



THE STUDY OF PLEURAL FLUID LACTATE DEHYDROGENASE /ADENOSINE DEAMINASE RATIO TO DIFFERENTIATE BETWEEN TUBERCULOUS AND PARA PNEUMONIC PLEURAL EFFUSION

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ABSTRACT

Background: Pleural Effusion is a commonly observed clinical manifestation which is associated with more than 50 recognized diseases and disorders. Pleural fluid lactate dehydrogenase (LDH) and adenosine deaminase (ADA) levels are often used to distinguish between tuberculous pleural effusion (TPE) and para pneumonic pleural effusion (PPE). This is challenging as the LDH level varies from normal to severely increased in PPE and a significantly elevated ADA is frequently measured in both conditions. In this study, we evaluated use of the pleural fluid LDH/ADA.

Material and Methods: This was a prospective observational case study. This study included 51 cases more than 18 years of age; presenting with unilateral pleural effusion meeting criteria of exudative pleural effusion to the OPD & IPD of Department of Medicine and Department Pulmonary Medicine S.R.N. Hospital Prayagraj, U.P. Following allotment, Pleural fluid aspiration was done, and patients were divided into 2 groups: tubercular and parapneumonic pathology. Pleural fluid sent for analysis (cytology, microbiology, and biochemistry). Pleural fluid LDH and ADA levels and LDH/ADA ratios were compared between two groups and results were obtained.

Results: out of 51 study subjects, 27 were diagnosed to have Tubercular pleural effusion and 24 were found to have parapneumonic pleural effusion. The median pleural fluid LDH, ADA and LDH/ADA ratio in TPE group were found to be 293.8 U/L, 59.8 U/L & 6.1 where as in PPE group it was found to be 503.3 U/L, 12.6 U/L and 40. Using LDH/ADA ratio as indicator of TPE an area under the ROC curve of 0.998 was obtained at cutoff value of 16.465. The sensitivity, specificity, positive predictive value and negative predictive value was found to be 96.4%, 100%, 100% and 95.8%.

Conclusion: The pleural fluid LDH/ADA ratio at cut-off level of 16.465 is highly effective in differentiating between TPE and PPE and can be used as a useful diagnostic aid for the clinicians and thus improving patient care.

KEYWORDS : Ldh; Ada; Tubercular Pleural Effusion; Parapneumonic Pleural Effusion

INTRODUCTION

Pleural effusion, which is a commonly observed clinical manifestation, is associated with more than 50 recognized diseases and disorders. A transudative pleural effusion occurs when systemic factors that influence the formation and absorption of pleural fluid are altered. An exudative pleural effusion occurs when local factors that influence the formation and absorption of pleural fluid are altered, in most parts of the world, subtypes of exudative effusions often seen in clinical practice include¹:

- Tuberculous pleural effusion (TPE),
- Parapneumonic effusion (PPE), and
- Malignant pleural effusion (MPE).

Parapneumonic effusions are associated with bacterial pneumonia, lung abscess, or bronchiectasis. They are subdivided into following 3 types

1. **Uncomplicated Para pneumonic Effusion-** Effusion in which patients respond to antibiotic treatment alone.
2. **Complicated Para pneumonic Effusion-** Non purulent appearing effusion requiring interventions such as Drainage.
3. **Empyema-** Effusion with frank pus (purulent in nature).

Transudative and exudative pleural effusions are distinguished by measuring the lactate dehydrogenase (LDH) and protein levels in the pleural fluid.

Exudative pleural effusions meet at least one of the following Light's Criteria, whereas transudative pleural effusions meet none:

1. Pleural fluid protein/serum protein >0.5
2. Pleural fluid LDH/serum LDH >0.6
3. Pleural fluid LDH > 2/3rd the normal upper limit for serum LDH

These criteria misidentify approximately 25% of transudates as exudates. If one or more of the exudative criteria are met and the patient is clinically thought to have a condition producing a transudative effusion, the difference between the protein levels in the serum and the pleural fluid should be measured. If this gradient is >31 g/L (3.1 g/dL), the exudative categorization by these criteria can be ignored because almost all such patients have a transudative pleural effusion¹

Among the exudative group, currently a definitive diagnosis of Tubercular Pleural effusion (TPE) is made based on the following criteria:

- (1) A positive AFB smear or positive cultures for MTB in pleural fluid and pleural tissue;
- (2) Chronic granulomatous inflammation in pleural tissue;
- (3) A clinical response to anti-tuberculosis treatment².

