 2009;54:43-54.

[17] Byers T, Wolf HJ, Wilbur A, Franklin WA, Braudrick S, Merrick DT, *et al.* Sputum cytologic atypia predicts incident lung cancer: defining latency and histologic specificity. *Cancer Epidemiol Biomarkers Prev* 2008;17:158-62.

[18] Tockman MS. Advances in sputum analysis for screening and early detection of lung cancer. *Cancer Control* 2000;7:19-24.

[19] Hirsch FR, Franklin WA, Gazdar AF, Bunn Jr PA. Early detection of lung cancer: Clinical perspectives of recent advances in biology and radiology. *Clin Cancer Res* 2001;7:5-22.

[20] Petty TL, Tockman MS, Palcic B. Diagnosis of roentgenographically occult lung cancer by sputum cytology. *Clin Chest Med* 2002;23:59-64.

[21] Frost JK, Ball WC Jr, Levin ML, Tockman MS, Baker RR, Carter D, *et al.* Early lung cancer detection: results of initial (prevalence) radiologic and cytologic screening in the Johns Hopkins study. *Am Rev Respir Dis* 1984;130:549-54.

[22] Holiday DB, McLarty JW, Farley ML, Mabry LC, Cozens D, Roby T, *et al.* Sputum cytology within and across laboratories. A reliability study. *Acta Cytol* 1995;39:195-206.

[23] Varella GM, Kittelson J, Schulte AP, Vu KO, Wolf HJ, Zeng C, *et al.* Multi-target interphase fluorescence in situ hybridization assay increases sensitivity of sputum cytology as a predictor of lung cancer. *Cancer Detect Prev* 2004;28:244-51.

[24] Belinsky SA, Liechty KC, Gentry FD, Wolf HJ, Rogers J, Vu K, *et al.* Promoter hypermethylation of multiple genes in sputum precedes lung cancer incidence in a high risk cohort. *Cancer Res* 2006;66:3338-44.25. Lam S, Shibuya H. Early diagnosis of lung cancer. *Clin. Chest Med* 1999;53-61.

[25] Kennedy TC, Proudfoot SP, Piantadosi S, Wu L, Saccomanno G, Pretty TL, *et al.* Efficacy of two sputum collection techniques in patients with air flow obstruction. *Acta. Cytol.* 1999;43:630-6

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