



Original Research Article

Evaluation of adenosine deaminase in serum and exudative pleural fluid for diagnosis of HIV seronegative tuberculous pleural effusions

Shyam Prasad B R¹, G Mahesh^{2,*}¹Dept. of Biochemistry, Government Medical College, Ananthapuramu, Andhra Pradesh, India²Dept. of Forensic Medicine, Government Medical College, Ananthapuramu, Andhra Pradesh, India

ARTICLE INFO

Article history:

Received 13-11-2019

Accepted 18-11-2019

Available online 14-12-2019

Keywords:

Adenosine deaminase
Extrapulmonary tuberculosis
Exudative pleural fluid HIV
seronegative

ABSTRACT

Introduction: Tuberculosis, both pulmonary and extra pulmonary pose great threat to individual health and community economy. The diagnosis of tuberculosis is currently made by a detailed medical history, clinical examination, radiological, microbiological, immunological, biochemical, molecular-biological and histological investigations. The current diagnostic methods either are relatively expensive, feasible, and insufficient or give erroneous results especially while diagnosing extra pulmonary tuberculosis. However recent trials have shown that enzymatic, evaluations in serum and pleural fluid have given excellent diagnostic results in diagnosis of extra pulmonary tuberculosis.

Aim: The current study was aimed to evaluate whether analysis of adenosine deaminase enzyme in serum and pleural fluid helps in diagnosis of HIV sero negative tuberculous pleural effusion.

Material and Methods: The study encompassed a total of 100 subjects of which 50 patients were known cases of tuberculous pleural effusion and remaining 50 patients served as control subjects Adenosine deaminase was estimated in serum and pleural fluids using kits and auto analyzer.

Results: The results were tabled and analyzed using MEDCALC statistical software and are in favor of the study, supporting adenosine deaminase as a reliable marker in diagnosis of sero negative pleural effusions.

Conclusion: The current study has indicated that promising positive results can be obtained by estimating levels of Adenosine deaminase enzyme in diagnosis of HIV sero negative pleural effusions. Further the study concludes that testing the adenosine deaminase levels in a cheaper, cost effective diagnostic tool and can be used in large community of individuals with minimal costs.

© 2019 Published by Innovative Publication. This is an open access article under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by/4.0/>)

1. Introduction

Tuberculosis (TB), a bacterial disease caused by Mycobacterium tuberculosis is one of the leading cause of morbidity and mortality from an infectious disease worldwide and is prevalent in all parts of the world and more so in developing and underdeveloped countries.¹ Pulmonary form of tuberculosis is the commonest form when compared to the extrapulmonary form that can literally affect any organ of the body.² Transmission of Tuberculosis usually occurs by the airborne spread of infectious droplets and droplet nuclei containing the tubercle bacilli.

The progression of Tuberculosis has disastrous effects on the economy of any country as the disease most often affects the economically productive age group.³

Early diagnosis and prompt management is a must for halt of infection and spread.⁴ A diagnostic approach routinely includes a detailed medical history, clinical examination, radiological, microbiological, immunological, biochemical, molecular-biological and or histological investigations. It can happen in HIV infected patients or older patients with cryptic miliary tuberculosis that sputum for acid fast bacilli cannot diagnose pleural effusions and most of the pleural effusions after thoracentesis are being treated based on cytology which can often be misleading. Hence a simple method to detect tuberculosis is the need of

* Corresponding author.

E-mail address: shyamcapt@yahoo.com (G. Mahesh).

the hour that will complement the existing Revised National Tuberculosis Control Program (RNTCP) in diagnosing extra pulmonary tuberculosis.

It is an established fact that evaluation of the enzymes such as Adenosine deaminase, lactate dehydrogenase, alkaline phosphatase and aminase in the serum and pleural fluids will help in classification and diagnosis of tubercular pleural effusions.⁵ Adenosine deaminase has been proven to have higher specificity and sensitivity and be a good marker for tuberculosis. Adenosine Deaminase (also known as adenosine amino hydrolase, or ADA) is an enzyme involved in purine metabolism. It is needed for the breakdown of adenosine from food and for the turnover of nucleic acids in tissues. Its primary function in humans is the development and maintenance of the immune system.

In the circumstances cited above, the current study is aimed at studying the usefulness of Adenosine deaminase in serum and pleural fluids⁶ as a diagnostic tool for HIV seronegative tuberculous pleural effusion.⁷

2. Materials and Methods

The current prospective study was conducted in the Department of Biochemistry, Government Medical College and General Hospital Anantapuramu from January 2018 to August 2018. Ethical clearance was obtained from the institutional ethics committee of this institution.

