International Journal of Applied Research 2016; 2(7): 751-753

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR2016; 2(7): 751-753
www.allresearchjournal.com
Received: 19-05-2016
Accepted: 20-06-2016

Smita D Rajani
Microcare Laboratory, 105, Manthan point, Unapani road, Surat, Gujarat, India.

Dr. Rajesh Soni
Associate Professor, Department of Microbiology, Hind institute of Medical Science, Sitapur, Ataria, Mou. (UP), India.

Dhanji P Rajani
Microcare Laboratory, 105, Manthan point, Unapani road, Surat, Gujarat, India.

Correspondence
Smita D Rajani
Microcare Laboratory, 105, Manthan point, Unapani Road, Surat, Gujarat, India.

Diagnostic significance of adenosine deaminase (ADA) activity in patients of pulmonary tuberculosis with and without pleural effusion

Smita D Rajani, Rajesh Soni and Dhanji P Rajani

Abstract
Tuberculosis (TB) is one of the oldest and commonest infectious diseases also known as "master of Death". Pulmonary TB (PTB) and TB with pleural effusion remains a diagnostic challenge. Adenosine deaminase (ADA) is an enzyme of purine catabolism which is an inexpensive and easy test in early routine evaluation of patients with pleural effusion.
Aim: To assess the diagnostic level of ADA in TB patients with and without pleural effusion and in non-tuberculosis pleural effusion.
Materials and method: Study comprised of 120 subjects of which 30 are healthy controls and 30 are confirmed cases of pulmonary TB without effusion, 30 are PTB with effusion and 30 are non-TB effusion patients. Age group range was from 25- 75 years. Estimation of serum and pleural fluid Adenosine deaminase by Giusti and Galanti method of enzymatic analysis**.**
Results: Serum ADA levels in pulmonary tuberculosis (55.07 $\pm$ 11.04) patients and pulmonary tuberculosis with pleural effusion (44.01 $\pm$ 7.83) were significantly higher (p<0.001) when compared with healthy controls (18.09 $\pm$ 6.15). Pleural fluid ADA levels were significantly higher (p<0.0001) in pulmonary tuberculosis with pleural effusion (82.63 $\pm$ 12.02) than in non-tuberculosis pleural effusion (27.71 $\pm$7.81).In the present study, the mean pleural fluid ADA were significantly higher as compared to mean serum ADA in pulmonary tuberculosis with pleural effusion (p<0.0001) and in non-tuberculosis pleural effusion (p<0.0008).
Conclusion: ADA level in serum as well as in pleural fluid in the diagnosis of pulmonary TB with or without pleural effusion is a very sensitive, specific, inexpensive, rapid, easily available & reliable investigation.

Keywords: Adenosine deaminase, pulmonary TB, pulmonary TB with pleural effusion, non-tuberculosis pleural effusion

Introduction
Tuberculosis (TB) is one of the most ancient diseases of mankind and has co-evolved with humans for many thousands of years. It is a major cause of morbidity and mortality throughout the world. One-third of the world's population is infected with the TB bacillus [1].
TB is categorised as pulmonary tuberculosis and extra-pulmonary tuberculosis. Diagnosis of pulmonary tuberculosis is confirmed mainly by sputum examination of AFB. However, the diagnosis of extra pulmonary tuberculosis requires investigation of pleural fluid biochemistry, cytology and pleural biopsy. Positivity for AFB and Histopathological (HP) study of pleura is very low and culture is very time consuming. ELISA, PCR & Interferon are very expensive tests [2].
Adenosine deaminase (EC 3.5.4.4), called ADA is an enzyme of purine catabolism which catalyses the pathway from adenosine to inosine [3]. Its distribution in the human organism is ubiquitous [4], but its physiologic role is especially important in lymphoid tissue. Its level is ten times higher in lymphocytes than in erythrocytes [5], Estimation of ADA activity helps in early diagnosis and treatment of the patient and prevents the spread of disease in the community [6]. Therefore, this study was planned to determine the exact role of ADA in TB patients with and without pleural effusion. The results of this study will help to provide a clear picture of ADA for diagnosis & prognosis of TB, & hence future plans for TB can be better executed.

