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Original Research Article

Effect of Storage Time on Pleural Fluid Adenosine Deaminase Level

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Abstract

Background: It is essential to investigate why some clinicians are reporting low level of ADA (< 40 IU/L) in TB pleural fluid. We studied the effect of storage time at room temperature on pleural fluid ADA level.

Methodology: This study was a cross-sectional observational study conducted at a tertiary care hospital on patients with pleural effusion over a period of 18 months. ADA estimation in the fluid was done within one hour of collection as well as after 24 hours of storage at room temperature.

Results: The mean ADA level within 1 hour of collection was 43.19±19.47 IU/L which increased to 45.32±18.80 IU/L after 24 hours of storage at room temperature ($p<0.05$). In non-tuberculous pleural effusions, the mean ADA level within 1 hour of collection was 32.75±14.97 IU/L, which increased to 35.44±14.19 IU/L after 24 hours of storage at room temperature ($p<0.05$). In neutrophilic predominant exudative pleural fluid the mean ADA level within 1 hour was 34.25±20.71 IU/L, which increased to 38.50±21.61 IU/L after 24 hours ($p<0.05$). The mean ADA level increased after 24 hours only in rainy season from 40.27±14.56 IU/L to 43.50±16.13 IU/L ($p<0.05$).

Conclusion: Twenty-four hours of storage of pleural fluid at room temperature increased the ADA level but not enough to change the diagnosis from a non-TB pleural effusion to a TB pleural effusion. The factors affecting the increase in ADA level upon storage included the type of fluid (exudate), predominant cells (neutrophils), aetiology (non-tuberculous), and season (rainy).

Keywords: Storage, ADA Level, Seasons, Tuberculous, Non-Tuberculous, Pleural Effusions.

Introduction

Adenosine deaminase (ADA), also called adenosine aminohydrolase, is an important enzyme which has been involved in purine catabolism, i.e. it catalyzes the irreversible deamination of adenosine and deoxyadenosine into inosine and deoxyinosine respectively, releasing ammonia.[1] ADA is produced from the lymphocytes and is a widely studied marker for tuberculosis, both pulmonary and extrapulmonary. ADA activity can be seen in pleural fluid in tuberculous pleural effusion, CSF in tuberculous meningitis, and

peritoneal fluid in abdominal tuberculosis.[2,3]

Pleural fluid in tuberculous pleurisy is predominantly lymphocytic.[4,5] Though the ADA estimation in pleural fluid is not a good discriminator, its level is considerably higher in tuberculous patients. ADA levels may be elevated in other conditions such as malignancy, empyema, or rheumatoid pleurisy.[6] Studies indicate that ADA values above 70 IU/L are strongly suggestive of tubercular pleural effusion, while values below 40 IU/L excludes TB diagnosis.[2,7]

Though pleural fluid ADA level estimation is done routinely for diagnosis of TB pleural effusion and a cut-off of >40 IU/L is most commonly suggested for diagnosis of TB pleural effusion.[8-12] Many clinicians report an ADA level below 40 IU/L in patients with TB pleural effusion.[8-10] In our clinical experience, tuberculous pleural effusions invariably have ADA level ≥ 40 U/l.

It is essential to investigate why some clinicians are reporting low level of ADA in pleural fluid. It is possible that pleural fluid, soon after aspiration, is left at room temperature in the ward or laboratory for a prolonged period before analysis, thus affecting the ADA level in the fluid. We, therefore, to studied whether storage time of pleural fluid at room temperature could affect pleural fluid ADA level.

Methodology

This study was conducted as a cross-sectional observational study at the Department of Pulmonary Medicine and General Medicine of a tertiary care hospital in Central India on patients diagnosed with pleural effusion over a period of 18 months. All patients above 12 years of age, who consented to participate in the study were included. Patients with comorbid conditions like immunodeficiency, bleeding diathesis and local skin lesions, were excluded from the study. All patients fulfilling the inclusion criteria were enrolled using non-probability sampling.

After obtaining ethical approval from the Institute ethics committee, all patients who met the inclusion criteria were enrolled in the study. After explaining the purpose of the study to the patients in their local language, written informed consent was obtained from them. Participants were assured confidentiality, and option to opt out of the study was kept open to them. The cause of the pleural effusion was determined by history, clinical examination and laboratory investigations. Routine blood tests were performed in all patients.

