MS.ID-IJPBS-2888
International Journal of Pharmacy and Biological Sciences-IJPBS™ (2023) 13 (2): 104-107
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

Effect of 24 Hours Storage at Different Temperature on Pleural Fluid ADA (Adenosine Deaminase) Level

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Received: 12 Jan 2023 / Accepted: 10 March 2023/ Published online: 01 April 2023
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INTRODUCTION:

Adenosine deaminase, an enzyme produced from lymphocyte and involved in purine metabolism has been extensively studied as a biochemical marker in pleural fluid during investigation for tropical pulmonary eosinophilia. The test assay, low priced, quick, barely forwarded, and can be performed in most laboratories its major physiological role is related to the proliferation and distinction of lymph cell. Pleural fluid of tuberculate start is generally lymph cell preponderant. Different cut off values of Adenosine Deaminase ranging from 30-100 IU/ L have been used in various studies with differing sensitivities and specificities.

Adenosine deaminase (ADA) is an enzyme in the purine salvage pathway that catalyzes the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine with the release of ammonia. It plays an important role in differentiating lymphoid cells and is present in abundance in active T-lymphocytes whose concentration is inversely proportional to the degree of differentiation. Its levels are ten times higher in T-lymphocytes than in erythrocytes. The enzyme activity increases during mitogenic and antigenic responses of lymphocytes, and T-lymphocyte blastogenesis can be inhibited by inhibitors of ADA. Likewise, a deficiency of adenosine deaminase is associated with severe defects in the cell-mediated and humoral arms of the immune system, predisposing the patient to opportunistic infections. Tuberculous effusion is the result of a cell-mediated immune response to the presence of Mycobacterium tuberculii and is characterized by the accumulation of activated T-lymphocytes and macrophages. Elevated levels of ADA in tuberculous effusions have been noted by several authors. ADA is now being recognized as a marker of cell-mediated immunity particularly as a marker of T-lymphocyte activation. Since ADA is increased in TB effusions and is an easy little-invasive investigation, it is frequently considered as a diagnostic aid in such cases with a sensitivity of 90 - 100% and specificity 89-100%. ADA levels have also been considered by several researchers to differentiate tubercular disease from non-tubercular [5-7]. The pleural fluid ADA levels are elevated in almost all patients with tuberculous pleuritis but not with other conditions even when associated with lymphocytic effusions. Despite earlier concerns about false-negative values in HIV-positive patients, ADA remains a sensitive marker for in patients with HIV.

METHODOLOGY:

This is an observational analytical study conducted in the Department of Biochemistry. All patients coming to the pulmonary Medicine and general Medicine, diagnosed with pleural effusion were included irrespective cause for pleural effusion. This study was carried out from December 2021 to November 2022. ADA pleural effusion cases were selected from the inpatient department of pulmonary medicine and general medicine ward, people's Hospital Bhopal (M. P). Cases provide informed and written consent about this study. This study was approved by the ethical and research committee of peoples Institute of Medical Science, Bhopal (M.P) to use human subject in the research. The Patients voluntary participated in the study.

Subjects:
All patients selected for the study based on the inclusion and exclusion criteria coming from time between December 2021 to June 2022 in pulmonary medicine and general medicine ward for pleural effusion.

AIM AND OBJECTIVES:
-To study the effect of storage time 24 hours at temperature (2 to 8 ℃ and 37 ℃) on Adenosine Deaminase level in pleural fluid.
-To determine pleural fluid Adenosine Deaminase levels after 24 hours of storage at 37°C.
-To determine pleural fluid Adenosine Deaminase levels after 24 hours of storage at 2 -8 ℃ -To compare pleural fluid ADA values of temperatures both 2-8 ℃ and 37 ℃ after 24 hours of storage.

Statistical analysis
All the data was entered in Ms-Excel, and analysis was performed using IBM SPSS ver. 20 software. Analytical and frequency distribution were used to prepare tables. The factors affecting ADA level were assessed using the 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.

