Original Research Article

Clinical study of diagnostic role of adenosine deaminase in pleural effusions

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Abstract

Introduction: The diagnosis of tubercular pleural effusion is critical, because the earlier the diagnosis, the better the result. Biochemical markers aid in the early detection of tubercular pleural effusion. Such include adenosine-deaminase (ADA), interferon, and lysozyme. Amongthese, ADA determination appears to be the most promising due to its speed and low cost. The current study is being conducted to determine the diagnostic role of ADA in patients hospitalised with pleural effusion at hospitals affiliated with Kurnool Medical College, as well asto correlate the level of ADA in tubercular and non-tubercular pleural effusion. **Methodology:** The current prospective trial will last a year, from September 2020 to December 2021. All patients over the age of 18 who presented with clinical symptoms of pleural effusion and provided informed consent for the trial were included. A thorough history was taken, a physical examination was performed, and any necessary regular investigations were carried out. **Results and Discussion:** In our study, the mean SD in TBPE is 79.17, with a cutoff value of 40 U/L and sensitivity of 71%, specificity of 100%, PPV of 100%, and NPV of 40%. **Conclusions:** The levels of ADA in pleural fluid were considerably higher in tubercular pleural effusion. As a result, our findings support the concept that pleural fluid ADA estimate is particularly important in establishing an accurate and early diagnosis of pleural effusion owing totuberculosis.

Key words: ADA; Tubercular pleural effusion, TBPE

Introduction

The pleural space is defined as the gap between the parietal pleura, which covers the chest wall, and the visceral pleura, which covers the lung. It includes a few millilitres of fluid that functions as a lubricant between the two surfaces in a healthy person. The abnormal collection of fluid in the pleural space is referred to as pleural effusion. Despite the fact that it has little clinical indications. It should be regarded asa universal symptom of a serious condition[1].

Pleural effusions are classified into two categories[1]. effervescent [2] It is transudative. When the pleural surfaces or capillaries are damaged, exudative pleural effusions form. When the systemic variables regulating fluid generation or absorption are changed, transudative pleural effusion develops. It is the second most common clinical manifestation of tuberculosis, accounting for 15% of all tuberculosis cases[2].

Tubercular pleural effusion must be diagnosed since tuberculosis is a curable cause of exudative lymphocytic pleural effusion. And the earlier the diagnosis, the better the outcome[1].

Pleural aspiration and biopsy are required for the investigation of a pleural effusion. Because the number oforganisms in the pleural fluid is quite low in most cases of tubercular pleural effusion. As a result, standard approaches for detecting Mycobacterium Tuberculosis (MTB) are useless. Only around 60% of the time does a pleural biopsy reveal granulomatous inflammation. In the majority of cases, the available tests and procedures for confirming its cause are useless.

The use of biochemical markers such as Adenosine Deaminase (ADA), -interferon, and lysosome has substantially improved the early detection of tubercular pleural effusion. Because of its ease - speed and cost effectiveness, determining the ADA level in pleural fluid appears to be the most promising for the diagnosis of tubercular pleural effusion[3,4].

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ADA is an enzyme that catalyses the purine salvage pathway's conversion of adenosine to inosine and deoxyadenosine to deoxyinosine with the emission of ammonia. The cellularity of activated T-lymphocytes in the pleural compartment is reflected by ADA activity. It is found in patients with exudative lymphocytic pleural effusion. Pleural effusions caused by parapneumonia, tuberculosis, malignant pleural effusion, lymphoma, and VLE empyema are examples[4].

The current study was carried out to learn about the diagnostic role of ADA in patients hospitalised with pleural effusion at Kurnool Medical College & Hospital, Kurnool, as well as the correlation of ADA levelsin tubercular and non-tubercular effusion.

Objectives of the present study

- To study the role of ADA in pleural fluid as a diagnostic biochemical marker.
- Correlating the level of ADA in tubercular effusion and non tubercular effusion.
- To differentiate between tubercular effusion from non tubercular effusionbased on ADA levels.

Materials and methods

The study was conducted at hospital attached to Kurnool Medical College) from Septmber 2020 to December 2021. Final diagnosis was based on clinical, radiological and other laboratory findings.