Patients diagnosed with pleural effusion underwent pleural fluid aspiration and pleural fluid analysis. Pleural fluid was stored at room temperature for 24 hours. Since ambient temperature varies with seasons, are decided to analyze ADA levels at various seasons. Seasons in which pleural fluid aspiration and analysis was done was noted. The seasons were classified according to the Indian Meteorological Department classification into summer (March to June), winter (December to February), rainy season (July to September), and post-monsoon (October to November). Pleural fluid was analysed to assess the type of fluid, predominant cells, protein, albumin, and LDH levels. The fluid sample was divided into two parts, the first part underwent ADA estimation within one hour of collection, and the second part after 24 hours of storage at room temperature. Pleural fluid ADA was measured using a biochemistry semi-automated analyzer (STAR21PLUS™) developed by Aspen Diagnostic Pvt. Ltd. The kit used ADA assay kid LiquiMAX ADA Kinetic™ based on Kinetic Method.

Statistical Analysis

All the data were entered in MS-Excel, and analysis was performed using IBM SPSS ver. 20 software (Illinois, Chicago). Factors affecting ADA level were assessed using chi-square test (for categorical variables) and ANOVA or T test (for continuous variables). A p-value of less than 0.05 was considered statistically significant.

Results

The study was conducted on a total of 74 patients presenting with pleural effusion with mean age of 44.05±18.75 years

Table 1: Distribution of patients according to baseline variables

Baseline variables		Frequency (n=74)
Age (years)	<20	6 (8.1%)
	21-30	20 (27.0%)
	31-40	8 (10.8%)
	41-50	12 (16.2%)
	51-60	11 (14.9%)
	>60	17 (23.0%)
Sex	Male	57 (77.0%)
	Female	17 (23.0%)
Type of fluid	Exudate • Neutrophilic • Lymphocytic	54 (73.0%) 4 (7.4%) 50 (92.6%)
	Transudate	20 (27.0%)
Predominant cells in fluid	Neutrophilic	17 (23.0%)
	Lymphocytic	57 (77.0%)
Hemorrhagic effusion		6 (8.1%)
Diagnosis	Tuberculosis	38 (51.4%)
	Non-Tubercular • Cardiac failure • Liver disease • Renal failure • Malignancy • Synpneumonic • Hypothyroidism	36 (48.6%) 7 (9.5%) 8 (10.8%) 8 (10.8%) 10 (13.5%) 2 (2.7%) 1 (1.4%)

Most patients with pleural effusion were in the age group of 21 to 30 years (27%). They were predominantly men, with a male-to-female ratio of 3.4:1. Pleural fluid was exudative in 73% of patients. Lymphocytes were the predominant cells in most patients (77%), and neutrophils predominated in the remaining 23% of cases. Of the 54 exudative effusions, 50 were predominantly lymphocytic (92.6%). Pleural effusion was tuberculous in 51.4% of cases. The aetiologies of non-tuberculous effusions were malignancies (13.5%), renal failure (10.8%), liver disease (10.8%), heart failure (9.5%), pneumonia (2.7%), and hypothyroidism (1.4%).