Material and Methods

This study was carried out during July 2013-July 2014 at the Microcare Laboratory & Tuberculosis Research Laboratory, Surat. The laboratory is accredited for carrying out culture and Drug Susceptibility Testing (DST) by the Central TB Division, Ministry of Health and Family Welfare, Govt. of India. Present study comprised of 120 subjects of which 30 are clinically, radiologically and microscopically confirmed cases of pulmonary TB without effusion(group 1), 30 are PTB with effusion(group 2), 30 are non-TB effusion patients(group 3) and 30 are healthy controls (group 4). In all the above mentioned samples, the role of Adenosine deaminase (ADA) enzyme activity is carried out. Age group range was from 25- 75 years. The controls and patients voluntarily participated in the study. Informed consent was taken from controls and cases before collecting the blood and pleural fluid samples.

Specimen: - Serum
-Pleural fluids & other body fluids such as pericardial, ascitic fluids.

Collection & Preparation of Specimen

Collect specimen prior to use of antimicrobial agents. Do not use haemolysed, contaminated or turbid specimen.
ADA is reported to be stable in serum for 3 days at 2-8ºC& in biological fluids for 2 days at 2-8ºC as after this, ammonia may be released in the samples even without any microbial contamination. It is recommended to use fresh specimen for testing.

Adenosine deaminase activity assay

ADA was assayed on the same day as collection of samples. ADA activity was measured by a spectrophotometric method described by Guisti and Galanti[7], using ADA-MTB(R) kit from Microxpress a division of Tulip diagnostics (P) Ltd[8].

Principle: Adenosine deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue coloured indophenols complex formedis directly proportional to the amount of ADA present in the sample.

Normal range: in serum, pleural, pericardial and peritoneal fluid.
Normal - < 40 U/L
Suspect – 40 - 60 U/L
Positive - > 60 U/L
Linearity: The procedure was linear up to 150 U/L if values exceed, the sample was diluted with distilled water and the assay was repeated.

Result

Table 1: Categorisation of study population in different groups (n = 120).

Study Group	No. of Patients	Nature of Group
1	30	Pulmonary TB
2	30	Pulmonary TB with pleural effusion
3	30	Non TB with pleural effusion
4	30	Healthy controls

Table 2: Comparison of mean values of ADA (u/l) in serum in different groups.

Groups	Mean ADA ± SD	P Value
1	55.07±11.04	<0.0001*
2	44.01±7.83	
3	21.91±5.34	
4	18.09±6.15	

*P<0.05 was considered to be significant**.**

Serum ADA levels in group 1 (55.07 ± 11.04) patients were significantly higher (p<0.001) when
Compared with group 4 (18.09 ± 6.15). At the same time significant difference was found when compared with groups 2 and 3 (p<0.001).
Serum ADA levels in group 2 (44.01 ± 7.83) patients were significantly higher (p value <0.001) when compared with group 4 (18.09 ± 6.15). Also, significant difference was found when compared with group 1 and 3 (p<0.001).
Serum ADA levels in group 3 (21.91 ± 5.34) patients were higher but not statistically significant (p>0.05) when compared with group 4 (18.09 ± 6.15).

Table 3: Mean value of ADA (u/l) in pleural fluid in groups b &c.

Groups	Mean ADA ± SD	P Value
2	82.63 ±12.02	<0.0001*
3	27.71 ±7.81	

*p-value <0.0001, highly significant.

