

## Role of Adenosine Deaminase in Pleural Effusions and its Cytological Correlation.

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### ABSTRACT

**Background:** Diagnosis of pleural diseases creates difficulty due to overlapping features of various benign and malignant conditions. However pleural fluid cytology of closed pleural biopsy specimen is most commonly used procedure to diagnose various pleural diseases in developing country like India. Present study was conducted to establish diagnostic utility of ADA in Pleural Fluids and its correlation with cytological findings. **Methods:** The present study includes 100 samples of pleural fluid samples taken from patients coming to the Department of Chest and TB, Government Medical College and Hospital, Amritsar with the complaint of pleural effusion. **Results:** For ADA levels in pleural fluid Sensitivity is 92%, Specificity is 81.33%, Positive predictive value is 62.16% and Negative predictive value is 96.83%. For lymphocyte count in pleural fluid Sensitivity is 100%, Specificity is 16.67%, Positive predictive value is 31.25 % and Negative predictive value is 100%. In Combination of ADA and lymphocyte count to diagnose tubercular pleural effusion Sensitivity is 100%, Specificity is 77.27%, Positive predictive value is 82.14 % and Negative predictive value is 100%. **Conclusion:** Measurement of ADA level in pleural fluid in combination with the differential count of pleural fluid will give best results to categorize and to rule in the diagnosis of tubercular pleural effusion.

**Keywords:** Adenosine Deaminase, Cytology, Effusion, Pleural Fluid.

### INTRODUCTION

Pleural Effusion is an abnormal accumulation of fluid in the pleural cavity and remains the most common manifestation of pleural pathology.<sup>[1]</sup> Pleural effusions may be asymptomatic but if large produce breathlessness or pain, or both. Breath sounds are reduced on the affected side, and the percussion note is stony dull.<sup>[2]</sup> Pleural effusion is the end result of many pulmonary as well as other systemic diseases. It requires diagnostic and/or therapeutic tapping in most cases to make a final etiological diagnosis. Adenosine Deaminase activity (ADA) analysis in effusion as well as in serum are in practice as this is the cheapest test with high diagnostic value. Adenosine Deaminase is an enzyme in the purine salvage pathway required for

converting adenosine to inosine.<sup>[3]</sup> It is needed for the breakdown of nucleic acids in tissues. ADA value above 40 IU/L are known to well correlate with tubercular pathology in various studies and help to differentiate between tubercular and nontubercular exudative pleural effusion.<sup>[4]</sup> Pleural effusions accompany a wide variety of disorders of the lung, pleura, and systemic disorders. Therefore, a patient with pleural effusion may present not only to a pulmonologist but to a general internist, rheumatologist, gastroenterologist, nephrologist, or surgeon. To treat pleural effusion appropriately, it is important to determine its cause. With knowledge of the pleural fluid cytology, biochemistry, and clinical presentation, an etiological diagnosis can be established in approximately 75% of patients.<sup>[5]</sup> There has been a need to study the diagnostic efficacy of ADA in pleural effusion and its cytological correlation at the cost effectiveness of each test. So this study was done to study the diagnostic utility of ADA in Pleural Fluids and its correlation with cytological findings.

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## MATERIALS AND METHODS

The present study includes 100 samples of pleural fluid samples taken from patients coming to the Department of Chest and TB, Government Medical College and Hospital, Amritsar with the complaint of pleural effusion. All clinically diagnosed cases of pleural effusion irrespective of age and sex were included in the study. All patients who were diagnosed as a case of active tuberculosis and are on anti-tubercular treatment were excluded. The site of aspiration was located clinically or radiologically. After preparation of the site, the needle was inserted in the appropriate intercostal space along the upper border of the rib and the pleural fluid was aspirated. The fluid received was sent to the laboratory for examination. Physical examination of the fluid was done to note colour, appearance and presence of any sediment. The fluid received was subjected for ADA level measurements and cytological examination. The data collected was analysed according to the standard statistical methods to reach a conclusion. All statistical analyses were performed using IBM SPSS Statistics version 19. A 2 tailed p value of < 0.05 was taken to be statistically significant.

## RESULTS

**Table 1: Types of pleural effusion**

Type of pleural effusion	No. of patients (n=100)	Mean ADA Levels (gm/dl) $\pm$ SD	Mean Protein (gm/dl) level $\pm$ SD
Exudates	80	47.49813 $\pm$ 29.26052	3.85 $\pm$ 0.347491
Transudate	20	20.26 $\pm$ 13.34029	2.2 $\pm$ 0.439318
Total / P value	100	0.0001	0.0001

**Table 2: Total leucocyte count**

Total Count	Exudative	Transudative	Total
LESS THAN 1000	2	20	22
1000 & MORE THAN 1000	69	0	69
MEAN $\pm$ STD	1387.21 $\pm$ 342 $\pm$ 223	251.5385 $\pm$ 139.6615	91

**Table 3: Distribution of pleural disease and appearance of pleural fluid**

Appearance of pleural fluid	Pleural disease				Total
	Malignant effusion	Tuberculous effusion	Non malignant effusion	OTHERS	
Straw	1	13	29	2	45
Yellow	0	6	11	1	18
Dark yellow	0	1	2	1	4
Haemorrhagic	2	5	24	2	33
Total	3	25	66	6	100

There were 73 males and 27 females. The mean age was 43.02  $\pm$  17.73 years. The mean age among men was 42.05  $\pm$  16.64 years and in women were 45.62  $\pm$  20.15 years. Majority of the patients were in the age group of 21-40 years and 41-60 years. The mean quantity of pleural fluid was 10.88  $\pm$  7.14 ml. Most of the patients came with chief complaint of fever, cough, dyspnea and chest pain. Out of 100 patients 10 were smokers and 11 were alcoholic.