The criteria for arriving at the diagnosis of diseases associated with pleural effusion were-

1. Typical clinical history and physical examination.
2. Radiological picture along with confirmation by diagnostic pleural fluid aspiration with laboratory pleural fluid analysis.

The study comprised of fifty patients of pleural effusion. All the patients underwent a thorough physical examination, urine analysis, complete blood counts and pleural fluid analysis. Pleural fluid was analysed for protein, sugar, cell type and cell count. Pleural fluid AFB and malignant cells were done wherever appropriate. Sputum examination of AFB (both under RNTCP and microbiology) done

in cases where patient brought out sputum. Lymphnode biopsy for fine needle aspiration cytology were done in cases where there was lymphadenopathy. Tests for tuberculosis – pleural fluid AFB; TB ELISA; fluid culture for M.tuberculosis and pleural biopsy were considered.

Out of 60 cases of pleural effusion studied –

- 27 cases: Tuberculous pleural effusion
- 12 cases: Malignant pleural effusion
- 7 cases: Empyema thoracis
- 7 cases: Parapneumonic effusion
- 7 cases: Transudates

Pleural fluid was collected from all the patients. The samples were stored at zero degree in the refrigerator. The estimations were carried within 72 hours of the sample collection. Adenosine deaminase is studied in all thepatient groups.

Method[12]

Statistical analysis

1. Comparison of means of two groups was done by students 't' test.
2. Level of significance: P<0.05, P<0.01 significant, P<0.001 – Highlysignificant.

Ethical clearance was obtained from ethical committee of the institution.

Results

Our study group includes 60 patients with 34 male patients and 16 females. Maximum number of includes in the age group of 21-50. Most of the patients presented with symptoms of fever, cough, breathlessness, chest pain and hemoptysis, maximum number of patients presented with symptoms of cough and breathlessness with the percentile level of 83.34% and 78% respectively.

Table – 1: Age Distribution

Age in years	No. of cases	Percentage
11 - 20	2	3.33
21 -30	9	15.0
31-40	13	21.6
41-50	12	20.0
51-60	5	8.3
61-70	8	13.3
71-80	8	13.3
>80	3	5
Total	**60**	**100**

Table – 2: Sex Distribution

Sex	No. of cases	Percentage
Male	39	65.0
Female	21	35.0

Table – 3: Age and Sex Distribution

Age	Sex		Total
	Male	Female	
11 - 20	0	2	1
21 -30	7	2	8
31-40	8	3	11
41-50	10	2	11
51-60	3	3	4
61-70	5	4	7
71-80	4	3	6
>80	2	2	2
Total	**39**	**21**	**60**

Table – 4: Symptomatic Presentation

Symptoms		No. of cases	Percentage
Fever	Present	29	48
	Absent	31	51.6
Cough	Present	50	83.34
	Absent	10	16.66
Hemptopysis	Present	7	11.66
	Absent	53	88.33
Chest Pain	Present	28	46.66
	Absent	32	53.33
Breathlessness	Present	44	73.33
	Absent	16	26.66
Loss of Weight	Present	21	35
	Absent	39	65

Chest X-RAY features

On chest X-ray examination maximum number of patients presented with signs of fibrosisand consolidation with underlying pleural effusion (51.66% and 20.0% respectively).

Table – 5: Chest X-RAY features

Fibrosis	No. of cases	Percentage
Absent	31	51.66
Collapse	7	11.66
Consolidation	12	20.0
Fibrosis	6	10
Lung collapse	2	3.33
Mass lesion	3	5
Total	60	100.0

Table – 6: Chest X-RAY Signs

Signs		Frequency	Percentage
Cavity	Present	4	6.66
	Absent	56	93.33
Consolidation	Present	3	5
	Absent	57	95
Mass Lesion	Present	5	8.33
	Absent	55	91.66

Moderate to massive amount of pleural effusion noted in most of the cases with minimum pleural effusion in some cases. On diagnostic aspiration most of the cases showed straw colour effusion (around 63.33%), 20% cases showed haemorrhagic and 16.66 % showed purulent.