However, in most studies, an ADA level ≥ 40 U/L in lymphocytic exudates obtained via thoracentesis has been the most widely accepted indicator for a diagnosis of tuberculous pleural effusion³. A similar or even higher ADA level has been reported often in parapneumonic effusion as well. So sometimes ADA value alone may be misleading in differentiating tuberculous & parapneumonic effusion. Similarly, an elevated pleural fluid LDH may be present in tuberculous & parapneumonic effusion, malignant pleural effusion, and it may vary from normal to extreme values, so the sensitivity of LDH is low for identifying parapneumonic

effusion¹, Although parapneumonic effusion can be confirmed by pleural exudate in patients with bacterial pneumonia, lung abscesses or bronchiectasis, as well as the absence of disease specificity in pleural effusion biomarkers⁴ yet making a definitive diagnosis of parapneumonic effusion is always challenging as such patients may or may not present with symptoms and signs of pneumonia. . So, the aim of study is to establish pleural fluid LDH/ADA ratio as a new parameter to differentiate between tuberculous & parapneumonic effusion.

MATERIAL AND METHODS

Study Design

Analytical Observational Case Study.

Selection of Cases

All adult Cases > 18 years of age of either sex presenting to OPD & IPD of **Department of Medicine and Pulmonary Medicine S.R.N. Hospital Prayagraj**, as a case of pleural effusion was the source of our data.

Duration Of Study

June 2020 to June 2021. (Total 12 Months)

Inclusion Criteria

1. Cases with unilateral pleural effusion meeting the criteria of exudative pleural effusion.

Exclusion Criteria

1. Patients of bilateral pleural effusion.
2. All cases of unilateral pleural effusion with findings consistent with transudative pleural effusion
3. Patients having presence of malignant cells in pleural aspirate.

Study Procedure

All the selected patients by the above criteria were enrolled in study. Detailed history and physical examination were recorded. Complete routine investigations along with required radiological investigations were done. Pleural fluid aspiration followed by complete laboratory evaluation was done. Patients were divided into tuberculous and parapneumonic group. Pleural fluid LDH/ADA ratio was calculated. Results were analyzed by applying appropriate statistical tests.

Statistics

All the collected data was entered in an excel spreadsheet. Quantitative data was expressed in Mean and Standard deviation. Qualitative data was expressed in number and frequency. Sensitivity, specificity, positive predictive value, negative predictive value was calculated. The above statistical procedures were done with the help of the statistical package namely IBM SPSS statistics -20. The P-values less than or equal to 0.05(P≤0.05) were considered as statistically significant.

RESULTS

In this study a total of 51 subjects were recruited. The laboratory results were obtained from the Department of Biochemistry and Department of Microbiology; MLN Medical College, Prayagraj. **Out of the 51 subjects; 24 were found to have Parapneumonic pleural effusion and 27 were found to have tubercular pleural effusion.** The study subjects were described in respect of their demographic profiles and clinical variables of continuous variables were analysed and interpreted by a student independent “t” test. The discrete variables were described and interpreted by an appropriate nonparametric test. The above statistical procedures were undertaken with the help of the statistical package namely IBM SPSS statistics -20. **The P-values less than or equal to 0.05(P≤0.05) were considered as statistically significant.**

Table 1: Distribution Of Subjects With Pleural Effusion According To Age

Age group	Tuberculous pleural effusion		Parapneumonic pleural effusion		Total	
	Frequency	%	Frequency	%	Frequency	%
20-29	5	9.8	5	9.8	10	19.6
30-39	3	5.9	5	9.8	8	15.7
40-49	7	13.7	1	2.0	8	15.7
50-59	7	13.7	5	9.8	12	23.5
60-69	5	9.8	8	15.7	13	25.5
Total	27	52.9	24	47.1	51	100.0
Mean± SD	45.6±14.0		46.7±16.4		46.1±15.0	
Significance	“t” =0.262, Df=49, P=0.795					Range=20-69 years

Table - 1 Illustrates socio demographic characteristics of 51study subjects who participated in the study. The range of the age was 20-69 years. Maximum study subjects were in the age group of 60-69 years (25.5%). The mean age of subjects with tubercular pleural effusion was found to be 46±14.7 years and with Parapneumonic pleural effusion was 46.7±16.4 years. The difference between them was not statistically significant (P>0.05).