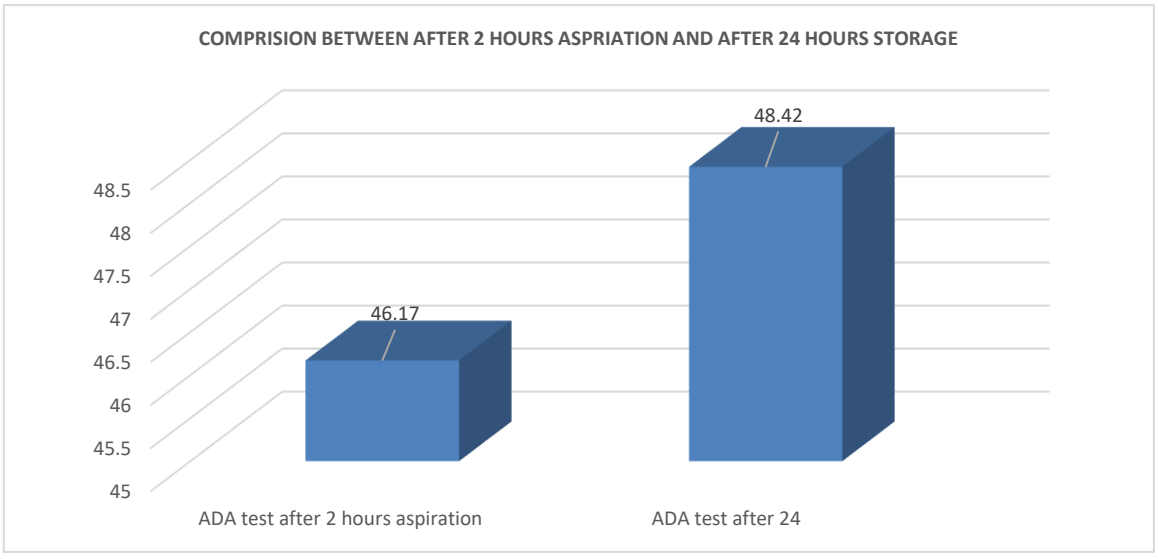
RESULT:
was conducted on a total no. of 42 patients presenting with pleural effusion in Department of Pulmonary Medicine and General Medicine and test done in Department of Biochemistry at People's college of medical science and research Center. The findings of the study are tabulated as under-total 42 patient with effusion were enrolled. Mean value of ADA after 24 hours storage at 37℃ was 47.95IU/L and at 2 to 8 ℃ was 48.43 IU/L. The difference in pleural fluid ADA level upon storage at 37℃ and 2 to 8℃ was not significant (P value >0.05). The mean value of ADA after 2 hours of aspiration was 46.17IU/L and after 24 hours was 48.42IU/L. The difference in pleural fluid ADA levels upon storage for 2 hours and 24 hours is insignificant. (p value >0.05) The difference in pleural fluid ADA levels upon storage for 2 hours and 24 hours is insignificant. (p value >0.05) and at temperature 37 ℃ and 2 to 8 ℃ are no significant difference in ADA level. (p value >0.05).