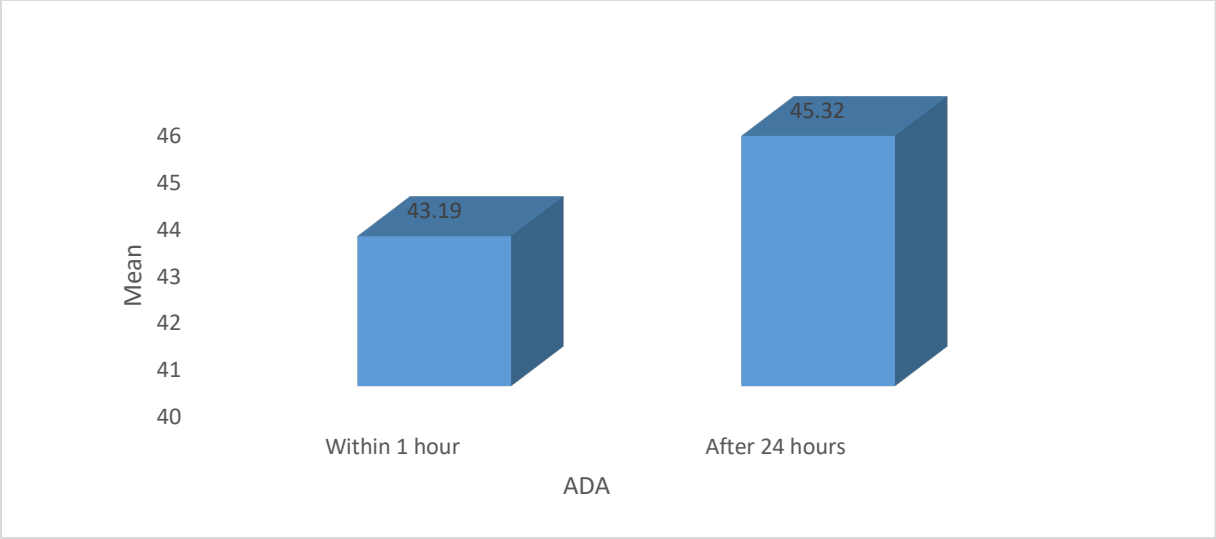

Figure 1: Effect of storage on mean ADA level in pleural effusion

The mean ADA level within 1 hour of collection was 43.19±19.47 IU/L. After 24 hours of storage at room temperature the mean ADA level increased significantly to 45.32±18.80 IU/L ($p<0.05$).

Table 2: Effect of etiology on mean ADA level

Etiology	ADA within 1 hour	After 24 hours	P value
	Mean	Mean	
Tubercular (n=38)	53.08±18.16	54.68±17.95	0.11
Non Tubercular (n=36)	32.75±14.97	35.44±14.19	**0.046**
P value	**0.001**	**0.001**	

The mean value of ADA in tuberculous pleural effusions was 53.08±18.16 IU/L within 1 hour of collection and increased significantly to 54.68±17.95 IU/L after 24 hours of storage at room temperature, but the observed difference was not significant ($p>0.05$). In non-tuberculous pleural effusions, the mean ADA level was 32.75±14.97 IU/L within 1 hour of collection and increased significantly to 35.44±14.19 IU/L after 24 hours of storage at room temperature. The mean ADA level was significantly higher in tuberculous pleural effusions compared with non-tuberculous pleural effusions both within one hour of collection and after 24 hours of storage ($p<0.05$).

Table 3: Comparison of change in ADA level in exudative lymphocyte predominant and exudative neutrophil predominant pleural fluids

Predominant cells in exudative effusions	ADA within 1 hour	After 24 hours	P value
	Mean	Mean	
Neutrophils (n=4)	34.25±20.71	38.50±21.61	**0.03**
Lymphocytes (n=50)	47.44±19.90	49.30±19.69	0.08
P value	0.21	0.29	

In neutrophil-predominant exudative pleural effusions, the mean ADA level was 34.25±20.71 IU/L within 1 hour of collection and increased significantly to 38.50±21.61 IU/L after 24 hours of storage at room temperature. The mean ADA level increased significantly in neutrophil-predominant exudative pleural effusions ($p<0.05$) as a result of storage but not in lymphocyte-predominant exudative fluid ($p>0.05$).

Table 4: Effect of seasons on mean ADA level

Season	ADA within 1 hour	After 24 hours	P value
	Mean	Mean	
Summer(n=22)	38.27±18.59	40.23±16.36	0.21
Rainy(n=26)	40.27±14.56	43.50±16.13	**0.03**
Post-monsoon(n=9)	45.33±17.31	48.33±17.42	0.13
Winter(n=17)	52.88±25.42	53.12±24.34	0.89
P value	0.095	0.171	

Mean ADA level increased significantly after 24 hours in the rainy season from 40.27±14.56 IU/L to 43.50±16.13 IU/L ($p<0.05$). The mean ADA increase in summer, post-monsoon and winter seasons was statistically not significant ($p>0.05$).

Table 5: Effect of etiology and season on mean ADA level

Etiology	Season	ADA within 1 hour	After 24 hours	P value
		Mean	Mean	
Tubercular (n=38)	Summer(n=12)	43.54±14.97	45.38±13.22	0.25
	Rainy(n=11)	52.73±10.59	54.64±14.32	0.38
	Post-monsoon(n=5)	56.00±11.48	59.75±14.24	0.34
	Winter(n=10)	64.70±24.56	64.80±23.37	0.95
Non-Tubercular (n=36)	Summer(n=10)	30.67±21.48	32.78±18.29	0.51
	Rainy(n=15)	31.13±9.34	35.33±12.22	**0.03**
	Post-monsoon(n=4)	36.80±17.19	39.20±14.85	0.31
	Winter(n=7)	36.00±15.92	36.43±14.45	0.91

Mean ADA level increased significantly after 24 hours in rainy season in non-tubercular pleural effusion ($p<0.05$).

