A CLINICO-PATHOLOGICAL STUDY ON CYTOLOGICAL SCREENING OF BRONCHOALVEOLAR LAVAGE AND SUBSEQUENT CORRELATION WITH ADENOSINE DEAMINASE, GENEXPERT MTB IN SUSPECTED SPUTUM NEGATIVE PULMONARY TUBERCULOSIS

Medical Science

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ABSTRACT

BACKGROUND: Sputum negative pulmonary tuberculosis is a world-wide burden on health budget. Clinico- radiologically suspected sputum negative pulmonary tuberculosis needs to be diagnosed using cytopathology along with ADA levels and GeneXpert MTB tests of BAL fluid obtained through bronchoscopy. **AIM:** To interpret the cytological screening of bronchoalveolar lavage and subsequent correlation with adenosine deaminase, genexpert mtb in suspected sputum negative pulmonary tuberculosis. **METHODS:** Patients visiting Pulmonary Medicine outdoor of R.G.KAR Medical College Hospital, who are clinico radiologically being suspected as pulmonary tuberculosis but are sputum smear AFB negative on two consecutive samples. The study group includes these patients who are willing to undergo bronchoscopy, in whom there are neither any medical contraindications nor other miscellaneous restraints i.e. the study group contains cases of pulmonary TB as well as other infectious malignant non-infectious lung diseases. **RESULT:** Of the 61 patients, 41(67.2%) patients were male and 20(32.8%) patients were female. The average age of male patients was 51.71 years and the average age of female patients was 39.35 years. The mean ADA level of BAL fluid in patients with Pulmonary Tuberculosis was 22.54 U/L (Normal level – less than 30 U/L) and mean ADA level of BAL fluid in patients without Pulmonary Tuberculosis was 4.76 U/L (Normal level – less than 30 U/L). **CONCLUSION:** Cytology of bronchoalveolar lavage along with adenosine deaminase and GeneXpert MTB are important diagnostic and screening tools in suspected sputum negative pulmonary tuberculosis. As it can detect different kinds of inflammatory and other types of cells, it can also help in choosing the appropriate therapeutic procedure / modality for a disease.

KEYWORDS

Cytological Screening, Bronchoalveolar, Genexpert MTB , Sputum Negative Pulmonary Tuberculosis

INTRODUCTION

Tuberculosis (TB), an important preventable and treatable cause of death, is a major health problem worldwide. Detecting patients with active pulmonary tuberculosis (PTB) disease is an important component of TB control as early appropriate treatment renders these patients non-infectious and interrupts the chain of transmission of TB. As per the guidelines of Revised National Tuberculosis Control Programme (RNTCP) of Government of India, the diagnosis of PTB is based on sputum smear examination. Sputum microscopy is a highly specific test, a low-cost, appropriate technology and is an essential component of the directly observed treatment, short-course (DOTS) strategy of the World Health Organisation (WHO) where a case detection rate of 70% or more is aimed at¹. However, in patients with a compatible clinico-radiological picture, sputum smears do not reveal acid-fast bacilli (AFB) in all patients. Smear negative-sputum culture positive state has been observed in 22% to 61% of the cases². Mycobacterial cultures take much time for confirming the diagnosis and thereby a valuable time is lost. The role of bronchoalveolar lavage (BAL) in making a definitive diagnosis of suspected sputum-negative PTB does arise.

Nowadays, BAL is obtained by the use of flexible fibre-optic bronchoscopy (FFB). BAL is easily performed and well tolerated procedure, is able to provide cellular contents, cellular products from the lower respiratory tract. Levels of biochemical constituents of the fluid like adenosine deaminase (ADA) can also be studied in it. BAL also provides fluid for genetic/molecular analysis by using GeneXpert MTB. Various studies, research works have shown the interpretation of BAL fluid cytology as an important diagnostic and screening tool in diffuse, infiltrative, interstitial, obstructive and restrictive diseases of the lung including suspected sputum smear negative pulmonary tuberculosis (SSN-PTB). The total and differential cell counts (macrophages, WBC's, plasma cells) of BAL fluid vary in infectious and non-infectious diseases of the lung. Cytological assessment of the fluid can detect malignant cell. In this connection, it is important to note that cytology from bronchial washings has a positive diagnostic yield in 45.1% of cases in detecting malignant cell. BAL fluid cytology has 93.44% sensitivity & 100% specificity as compared to trans-bronchial biopsy in diagnosing lung cancers³.