In the present study, the mean pleural fluid ADA (82.63 ±12.02) was higher as compared to mean Serum ADA (44.01 ± 7.83) in group 2. This was highly significant (p <0.0001). Similarly, the mean pleural fluid ADA (27.71 ±7.81) was higher than mean serum ADA (21.92 ± 5.33)in group 3 and was highly significant p-value <0.0008.But, the values of ADA in serum and pleural fluid in group 2 samples were higher as compared to Group 3 samples.

Table 4: Comparison of mean serum ADA and pleural fluid ADA within groups b and c.

Groups	Parameter	Mean ADA ± SD	P- Value
2	ADA serum	44.01±7.83	<0.0001*
2	ADA pleural fluid	82.63±12.02	
3	ADA serum	21.91±5.34	< 0.0008*
3	ADA pleural fluid	27.71±7.81	

*p -value < 0.05 considered significant

Discussion

In the present study there was statistically significant increase (p<0.001) in mean serum ADA levels in pulmonary TB (55.07 ±11.04), pulmonary TB with effusion cases (44.01±7.83) as compared to healthy controls (18.09±6.15) and non-TB pleural effusion patients (21.91±5.34). These findings are in accordance with the studies made by -Meena Verma *et al.*, in 2004 studied 100 patients of which 53 were suffering from pulmonary tuberculosis and 35 normal healthy control subjects. The mean serum ADA activity in pulmonary TB patients was 35.5 ± 6.93 U/L as compared to 16.20 ± 2.85 U/L in control group, showing highly significant (P<0.001) difference. ADA activity was highest in tuberculosis than compared to controls [9]. K.Srinivasa Rao *et al.* in 2010 found that in diagnosis of pulmonary TB, serum ADA showed high percent positivity of 88% followed by chest X-ray 76%, ESR 72%, Sputum AFB 63%,

Mantoux 61%. He also reported high serum ADA levels in pulmonary TB as compared to non-tubercular pulmonary diseases [10]. Saeed Amniafshar *et al.*, in 2004 evaluated 51 cases of active pulmonary tuberculosis, 50 of healthy controls. Mean serum ADA level in pulmonary tuberculosis (42.4 ± 21.5 IU/L) and was significantly more than controls (26.6 ± 8.21 IU/L) (p<0.0001) [11].

In pathological conditions, the clearance capacity of lungs is decreased leading to increased numbers of cells in pleural fluid and the recirculation of activated T cells may cause a high serum ADA activity in patients with pulmonary disease [12].

Also, in the present study serum ADA levels in non-tubercular pleural effusion were higher as compared to healthy controls but not statistically significant (p>0.05). In this study, findings seem to confirm that ADA activity is a useful parameter for the diagnosis of tuberculosis and tubercular effusion. The mean levels of pleural fluid ADA in tubercular pleural effusion (82.63 $\pm$12.02) were higher significantly (p<0.0001) as compared to pleural fluid ADA levels in non-tubercular pleural effusion (27.71 $\pm$ 7.81). Kaisemann *et al.*, in 2004 concluded that ADA determination in pleural fluid is a sensitive and specific method for diagnosis of pleural TB and its use can preclude need for pleural biopsy in initial workup of pleural effusion of patients [13]. Bharat Kumar Gupta *et al.*, in 2010 determined ADA activity in 96 pleural fluid samples comprising of tubercular and non-tubercular pleural fluid samples and found that pleural fluid ADA levels were significantly higher in TB pleural fluid as compared with non TB pleural fluid [14].

Thus, from present study results, it is observed that serum & pleural fluid ADA measurement plays a very important role in diagnosis of PTB without pleural effusion & with effusion respectively but, current study has certain limitations like small sample size which may be because of the knowledge or attitude of the patients with their condition. Therefore, it is suggested that a large sample size study in which different groups of PTB patients with their different categories like new cases, defaulters, relapse or failure, MDR should be taken & ADA values should be assessed which will give the exact role of ADA not just only in diagnosis of PTB but also in the prognosis of the disease. Measurement of ADA values is definitely having a very important role in the diagnosis of PTB patients with and without pleural effusion, thus giving a sensitive, specific, reliable, inexpensive, rapid, noninvasive and easily available investigation in a disease like TB for which whole nation is deeply concerned.