The study encompassed a total of 100 subjects of which 50 patients were known cases of HIV sero negative tuberculous pleural effusion and remaining 50 patients served as control subjects.

Patients were apprised of the purpose of study and written consent was taken prior to commencement of study. 5 ml of fasting venous sample and 10 ml of Pleural fluid aspirated using standard methods were collected under aseptic precautions as per standard procedures after explaining the procedure, taking consent and using standard methods of sample collection were used as samples for the study.

All the patients were in the age group of 15–55 years of random reporting in the hospital irrespective of sex criteria.

The patients were included on the criteria of confirmed diagnosis of tubercular pleural effusion and are HIV sero negative. Patients of confirmed diseases like HIV, brucellosis and other disease of altered adenosine deaminase levels were excluded and Adenosine deaminase was estimated using Guisti. G and Galanti Method. with Erba semi auto analyzer

The data obtained from study is recorded in a pretested proforma and results were analysed using appropriate statistical methods and MEDCALC software.

3. Results

3.1. Age

It is observed from Table 1 that the mean and standard deviation of age of cases in cases were 35 ± 15.32 and 42.2 ± 13.34 in controls respectively. This is in accordance with the incidence of tuberculosis which showed the age of tuberculosis pleural effusion as common in adults.

3.2. Levels of Adenosine deaminase

Adenosine deaminase levels were significantly elevated in serum and pleural fluid all cases (100%) when compared to controls (Table 2, Figures 1 and 2). The mean serum adenosine deaminase levels in cases is 76.25 ± 11.159 IU / L with p value < 0.0001 and is statistically highly significant when compared to controls which is 15.504 ± 5.437 IU / L. The mean of pleural fluid adenosine deaminase levels in cases is 85.112 ± 18.281 IU / L and in controls it is 13.876 ± 6.192 IU / L and the p value is < 0.0001 which is statistically highly significant. The serum and pleural fluid adenosine deaminase shows a sensitivity and specificity of 100%. Both positive predictive value and negative predictive value were also 100%.

Earlier studies mentioned below show similar results

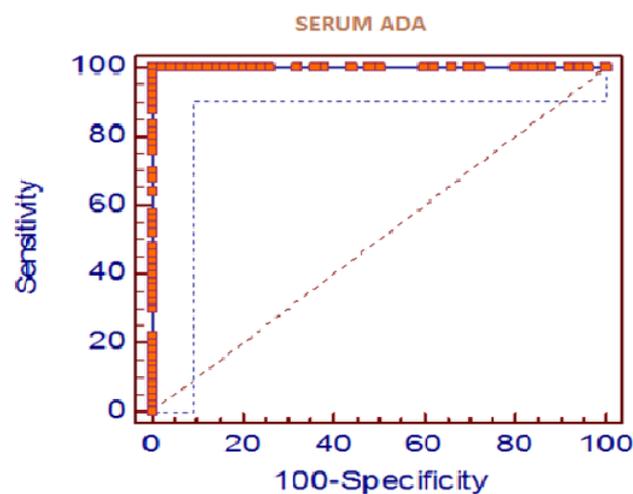


Fig. 1: Receiver operating characteristics Curve (Serum Adenosine Deaminase)

4. Discussion

Our findings confirm that serum and pleural fluid adenosine deaminase is a very good parameter for diagnosis of tuberculous pleural effusion. The Adenosine deaminase value in tuberculous pleural effusion is clearly higher than in non-tuberculous group ($p < 0.001$, highly significant).