Table – 7: Amount of Effusion

Pleural Effusion		Frequency	Percent
Amount ofEffusion	Moderate	48	80.0
	Min	2	3.22
	Massive	10	16.66

Table – 8: Pleural Effusion (Gross Appearance)

Pleural effusion	Frequency	Percentage
Straw	38	63.33
Haemorrhagic effusion	12	20.0
Purulent	10	16.66

Age and sex wise distribution

Among the patient group studied tubercular pleural effusion (TBPE) showed male to femaleratio of 13:7 with 20 male patients and 5 females and non tubercular pleural effusion (NTBPE) showed male to female ratio of ~7:5 with 14 males and 11 females.Among the TBPE groups males corresponds to 80% and females 20%. Among the NTBPE groupsmale corresponds to 58% females corresponds to 42%.

Table – 9: Sex wise distribution of TBPE and NTBPE

Diagnoses	Sex		Chi Square Test
	Male	Female	
TBPE	28	9	3.31, P<0.12, NS
Non-TBPE	17	14	

Table – 10: Sex wise comparison of effusion of different etiologies

Diagnoses	Sex		Total
	Male	Female	
TBPE	20	6	26
MALPE	4	8	12
EMPTH	5	2	7
PMNPE	6	3	9
TRANS	4	2	6
Total	39	21	60

In our study group TBPE comprised of 29 male patients and 9 females (33.33% and 10%), malignant pleural effusion showed 4 male and 8 females patients with (6.6% and 13.33%), Empyema thoracic and parapneumonic effusion both showed 5 male and 2 female with (8.33% and 3.33% respectively). Transudative effusion found 4 male and 2 females patients
i.e. 6.6% and 3.33 % respectively.

Table – 11: Comparison of age wise incidence

Diagnoses						
Age	TBPE	MALPE	EMPTH	PMNPE	TRANS	Total
11 - 20	0	1	0	0	0	1
21-30	6	2	2	1	0	8
31-40	4	4	1	2	1	12
41-50	7	2	0	1	2	11
51-60	3	0	1	1	0	4
61-70	2	1	1	3	2	7

71-80	3	2	1	0	1	6
>80	1	0	1	0	0	2
Total	26	12	7	8	6	60

When age wise incidence of pleural effusion studies tubercular pleural effusion (TBPE) occurred maximum among the age group of 21 to 50 years. Malignant pleural effusion among 31 to 50, empyema thoracic being equally distributed in the age group of >20 years. Parapneumonic effusion occurring in the age group of 31-70 years, transudative effusion inthe age group of 41 to 80 years.

Table – 12: Diagnosis

Diagnoses	Frequency	Percent
TBPE	26	43.33
EMPTH	8	13.33
MALPE	12	20
PMNPE	7	11.66
Trans	7	11.66
Total	60	100

Table – 13: Side of effusion

Diagnoses	Side of effusion			Total
	Right	Left	B/L	
TBPE	12	12	3	27
MALPE	7	5	0	12
EMPTH	4	1	2	6
PMNPE	2	4	1	7
TRANS	3	3	1	6
Total	28	25	7	60

Among TBPE group (25 patients) 48% were presented with right side pleural effusion, 41.6
% presented with left side pleural effusion and 11.66% bilaterally. Among NTBPE group(out of 27 patients) 44.44 % on the right side, 44.4 % on the left side and 11.11% bilaterally present.
NTBPE group includes malignant pleural effusion (MALPE), empyema thoracic (EMPTH), parapneumonic pleural effusion (PMNPE), and transudative effusion (TRANS).

Table – 14: Side of effusion

Pleural Effusion		Frequency	Percent
Side of effusion	Right	28	46.66
	Left	25	41.66
	B/L	7	11.66

Side effusion also deserves notice, when total pleural effusion were studied, it was 46.66 %on right side 41.66 % on left side and 11.66 % bilateral.

Table – 15: Investigations

Investigations		Frequency	Percent
Chest X-ray	Right sided pleural effusion	27	45
	eft sided pleuraleffusion	22	36.66
	B/L pleural effusion	5	8.3
USG thorax	Negative	52	86.66
TB ELISA	Negative	51	85
TB culture	Negative	53	88.33
Pleural biopsy	Negative	50	100.0

Pleural fluid profile and investigations

When total cell count correlated, counts are considerably elevated in TBPE compared to NTBPE. Erythrocyte sedimentation rate (ESR) is equally elevated in both TBPE andNTBPE.