In our present study, we selected a group of patients who have been clinico-radiologically suspected to be pulmonary tuberculosis but are sputum/smear negative on two consecutive samples i.e. the study group contains cases of pulmonary TB as well as other infectious/malignant/non-infectious lung diseases. Then with their

due consent and taking into consideration any contraindications/restraints, as per standard guidelines of RNTCP, bronchoscopy were performed in these patients and BAL fluids collected. Detailed cytological studies of these fluids were done along with measuring ADA levels and testing GeneXpert MTB in those BAL fluids. BACTEC culture of BAL fluid for demonstrating Mycobacterium Tuberculosis bacilli in it were done as the gold standard test. Firstly, the present study emphasized on the utility/diagnostic yield of bronchoalveolar lavage fluid cytology in the study population i.e. suspected sputum negative pulmonary tuberculosis cases (study group consisting of pulmonary tuberculosis & other infective / malignant / non-infective lung diseases). Secondly, this study showed the correlation between the results of BAL fluid cytology on one hand and ADA levels with GeneXpert MTB on the other in the study group/population by statistical analysis of data. Thirdly, this study screened all the patients for any lung pathologies like bronchogenic carcinoma, connective tissue disorders, interstitial lung disease (ILD), pneumonia, alveolar proteinosis, pneumoconiosis, diffuse alveolar haemorrhage etc., other than pulmonary tuberculosis and see their statistical distribution in the study group as pulmonary tuberculosis can mimic all pathological considerations of the lung and can present in various forms and appearances. And lastly this study compared the levels of ADA in BAL fluid in tuberculous and non-tuberculous cases.

- To interpret the results of cytological screening of bronchoalveolar lavage in the study population by detecting inflammatory cells, malignant / atypical cells, epithelial cells, giant cells, macrophages, granulomas, intra-nuclear & intracytoplasmic inclusion bodies, microorganisms, non-cellular elements etc in the fluid.
- To study the immunopathology i.e. the role of inflammatory cells in BAL fluid of various pulmonary diseases in the study population including sputum negative pulmonary tuberculosis.
- To study the correlation between the results of BAL fluid cytology on one hand and ADA levels with GeneXpert MTB in the BAL fluid on the other in the study group / population by statistical analysis of data.
- To compare the levels of ADA in BAL fluid in patients with and without pulmonary tuberculosis.

MATERIALS & METHODS

Across sectional study was done in collaboration with Department of Pathology Department of Pulmonary Medicine & Department of Biochemistry- all of R.G.KAR Medical college Hospital & in RNTCP

accredited laboratories of outside.

Different cytological profiles of bronchoalveolar lavage fluid in different patients, positive/negative findings of GeneXpert in BAL fluids of different patients, different levels of adenosine deaminase in BAL fluid of different patients.

INCLUSION CRITERIA:

1. Clinico-radiologically suspected cases of pulmonary tuberculosis who are sputum smear negative.
2. Patients willing to undergo bronchoscopy.
3. And lastly, patients who are willing to participate in this study.

EXCLUSION CRITERIA:

1. Patients not willing to undergo bronchoscopy.
2. Patients not willing to participate in this study.
3. Patients in whom bronchoscopy is contraindicated due to adverse medical/surgical history/illness/existing conditions.
4. Miscellaneous restraints.

SAMPLE DESIGN: Cases of clinico-radiologically suspected sputum negative pulmonary tuberculosis are selected by simple random sampling as per the inclusion & exclusion criteria.

METHODS OF DATA COLLECTION: Written consent for participating in this study was taken from the patients at first. Case histories were taken, examinations were done & the same were recorded in appropriate forms. BAL fluids were obtained from the Department of Pulmonary Medicine of R.G.KAR Medical College Hospital. Thereafter detailed cytological study of BAL fluid was done in the Department of Pathology of R.G.KAR Medical College Hospital & was recorded in cytological report forms. BAL fluid ADA levels were measured in the Department of Pathology of R.G.KAR Medical College Hospital. GeneXpert MTB were done in Department of Microbiology of R.G.KAR Medical College Hospital. BACTEC cultures of BAL fluids were outsourced from RNTCP accredited laboratories. These data were recorded in another form. Thereafter all data were compiled, tabulated and statistically analysed. After that inference was drawn.

OBSERVATION & RESULTS

In our study, 41(67.2%) patients were male and 20(32.8%) patients were female. The average age of male patients was 51.71years and the average age of female patients was 39.35years.

It was found that 2 patients were under 11-20years of age range, 15 patients were under 21-30years of age range, 4 patients were under 31-40years of age range, 12 patients were under 41-50years of age range, 10 patients were under 51-60years of age range, 16 patients were under 61-70years of age range and 2 patients were under 71-80years of age range.

Our study showed that 27 cases had Pulmonary Tuberculosis (PTB), 11 cases had Pneumonia, 16 cases had Malignancy and 7 cases had Interstitial Lung Disease (ILD). 54 patients had Persistent Cough, 42 patients had Low grade fever, 48 patients had Haemoptysis and 24 patients had Shortness of breath.

We found that the mean ADA level of BAL fluid in patients with Pulmonary Tuberculosis was 22.54 U/L (Normal level – less than 30 U/L) and mean ADA level of BAL fluid in patients without Pulmonary Tuberculosis was 4.76 U/L (Normal level – less than 30 U/L).

It was found that the mean total cell count of BAL fluid of patients with Pulmonary Tuberculosis was 504.44/cmm (Normal range – 100 to 180/cmm) and mean total cell count of BAL fluid of patients without Pulmonary Tuberculosis was 216.47/cmm (Normal range – 100 to 180/cmm).

We also found that the mean number of lymphocytes in BAL fluid of patients with pulmonary tuberculosis (PTB) was 399.88/cmm and mean number of lymphocytes in BAL fluid of patients without pulmonary tuberculosis (PTB) was 29.75/cmm.