**Table 4: Sensitivity and Specificity of ADA Pleural fluid**

ADA level	Tubercular	Non Tubercular	Total
> 40 IU/L	23	14	37
$\leq$ 40 IU/L	2	61	63
Total	25	75	100

Sensitivity = 92%

Specificity = 81.33%

Positive predictive value = 62.16%

Negative predictive value = 96.83%

**Table 5: Cytology Sensitivity and Specificity of Lymphocyte counts for Pleural Fluid**

Lymphocyte Percentage	Tubercular Effusion	Non Tubercular	TOTAL
>50%	25	55	80
$\leq$ 50%	0	11	11
TOTAL	25	66	91

Sensitivity = 100%

Specificity = 16.67%

Positive predictive value = 31.25%

Negative predictive value = 100%

**Table 6: The sensitivity and specificity of ADA and lymphocyte counts to diagnose tubercular pleural effusion**

ADA Levels (Pleural Fluid)	Tubercular Effusion	Non Tubercular	TOTAL
> 40 u/l and > 50% Lymphocytosis	23	5	28
$\leq$ 40 u/l and $\leq$ 50% Lymphocytosis	0	17	17
Total	23	22	45

Sensitivity = 100%

Specificity = 77.27%

Positive predictive value = 82.14%

Negative predictive value = 100%

## DISCUSSION

In the present study the mean quantity of pleural fluid was 10.88  $\pm$  7.14 ml. Most of the patients came with chief complaint of fever (56), cough (47), dyspnea (46) and chest pain (16). These findings are compatible with the study done earlier by Moudgil et al. Alaarag AH et al,<sup>[7]</sup> in their study showed that the most prevalent complaint was cough (100%)

followed by expectoration in 83.3% patients, dyspnea in 80% patients, This indicates that fever and cough are the most common symptom in all chest diseases and are the most common symptom that brings the patients to physicians to seek medical advice.<sup>[8]</sup>

Out of 100 patients, 10 were smokers and 11 were alcoholic patients. 5 HIV positive patients also have also been reported in our study. Out of 100 patients 73 cases of pleural effusions were exudative and 19 patients were of transudative pleural effusion. The mean ADA value for exudative pleural effusion is 47.49813 gm/dl and for transudative pleural effusion is 20.26gm/dl. The mean protein value for exudative pleural effusion is 3.85 gm/dl and for transudative pleural effusion are 2.2 gm/dl. By conventional criteria, this difference is considered to be extremely statistically significant. Kushwaha, et al,<sup>[9]</sup> in their study also observed 82% cases as exudative and 18% as transudative.

Among our 100 cases, 11 cases of exudative pleural effusion had less than 1000 total leucocyte count, 69 cases had more than 1000 total leucocyte count; whereas in transudative pleural effusions 20 cases had total leucocyte count less than 1000. Kushwaha, et al,<sup>[9]</sup> also stated that overall 52.44% of exudative effusions had TLC greater than 1000 cells/cu.mm. It was noted that 96.88% of tuberculous effusions had more than 50% lymphocytes, 81.25% had protein greater than 5 gm/dl.

In India tubercular effusion is the commonest cause of all exudative effusions. This is similar to the observation in another study from India by Maldhure et al,<sup>[10]</sup> where they showed that the tubercular effusions constitute 66% of the effusions, malignancy 15%, and parapneumonic effusion 4.8%. This observation is different from that of the West where the incidence of parapneumonic effusion and malignant effusion are much higher compared to that of tubercular effusion. This is consistent with the fact that India has a high prevalence of tuberculosis in the general population. On some patients special investigations were done to get definite diagnosis. Sputum for AFB was positive in 16 patients, CBNAAT test was positive in 7 patients; montoux test was positive in 4 patients and one patient showed blood culture positive for klebsiella. These are similar to the finding of Valdes et al.<sup>[11]</sup>

In our study Mean ADA levels were  $35.57 \pm 22.79$  in non malignant effusions,  $36.2 \pm 3.93$  in malignant effusions,  $74.88 \pm 29.04$  in tuberculous effusions and  $30.73 \pm 20.04$  in case of other effusions. The mean ADA were high in the 2 Indian studies done by Rajendra Prasad et al,<sup>[12]</sup> and Gilhotra et al,<sup>[13]</sup> with the mean ADA level ranging between  $76.8 \pm 23.8$  IU/L -  $95.8 \pm 57.5$  IU/L.