Pleueral fluid proteins and sugars

When pleural fluid proteins in different age group among 60 cases were studied 27 cases of TBPE showed mean protein level to be at 4.21 and that of NTBPE at 4.04. When pleural fluid sugars are compared the patients with TBPE showed mean sugar level to be at 66.69 and that of NTBPE to be at 88.02.

Table – 16: Pleural fluid profile and investigations

Investigations	Diagnoses				Statistical Analysis Unpaired ttest
	TBPE		Non-TBPE		
	Mean	Std Deviation	Mean	Std Deviation	
TC	15443.61	9522.36	11352.20	4562.68	1.16, NS
ESR	43.28	21.58	48.96	20.11	0.78, NS
Cell Count	1731.00	1391.65	900.88	941.33	2.4, P<0.01
Pleural fluid protein	4.21	1.17	4.12	1.19	0.46, NS
Pleural fluid sugar	66.69	43.76	88.02	56.21	1.54, NS
Adenosinedeaminase	79.19	58.45	39.17	25.89	3.11, P<0.003
Cell Percentage	74.20	14.30	67.64	18.10	2.89, P<0.001

Table -17: Cell type wise comparison

Cell Type	Diagnoses	
	TBPE	Non-TBPE
Lymphocytic cells	19	16
Polymorphonuclear cells	11	9
Mesothelial cells	0	8

When cell type was compared TBPE showed maximum of lymphocytic and polymorphonuclear type of cells. Where as NTBPE group showed all the cell type i.e. lymphocytic, polymorphonuclear and mesothelial cells.

Pleural fluid ADA

When the level of ADA in TBPE and NTBPE were compared. In TBPE ADA level were in the range of 34.2 - 278 with mean value of 79.19. Among NTBPE group patients with malignant pleural effusion had the ADA level of 11 to 26, patient with parapneumonic effusion had the ADA level of 18.7 to 62.7, patients with empyema thoracic had ADA level of 48.3 to 116 and those with transudative effusion had ADA in the level of 10 to 63.4.

Table – 18: Pleural fluid ADA

Diagnoses		ADA			groups compared	t value	P Value
		Range	Mean	SD			
TBPE		34.2 - 278	69.77	59.75	A Vs B	4.16	, P<0.003
NONTB	B1. MALPE	11 - 25	18.35	5.36	A VS B1	3.11	P<0.003
	B2. PMNPE	18.5 –62.88	42.62	16.69	A Vs B2	1.11	NS
	B3. EMPTH	48.5 - 117	75.96	26.29	A Vs B3	0.12	NS
	B4. TRANS	10 - 63.4	29.36	20.73	A VS B4	3.41	P<0.004
	B.TOTAL	10 - 118	38.17	26.51			

Discussion

Exudative lymphatic pleural effusions are a common occurrence in clinical practise, although theycan be difficult to diagnose because of their lymphatic nature. In impoverished nations such as India, TB is one of the most common causes of exudative pleural effusions. Obtaining a definitivediagnosis of tubercular pleural effusion is time-consuming and complicated. The culture of Mycobacterium tuberculosis is the gold standard for diagnosing tuberculosis-associated pneumonia. However, the production of tubercular pleural fluid is extremely modest. Other advanced diagnostic procedures include polymerized chain reaction (PCR), ligase chain reaction (LCR), and restriction fragment length polymorphism (RFLP), among others (RFLP). However, they are more expensive. The most widely acknowledged method of ADA estimate is because it issimpler, less expensive, and easier to diagnose.

Adenosine deaminase is a purine metabolism enzyme that catalyses the hydrolytic and irreversible deamination of deoxyadenosine to adenosine and then to inosine. It is found in the cytoplasm of all animals. Cell mediated immunity is thought to be indicated by the presence of this protein, which is found predominantly in T cells and macrophages. According to Piras et al. (1978)[5], it is associated with lymphocyte proliferation and differentiation. Patients with disorders in which cellular immunity is increased, such as typhoid fever, infectious mononucleosis, Mediterranean spotted fever, and tuberculosis, have elevated enzyme levels in their serum, according to research. Piras was the first to demonstrate the practicality of ADA, back in 1975. A large number of studieshave been conducted since the first proposal by Piras et al[5] to demonstrate the utility of ADA inthe diagnosis of tuberculous pleural effusion. Valdes et al[6] 1993; Sharma 1991[7] are examplesof such studies.