Conclusion

Serum ADA was significantly higher in pulmonary TB patients and pulmonary TB with pleural effusion patients compared to healthy controls and non TB pleural effusion. Pleural fluid ADA was significantly higher in pulmonary TB with pleural effusion patients compared to non-TB with pleural effusion patients. Pleural fluid ADA was significantly higher than serum ADA levels in pulmonary TB with pleural effusion and in non-TB with pleural effusion patients.

Thus serum ADA and pleural fluid ADA plays a very important role as an investigation in the diagnosis of PTB with or without effusion. Serum and pleural fluid ADA level measurement in the diagnosis of pulmonary TB without pleural effusion and pulmonary TB with pleural effusion respectively is a very sensitive, specific, inexpensive, rapid, easily available & reliable investigation.

References

1. Global Tuberculosis Control, A short update to the report, World health organization, 2009. WHO/HTM/TB/2009.426
2. Light RW. Useful tests on the pleural fluid in the management of patients with pleural effusions. Curr Opin Pulm Med. 1999; 5:245-52.
3. Martin DW Jr, Gelfand EW. Biochemistry of diseases of immune development. Ann Rev Biochem. 1981; 50:845-77.
4. Van Der Weyden MB, Kelley WN. Human adenosine deaminase: distribution and properties. J Biol Chem. 1976; 251:5448-56.
5. Gajdos A. Familial immune deficit and genetic deficiency of ADA of nucleotide phosphorylase. Nour press Medicine. 1978; 7(11):929-34.
6. Devkota KC, Shyam BK, Sherpa K, Ghimire P, Sherpa MT, Shrestha R,*et al*.Significance of adenosine deaminase in diagnosing tuberculous pleural effusion. Nepal Med Coll J. 2012; 14(2):149-152.
7. Giusti G. Adenosine deaminase. In: Bergmayer HU. Methods of Enzymatic analysis 2nd edition. New York, Academic press Inc 1974, 1092-1099.
8. Adenosine deaminase assay kit, package insert. ADA-MTB(R) kit from Microxpress a division of Tulip diagnostics (P) Ltd. ADA/1206/TP/VER-1.
9. Verma M, Narang S, Moonat A, Verma A. Study of Adenosine deaminase activity in pulmonary tuberculosis. Indian Journal of Clinical Biochemistry.2004; 19(1):129-131.
10. Srinivasa Rao K, Anand Kumar H, Rudresh BM, Srinivas T, Bhat KH.A Comparative study and evaluation of serum adenosine deaminase activity in the diagnosis of pulmonary tuberculosis. Biomedical Research. 2010; (2):189-194.
11. Aminiafshar S, Alimagham M, Maryam Keshtkar J, Latif G, Haghighat B, Mitra Keshtkar J, *et al*. Serum Adenosine deaminase level as an Indicator of Pulmonary Tuberculosis activity versus other infectious diseases. National Research Institute of Tuberculosis and Lung Disease, Iran. Tanaffos. 2004; 3(12):19-23.
12. Fonts BM, Pego A, Lima MA, Gaspar EV, Robalo CA. Serum and pleural Adenosine deaminase: correlation with lymphocyte populations. Chest. 1990; 97:605-610.
13. Kaisemann MC, Kritski AL, Pereira MFC, Trajman A. Pleural fluid adenosine deaminase detection for the diagnosis of pleural tuberculosis J Bras Pneumol. 2004; 30(6).
14. Gupta BK, Bharatb V, Bandyopadhyay D. Sensitivity, Specificity, Negative and Positive Predictive Values of Adenosine Deaminase in Patients of Tubercular and Non-Tubercular Serosal Effusion in India. J Clin Med Res. 2010; 2(3):121-126.