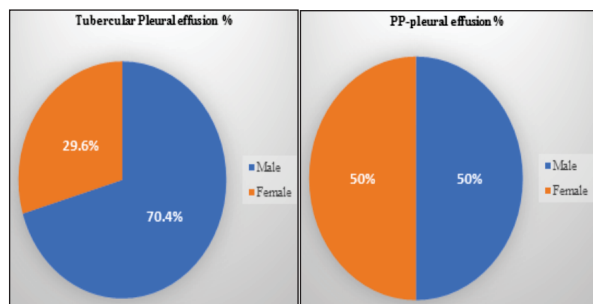


Figure 1 : Distribution of study subjects according to the gender

Figure-1 illustrates the distribution of cases according to gender. In this study it was deduced that 29.6% females and 70.4% males developed Tubercular pleural effusion and an equal number of males and females (50 %) developed para pneumatic pleural effusion.

Table 2: Comparison Of Serum Protein, Serum Albumin And Serum Ldh Between The Tubercular And Parapneumonic Pleural Effusion:

Serum	Tuberculous pleural effusion		Para pneumonic pleural effusion		Difference b/w means	“t”	DF	Significance
	Mean	SD	Mean	SD				
Protein	6.6	0.9	6.5	0.8	0.1	0.402	49	P=0.690
Albumin	3.6	0.6	3.7	0.5	0.1	0.500	49	P=0.619
LDH	426.6	123.0	578.0	376.1	151.4	1.978	49	P=0.054

Table -2 compares the Serum protein, serum albumin and serum LDH between the two groups. The mean serum proteins of both groups were 6.6±0.9 and 6.5±0.8. The mean serum albumins of both groups were 3.6±0.6 and 3.7±0.5. The mean serums LDH of both groups were 426.6±123.0 and 578±376.1. The differences between the means of three groups were not statistically significant (P>0.05).

Table-3: Comparison of LDH and ADA levels in pleural fluid to differentiate between TPE and PPE:

Variables	TPE		PPE		Difference b/w means	“t”	df	Significance
	Mean	SD	Mean	SD				
LDH	293.3	95.7	503.3	372.7	210.0	2.829	49	P<0.01
ADA	59.8	26.1	12.6	6.4	47.8	8.609	49	P<0.001

Table-3 shows the comparison of LDH and ADA values of the TPE and PPE group. The Mean value of LDH of TPE & PPE was 293.3 ± 95.7 and 503.3 ± 372.7 respectively. The mean values of ADA of TPE & PPE were 59.8 ± 26.1 and 12.6 ± 6.4 respectively. The difference of means of both groups were highly statistically significant ($P < 0.01$).

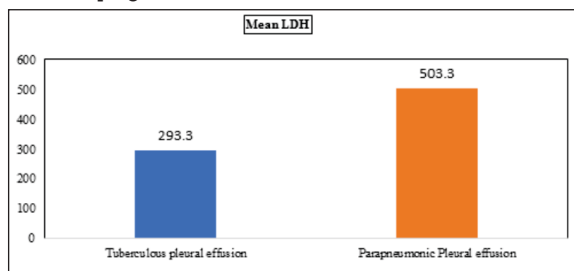


Figure 2: Comparison of LDH and ADA between TPE & PPE group

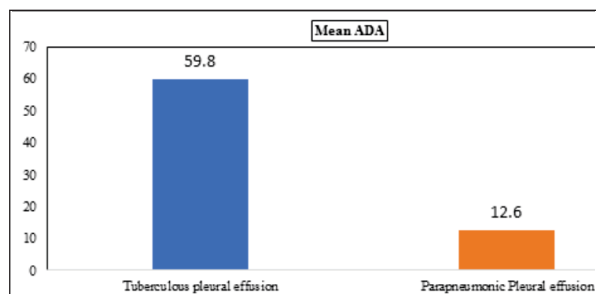


Figure 2: Illustrates a bar diagram showing the mean LDH and ADA value in the TPE and PPE group. The mean LDH value is 293.3 and 503.3 in TPE and PPE groups respectively. The mean ADA value is 59.8 and 12.6 in the TPE and PPE group respectively.