According to the findings of earlier investigations, an ADA level greater than 40U/L is believed to be indicative of tuberculosis infection. The greater the ADA number, the greater the likelihood of contracting tuberculosis. Increased ADA activity in pleural fluid in tuberculosis patients was mostlikely due to ADA being generated by cells in the pleural cavity, which was a reflection of local activities of selectively sequestered T-lymphocytes as part of the body's natural immunological response[8]. (Petterson and colleagues)

In the present study, the proteins and sugar levels in pleural fluid were measured, and it was discovered that the protein concentration was 4.211.17 (range 3.2-6.0 gm percent) and the sugar concentration was 66.6943.67 (range 68-238) in tubercular effusion, and the protein concentrationwas 4.121.19 (range 2.3 – 4.9 gm percent) and the sugar concentration was 88.02 56.21 (range 68-238) in non tuber (40-148).

An investigation by D.K. Gupta and colleagues[9] revealed that in all 30 cases of pleural effusion, the pleural protein concentration was greater than 3 gm percent, suggesting that the condition was severe.

In this study, the levels of adenosine deaminase in pleural fluid were compared in tubercular pleural effusions, malignant pleural effusions, empyema thoracis, parapneumonic effusions, and transudative effusions, among other conditions. When compared to the other groups, it revealed greater ADA values in tuberculous pleural effusions than the other groups. The mean ADA score in tubercular pleural effusion was 79.2759.45, according to the study. In contrast, when comparing pleural fluid to malignant effusion, a mean of 19.315.32 was found in the pleural fluid. Pleural fluid was found to be the most common cause of effusion in all other types of effusion.

Table-16 shows that the ADA was highly significant in this study of tubercular pleural effusion when compared to the other groups, indicating that the study was highly significant. Tubercular effusions with abnormally high levels of ADA may be caused by co-existing infections, poor hygiene, and malnutrition, among other factors. There is a high frequency of pulmonary tuberculosis in this region of the country[10]. The mean value of ADA activity in tubercular pleural effusions was higher (93.81 29.65 U/L) than in neoplastic effusions (13.02 9.66 U/L) in the investigations conducted by Inma Ocana et al. 3.65 x 30.83 U/L for lymphoma effusion and 5.914.70 U/L for various conditions In the tuberculous group, there was a statistically significant difference compared to the lymphoma group (P0.01).The ADA of the tubercular pleural fluid was 137.053.23 in this case. When compared to malignant 20.2 U/L with a range of 5 to 40 and empyema 94 U/L with a range of 16 to 222, Strankinga[11] demonstrated ADA activity at a mean of 115, which ranged from 79 to 175 whencompared to malignant. For the purposes of comparison, another cutoff value of 35 U/L was usedas a reference. According to the results of the statistical analysis, even with this number, we can suspect tuberculosis in the presence of a pleural effusion. The presence of higher levels validates the diagnosis.

Compared to results from other recent research, the values obtained in the current investigation are comparable; nevertheless, the overall predictive value of pleural fluid ADA levels of 40 was 100 percent diagnostic of tubercular pleural effusion, indicating that the patient had tubercular pleural effusion. ADA estimation in patients with tuberculous pleural effusion has been shown to have excellent sensitivity and specificity in previous research, and it can be concluded that this method aids in the early detection of cases and, consequently, the early administration of treatment to thesepatients.

The following are the study's limitations

The study had a limited sample size and was not randomised, so the results were not conclusive. The total amount of ADA was the iso enzymes (ADA-1 and ADA-2) were not taken into consideration independently, nor was their ratio studied in the study.

Conclusion

- All patients with tuberculous pleural effusion demonstrated increased ADA activity intheir pleural fluid.
- When compared to non-tuberculous pleural effusion, pleural fluid ADA activity was considerably higher in tuberculous pleural effusion.
- Pleural fluid levels that are abnormally high In the diagnosis of tuberculous pleuraleffusion, ADA demonstrated a very good sensitivity and specificity.
- Pleural fluid estimation ADA is a simple, inexpensive, quick, and fairly specific approach that aids in the early detection of tuberculous pleural effusions and can be incorporated in routine tuberculous pleural effusion investigations.

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Conflict of Interest

None

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