According to the literature pleural fluid adenosine deaminase (ADA) has got a good discriminative value in differentiating tuberculous effusions from malignant effusion and other effusions. Although a

pleural fluid ADA above 70IU/L is diagnostic of tuberculosis it has to be considered if the pleural fluid ADA is between 40 IU/L and 70 IU/L. An ADA level less than 40IU/L rules out pleural tuberculosis.<sup>[14]</sup>

In our study, non malignant effusion having 13 cases had pleural fluid ADA more than 40 IU/L, 53 cases had a pleural fluid ADA less than 40IU/L. Malignant effusion had pleural fluid ADA less than 40IU/L in 3 cases. In tuberculous effusions 23 cases had a pleural fluid ADA more than 40IU/L and 2 cases had pleural fluid ADA less than 40IU/L. In others 1 case had pleural fluid ADA more than 40IU/L and 5 cases had pleural fluid ADA less than 40IU/L. The p-value is  $< .00001$ . The result is significant at  $p < 0.05$  with Sensitivity = 92%, Specificity= 81.33%, Positive predictive value = 62.16% and Negative predictive value = 96.83%.

Lamsal et al,<sup>[15]</sup> studied the diagnostic utility of ADA activity in pleural fluid and serum of tuberculous and nontuberculous respiratory disease patients. Their study included 32 cases of active pulmonary TB, 29 cases of tuberculous pleural effusion, 13 cases of nontuberculous respiratory diseases, and 32 healthy individuals as a control group. Using the cut-off point of 25  $\mu$ l in serum in the diagnosis of TB, the sensitivity and specificity were 72.41 and 81.53%, respectively. Similar results were obtained by Rao et al,<sup>[16]</sup> who used 33  $\mu$ l as a cut-off point for serum ADA in the diagnosis of TB; the sensitivity and specificity were 98.06 and 95.35%, respectively. Hassanein et al,<sup>[17]</sup> used a cut-off point of 26.2  $\mu$ l in the diagnosis of pulmonary TB, with a sensitivity and specificity of 95 and 83.3%, respectively, with a positive predictive value of 79.2%.

Piras et al,<sup>[18]</sup> were one of the earliest workers who studied ADA activity in pleural effusion of different etiology and concluded that the tubercular pleural fluid ADA activity was significantly higher when compared to different non tubercular cases which is similar to this study.

All the 25 cases of tubercular effusions were lymphocytic pleural effusions. 55 of non tubercular effusions were lymphocytic and 11 effusions were neutrophilic pleural effusions. With the Sensitivity = 100%, Specificity= 16.67%, Positive predictive value = 31.25% and Negative predictive value = 100%. Our result was similar to the study done by Valdes L et al where they have encountered neutrophil predominant tuberculous effusion in only 6.7% of patients and only one malignant effusion had neutrophil predominant effusion (3%).<sup>[11]</sup>

Combination of pleural fluid differential count (lymphocytosis  $>50\%$ ) and ADA level ( $>40$ U/L) as well as (lymphocytosis  $<50\%$ ) and ADA level ( $\leq 40$ U/L) was also calculated and found that Sensitivity = 100% Specificity= 77.27% Positive predictive value = 82.14% and Negative predictive value = 100%. Pande K et al,<sup>[3]</sup> also observed a bit variable results. The sensitivity and specificity of

ADA alone to diagnose tubercular pleural effusion was 92% each and when lymphocytosis alone was considered sensitivity was 85% with specificity of 32% whereas the combined effect of both ADA with lymphocytosis was 100% sensitivity and 87% specificity, 83% positive predictive value and 100% negative predictive value respectively.

Castro et al,<sup>[19]</sup> studied 410 cases of lymphocytic pleural effusion. The negative predictive value of the ADA test was very high (99%) similar to the present case. They also stated that with the decline in the prevalence of tuberculous pleural effusion in some areas, the positive predictive value of pleural fluid ADA also declines but the negative predictive value remains high. Besides, lymphocyte count and ADA level in pleural fluid, even other parameters will also help to build up a conclusion towards diagnosis as stated by Liam et al.<sup>[20]</sup> This study showed that a higher proportion of patients with tuberculosis had lymphocyte predominant effusion and tuberculous effusion had higher lymphocyte percentage. Newer school of thoughts has been provided by a study done by Mohan et al suggesting that the combined use of adenosine Deaminase activity along with lymphocyte neutrophils ratio would provide a more efficient means for diagnosing tuberculosis pleuritis than the use of ADA alone.<sup>[21]</sup>

## CONCLUSION

Diagnosis of pleural diseases create difficulty due to overlapping cytological and histopathological features of various benign and malignant conditions. However, in developing countries like ours, where investigations and health facilities are inadequate and cost of treatment is often unaffordable, pleural fluid analysis and cytology should continue to be a first line investigation to screen out the pleural effusion cases, as it is a very convenient, costeffective and safe investigation. Measurement of ADA level in pleural fluid in combination with the differential count of pleural fluid will give best results to categorize and to rule in the diagnosis of tubercular pleural effusion. Also its combination with other investigations can further enhance its usefulness in diagnosing pleural lesions.

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