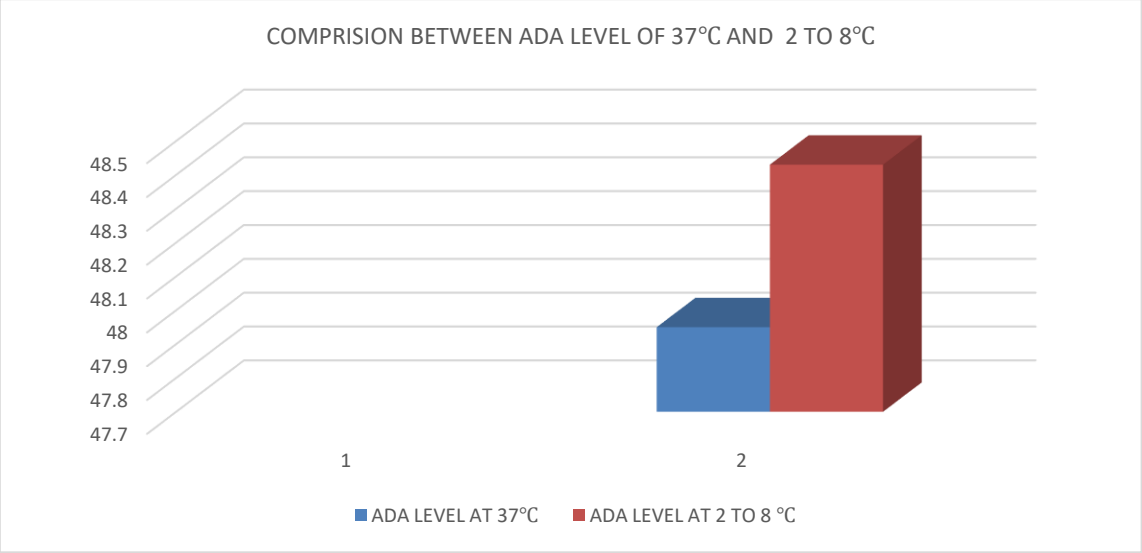
Table 1- Effect of storage on ADA levels in pleural effusion

	Mean	Std. Deviation	t test value	Df	P value
ADA test after 2 hours aspiration	46.17	21.88	1.088	41	.283
ADA test after 24	48.42	23.78			

Table -2 Comparison between ADA Level of 37℃ and 2 TO 8℃

	Mean	Std. Deviation	t test value	Df	P value
ADA test after 24 2 to 8 ℃	48.43	23.78	.276	41	.784
ADA test after 24 hours at 37	47.95	22.11			





DISCUSSION:

SOCIODEMOGRAPHIC VARIABLES

A total no. of 42 patient were enrolled in our study, with a mean age of 41.64 years. Literature suggests that HIV infection may affect the ADA levels. ADA levels have been reported to be much lower in patients of TB co-infected with HIV. ADA is secreted from the lymphocytes and T lymphocytes are deficient in HIV patients.[16] In the present study HIV status of all the patients was assessed, and all the patients were HIV-negative.

Although ADA is helpful in diagnosing tuberculous pleural effusions, ADA can also be detected in nontuberculous pleural effusions. ADA is secreted mainly by lymphocytes

EFFECT OF STORAGE ON ADA LEVELS

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 time and temperature on pleural fluid ADA levels. We analyzed ADA activity in pleural fluid first within 2 hours of collection and then after 24 hours of storage at temperature 37 ℃ and 2 to 8 ℃. Irrespective of the type of pleural fluid, cause of pleural effusions, and storage conditions.

The storage of pleural fluid at temperature 37 ℃ statically non - significant because P value is n P>0.05. The findings of our study are cordant with the Antonangelo L et al. (2006), who did not find any change in pleural fluid ADA activity up to 28 days after collection if stored at 4^0C or -20^0C [13]. Bielsa S et al. (2014) documented ADA activity in samples frozen at -800^0C 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.[34] In our study although pleural fluid ADA was statistically not significant.

CONCLUSION:

ADA estimation of After 2 hours of aspiration and after 24 hours storage, there is no effect of ADA level. Adequate storage of pleural fluid is essential as there is usually a delay (hours) in processing the samples after collection. Pleural fluid storage at temperature 37℃ for 24 hours and at temperature 2 TO 8 ℃ for 24 hours does not affect the ADA activity in time and temperature on pleural fluid ADA level.

Pleural fluid storage at different temperature for 24 hours does not affect the ADA activity enough to result in a change of diagnosis in etiology of pleural effusion. with no evidence of significant increases or decrease in enzyme activity that might distort the results.

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