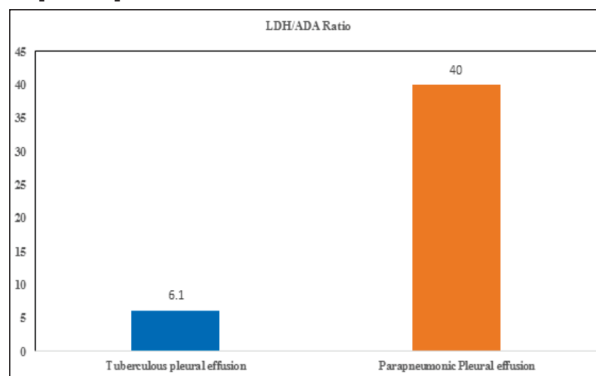


Figure 3: Comparison of LDH/ADA ratio between TPE & PPE group:

Figure-3 depicts a bar diagram showing mean LDH /ADA ratio values in TPE & PPE groups respectively. It was found that the Pleural fluid LDH/ADA ratio was 6.1 and 40 in the TPE and PPE group respectively. The ratio in the TPE group was significantly lower than in the PPE group. This ratio is found to have statistical significance ($p < 0.005$).

Table- 4: Sensitivity and specificity of LDH/ADA ratio for identification of TPE & PPE

AFB (Sputum)	LDH/ADA < 16.465 > 16.465		Total
	Positive	Negative	
Positive	27	0	27
Negative	1	23	24
Total	28	23	51

Sensitivity of the LDH/ADA = $27/28 = 96.4\%$
 Specificity of the test LDH/ADA = $23/23 = 100\%$
 Positive predictive value of the test LDH/ADA = $27/27 = 100\%$
 Negative predictive value of the test LDH/ADA = $23/24 = 95.8\%$.

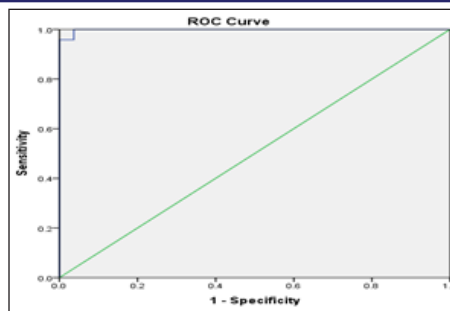


Fig-4: ROC curve for LDH/ADA ratio in differentiation between TPE and PPE.

Area under the curve:

Area	Std error	Significance	95% confidence interval	
0.998	0.003	$P < 0.001$	0.993	1.000

The Cut off value of LDH/ADA = 16.465 or 16.5.

Figure 4 - Illustrates the ROC curve for LDH/ADA ratio in differentiation between TPE and PPE. An AUC value of 0.998 was obtained using the same ratio. This ratio is highly predictive of TPE at cut off value of 16.465.

DISCUSSION

Currently, a definitive diagnosis of TPE is made on the basis of the following criteria:

- (1) A positive AFB smear or positive cultures for MTB in pleural fluid and pleural tissue
- (2) Chronic granulomatous inflammation in pleural tissue. And
- (3) A clinical response to anti-tuberculosis treatment².

In most studies, an ADA level ≥ 40 U/L in a lymphocytic exudate obtained via thoracentesis has been the most widely accepted indicator for a diagnosis of TPE³. It is difficult to make a differential diagnosis of PPE due to the variety of subcategories of PPE. Also; the absence of disease-specificity in pleural effusion biomarkers⁴ makes a definitive diagnosis of PPE challenging as such patients may or may not present with symptoms and signs of pneumonia.

The present study which has been done at our tertiary health care centre is devoted to find use of the pleural fluid LDH/ADA ratio as a new parameter to discriminate between Tubercular Pleural effusion and Parapneumonic effusion. Also in this study we have studied the diagnostic value of ADA and LDH levels in the pleural fluid for the diagnosis of tubercular and parapneumonic effusion.

The discussion of the study has been covered under the following headings:

1. Diagnostic value of estimation of LDH and ADA levels in the pleural fluid to differentiate between TPE and PPE.
2. Diagnostic value of estimating LDH/ADA ratio in discriminating TPE and PPE.
3. Advantages of using LDH/ADA ratio in discriminating TPE and PPE.
4. Limitations of this study.