Discussion

Determination of ADA in pleural fluid is usually performed in exudative pleural effusions. [2,8,9] Literature suggests that certain factors may affect the ADA level in the pleural fluid, and hence affect its diagnostic accuracy. So far, only a few studies have established such a relationship between storage conditions and levels of ADA. [13,14] Our study is unique because we stored the sample at room temperature, which is often seen in developing countries where electricity is erratic, or refrigeration facility is unavailable. A total of 74 patients with a mean age of 44.05±18.75 years were included in our study. Most of the patients with pleural effusion were aged between 21 and 30 years and were predominantly male (M: F - 3.4:1). Pleural effusion was tuberculous in 38 (51.4%) patients. Non-tuberculous pleural effusion was seen in 36 (48.6%) cases. Various factors, such as age, pleural fluid protein level, LDH, and smoking status have been shown to affect the ADA level in pleural fluid. We assessed the effect of storage at room temperature on pleural fluid ADA level. The ADA level in the pleural fluid was measured twice, once within 1 hour of collection and then after 24 hours of storage at room temperature, regardless of the type of pleural fluid, the cause of the pleural effusion or the storage conditions. The storage of pleural fluid at room temperature significantly increased ADA levels from a mean level of 43.19±19.47 IU/L measured within 1 hour of collection to 45.32±18.80 IU/L after 24 hours of storage ($p<0.05$).

Antonangelo *et al* (2006), however did not find any change in pleural fluid ADA activity up to 28 days after collection if stored at 4°C or -20°C.[13] Bielsa *et al* (2014) documented ADA activity in samples frozen at -80°C to be stable for 2.6 years, and after that, it decreased by 6 to 8 U/L to drop two (3.3%) tuberculous patients below the diagnostic ADA cutoff.[16]

In our study, although the rise in pleural fluid ADA was statistically significant after 24 hours of storage, it was not enough to result in a change in the diagnosis of the etiology of pleural effusion.

Mean ADA level in tubercular pleural effusions within 1 hour was 53.08±18.16 IU/L, which increased to 54.68±17.95 IU/L after 24 hours, but the observed difference was not significant ($p>0.05$). No TB pleural sample had an ADA≤ 40IU/L. In non-tubercular pleural effusions, the mean ADA level within 1 hour of collection was 32.75±14.97 IU/L, which increased to 35.44±14.19 IU/L after 24 hours of storage ($p<0.05$). The mean ADA level was significantly higher in tubercular pleural effusion as compared to non-tubercular pleural effusions, both at one hour and of 24 hours.

None of the previous studies has reported the effect of storage on ADA levels in tuberculous and non-tuberculous effusions. Our study is, therefore, unique in assessing such an association.

Most exudative pleural effusions were predominantly lymphocytic (n=50). The mean ADA level in neutrophilic exudative pleural fluid was 34.25±20.71 IU/L within 1 hour of collection and it significantly increased to 38.50±21.61 IU/L after 24 hours of storage ($p<0.05$). Although the mean ADA level was significantly higher in lymphocytic exudative pleural effusions respectively at 1 hour and after 24 hours of storage than in neutrophilic effusions , we did not observe a statistically significant increase in the concentration of ADA after 24 hours of storage.

To the best of our knowledge, none of the previous studies has assessed the effect of storage on the ADA level according to pleural fluid cell types.

Different seasons, owing to temperature variations, may affect the ADA level. The mean ADA level within 1 hour of collection in the summer season was 38.27±18.59IU/L which increased after 24 hours to 40.23±16.36 IU/L but the increase was statistically not significant ($p>0.05$). However, the mean ADA level increased significantly after 24 hours in rainy season from 40.27±14.56 IU/L to 43.50±16.13 IU/L ($p<0.05$). The mean ADA increase in post-monsoon and winter seasons was also not statistically significant ($p>0.05$).

Conclusion

Assessment of ADA in pleural fluid is an essential investigation in the diagnosis of tuberculous effusions and ruling out non-tuberculous effusions.

Twenty-four hours of storage of pleural fluid at room temperature increased the ADA level but not enough to change the diagnosis from a non-TB pleural effusion to a TB pleural effusion. Factors that increased the ADA level upon storage included the type of fluid (exudate), predominant cells (neutrophils), aetiology (non-tubercular), and season (rainy). Although, adequate storage of pleural fluid is essential following collection, pleural fluid storage at room temperature for several hours does not affect the ADA level enough to result in a change of diagnosis in the etiology of pleural effusion.

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