DISCUSSION

In a study (The Value of Bronchoalveolar Lavage in the Diagnosis of Sputum Smear-Negative Pulmonary Tuberculosis) conducted by Novin Nikbaksh et al ⁴ the study population consisted of 59.7% male

and 40.3% female subjects. The mean age was 52.6±19.1 years with range of 20 to 76 years. The patients aged 50 to 60 years (17.9%) were the ages most frequently affected. 35.5% cases were confirmed as tuberculosis by culture in their study.

In our study the most common symptom in the study group is persistent cough (54 cases) followed by haemoptysis (48 cases), low grade fever (42 cases) and shortness of breath (24 cases) respectively.

The mean ADA level of BAL fluid in pulmonary tuberculosis cases was 22.54 U/L as compared with a mean ADA level of 4.76 U/L in cases other than pulmonary tuberculosis in our study. Orphanidou D et al ⁵ showed that patients with pulmonary tuberculosis had significant higher activity in BAL fluid than patients with non tubercular lung diseases (p<0.001). In yet another study Wipa Reechaipichitkul et al ⁶ found the mean ADA activity in the bronchoalveolar lavage for the pulmonary tuberculosis, malignancy, and miscellaneous causes groups as 8.98 U/L(95% CI, 3.79-14.17), 7.63U/L (95% CI, 4.12-11.14), and 11.61 U/L(95% CI, 3.59-19.62), respectively.

Out of total 61 patients 27 cases were confirmed as pulmonary tuberculosis by BACTEC culture of BAL fluid in our study. In these 27 cases 24 were found to be GeneXpert MTB positive and 3 were negative in our study. Apart from these there was a case where GeneXpert MTB was positive and BACTEC culture was negative. Bactec culture was negative in 34 cases and both bactec culture and genexpert were negative in 32 cases. Sensitivity and specificity of BAL fluid genexpert are 88.9% & 94.1% respectively in our study. In a similar study Khalil KF et al ⁷ showed the sensitivity and specificity of BAL fluid genexpert to be 91.86% and 71.42% respectively.

In the pneumonia group of patients neutrophilic population (76.2%) was highly elevated in our study. Lymphocytes were in the normal range (8.2%). Alveolar macrophages were heavily decreased (15.6%). These were all cases of bacterial pneumonia as found in subsequent cultures in our study. There were also few epithelial cells and RBC's. Sang-Ho Choi et al ⁸ in their study found 80.5% neutrophils, 4% lymphocytes & 12% macrophages in BAL fluid of bacterial pneumonia cases.

In another group of patients alveolar macrophages were marginally decreased (76.7%), lymphocytes marginally increased (17.4%), neutrophils marginally increased (5.9%) in our study. Apart from these atypical cells with irregular, pleomorphic, hyperchromatic nuclei were also seen in those cases. Few epithelial cells were also found. Subsequently with the help of transbronchial biopsy (gold standard) malignancy was confirmed in those cases. The sensitivity of BAL fluid cytology in detecting malignancy was 100% in our study. Ayesha Ahmed et al ⁹ in their study found the sensitivity of BAL fluid cytology in detecting malignancy as 93.44%.

In the interstitial lung diseases group of patients alveolar macrophages were decreased (60%), lymphocytes were increased (21.4%), neutrophils were increased (18.6%) in our study. All these ILD cases were connective tissue diseases related in our study. Respiratory epithelial cells were also found. Boubacar Efares et al ¹⁰ in their study found 52.27% alveolar macrophages, 30.71% lymphocytes, 15.21% neutrophils & 1.57% eosinophils in connective tissue diseases related ILD cases.

Diseases such as malignancies, pneumonia, interstitial lung diseases were confirmed subsequently with the help of other relevant definitive investigations.

CONCLUSION

This present clinicopathological study conducted over one year at R.G.Kar Medical College Hospital showed the importance of bronchoalveolar lavage in suspected sputum negative pulmonary tuberculosis. In the bactec culture confirmed cases of pulmonary tuberculosis much higher levels of lymphocytic population were seen in the lavage as compared to the cases not diagnosed as pulmonary tuberculosis. The total cell count of BAL fluid, adenosine deaminase levels were much higher in pulmonary tuberculosis than in the cases not diagnosed as pulmonary tuberculosis. GeneXpert MTB was positive in most of the cases of pulmonary tuberculosis. Thus, there is a correlation between cytology, ADA levels and GeneXpert MTB in suspected sputum negative pulmonary tuberculosis.

Our study also detected other diseases which clinicoradiologically mimic pulmonary tuberculosis. Diseases such as malignancies, bacterial pneumonia, interstitial lung diseases (ILD) were confirmed subsequently with the help of other relevant definitive investigations. Bactec culture & GeneXpert MTB were negative; adenosine deaminase levels were low in the cases of malignancies, bacterial pneumonia, and interstitial lung diseases. Total cell counts of BAL fluid, lymphocyte count were lower than in the cases of tuberculosis. Atypical cells were noted in cases of malignancies. Neutrophilic population was predominant in the pneumonia group. A mixed inflammatory infiltrate was noted in the ILD group.

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