1) Diagnostic value of estimation of LDH and ADA levels in the pleural fluid to differentiate between TPE and PPE.

In this study we found out that mean LDH (503.3) value was higher in patients with parapneumonic pleural effusion and ADA value was higher in TPE (59.3). The differences of means of both groups were statistically highly significant ($P < 0.01$). This finding is supported by Morné J. Vorster et al¹ who concluded that ADA levels are most useful when there is a moderate to high suspicion of TB in patients with negative pleural fluid or biopsy cultures, and non-diagnostic histology.

The most accurate value of ADA was found to range between 40 and 60 U/L. In a study of 254 patients with pleural TB, 99.6% had ADA more than 47 U/L. Elevated ADA level might be considered as a confirmatory test justifying treatment initiation. Our finding is also supported by Jinlin Wang¹ et al who concluded that Use of the ADA level in pleural fluid has demonstrated high sensitivity and specificity for the differential diagnosis of TPE. This is contradictory to the data obtained by Zaric^{et al} who reported a poor specificity of 70.4% for the ADA level in diagnosing TPE, but an acceptable sensitivity of 89.2%.

2) Diagnostic value of estimating LDH/ADA ratio in discriminating TPE and PPE.

Pleural fluid lactate dehydrogenase (LDH) and adenosine deaminase (ADA) levels are often used to distinguish between tuberculous pleural effusion (TPE) and parapneumonic pleural effusion (PPE) but it can be challenging as the LDH level may vary from normal to severely increased in PPE¹. also; an elevated ADA is frequently measured in both conditions. so, In view of this limitations of using pleural fluid ADA and LDH levels alone as biomarkers for differentiating between TPE and PPE, in our study we combined the 2 parameters in an attempt to develop a predictor of TPE with acceptable specificity and sensitivity. In this study we found out that the mean ratios of LDH/ADA in TPE & PPE were 6.1 ± 3.6 and 40.0 ± 21.5 respectively. The ratio was found to be significantly higher in the patients with PPE. The difference of ratio between the two groups was found to be statistically significant ($P < 0.001$). This finding is supported by Jinlin Wang¹ et al who conducted a retrospective study in patients with pathologically-confirmed TPE ($n = 72$) and PPE ($n = 47$) and compared pleural fluid LDH and ADA levels and LDH/ADA ratios between the 2 groups. The study revealed a significantly lower pleural fluid LDH/ADA ratio in the TPE group compared with the PPE group ($P < .0001$).

3) Advantage of using LDH/ADA ratio in discriminating between TPE and PPE:

Most patients with TPE have chronic granulomatous inflammation in pleural tissue, and infiltration of mononuclear cells and macrophages. In patients with PPE, pleural tissues show acute inflammation and infiltration of neutrophil cells, with many pus cells. In our study, the pleural fluid LDH/ADA ratio was significantly lower in patients with TPE compared with those with PPE ($P < .0001$). Our finding is consistent with the finding of Jinlin Wang et al¹ who interpreted this can be due to differences in the pathological nature of the two conditions, increased LDH levels and corresponding increases in the LDH/ADA ratio in the three subgroups. These results indicate that the LDH/ADA ratio may be a useful indicator of pleural inflammatory responses.

4) Limitation of this study:

- This study has a smaller sample size. More prospective studies with larger sample sizes are required to establish results.

CONCLUSION

- Pleural effusion is a commonly observed clinical manifestation and is associated with more than 50 recognized diseases and disorders.
- It is of utmost importance to differentiate between TPE and PPE as misdiagnosis and delayed treatment can result in significant morbidity and mortality.
- An increased pleural fluid adenosine deaminase (ADA) level is frequently seen in TPE, which helps to discriminate it from PPE
- This study has provided evidence that the pleural fluid LDH/ADA ratio is a useful indicator to distinguish TPE from PPE.
- In This study we found out that LDH/ADA ratio was found to

be highly predictive of TPE at cutoff value of 16.465 and **Sensitivity; specificity, PPV and NPV** of the LDH/ADA ratio was found to be 96.4%, 100%, 100% and 95.8% respectively.

- The LDH/ADA ratio may also reflect the nature of pleural inflammation and the response to inflammation.

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