The study findings are supported by the following studies

Table 1: Age wise distribution of cases

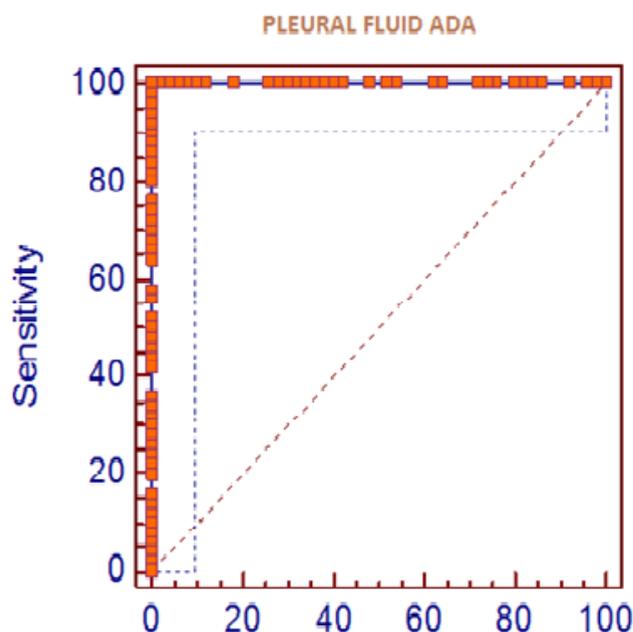
| Trait | Age Mean | Standard deviation | Standard error mean |
|--------------------------|-------------|--------------------|---------------------|
| Tuberculous effusions | 35 | 15.32 | 2.114 |
| Non-tuberculous effusion | 42.2 | 13.342 | 1.887 |

Table 2: Levels of Adenosine deaminase enzyme

| | Serum | | | Pleural fluid | | |
|--------------------------|--------|--------------------|---------------------|---------------|--------------------|---------------------|
| | Mean | Standard deviation | Standard error mean | Mean | Standard deviation | Standard error mean |
| Tuberculous effusions | 76.25 | 11.159 | 1.5162 | 85.112 | 18.281 | 2.5207 |
| Non-tuberculous effusion | 15.504 | 5.437 | 0.7688 | 13.876 | 6.192 | 0.8756 |

Table 3: Comparison of values obtained in various studies

| Pleural fluid Adenosine deaminase studies | | | |
|---|------------------------------|-------------------|---------|
| Year of study | Name of study | Mean \pm SD | P value |
| 2011 | Anand Patel et al | 114.1 \pm 61.36 | < 0.001 |
| 2010 | BharthKumar gupta et al | 67.34 \pm 22.85 | < 0.001 |
| 2010 | Zay Soe et al | 73.90 \pm 33.96 | < 0.001 |
| 2007 | Ashish Ananth RaoJadav et al | 80.66 \pm 49.05 | < 0.001 |
| 2006 | PC Mathur et al | 100 | < 0.001 |

**Fig. 2:** Receiver operating characteristics Curve (Pleural fluid Adenosine Deaminase)

Anand Patel and Susmitha Chowdary et al⁸, in their study have showed that adenosine deaminase activity of > 40 IU / L in tuberculous pleural effusion has a sensitivity of 97% and specificity of 93% and with > 35 IU/L as cut off value the sensitivity is 100% and specificity is 93%. They

had obtained a mean level of 114.1 ± 61.36 in tuberculous effusion and 20.3 ± 23.42 in non-tuberculous effusion.

Bharth Kumar Gupta et al,⁸ in 2010 have reported that the pleural fluid adenosine deaminase levels to be consistently increased and more than the cut-off (40U/L) in cases of exudative pleural effusions of tuberculous etiology. In cases with non-tubercular exudative pleural effusion the adenosine deaminase levels were found to be consistently below the cut-off. The values of mean for adenosine deaminase levels in pleural fluids of tuberculous and non-tuberculous groups obtained were 67.34 ± 22.85 and 18.60 ± 9.12 respectively. The negative predictive value of adenosine deaminase for the diagnosis of non-tuberculous etiology was 97.5%.

Zay Soe et al⁹ in a study in Malaysia, observed that the best cut off level of adenosine deaminase activity tested was at 42.5 IU/L when sensitivity was 87% and specificity was 89%. The mean adenosine deaminase levels in their study was 73.90 ± 33.96 . The positive predictive value (PPV) was 96% and NPV was 83%.

P.C. Mathur¹⁰ studied adenosine deaminase activity in 50 patients with pleural effusion and found sensitivity and specificity of 100% at a level of 40 IU/L cut off value. Adenosine deaminase Level in tuberculous pleural effusion ranged from 45-160 U/L with a mean level of 100 U/L while in non- tuberculous group it ranged from 5 to 33 U/L with the mean of 18 U/L ($p < 0.001$, highly significant) The sensitivity and specificity for diagnosing tubercular effusion was 100% and 94.6% with positive and negative predictive values of 95.5% and 100% respectively.

The serum levels of adenosine deaminase in tuberculous effusions are in accordance with the studies of Jadhav et al¹¹ who observed a mean \pm SD of 38.58 ± 22.81 in serum.

In another study by Meena Verma et al.¹² the mean serum level of adenosine deaminase was 39.97 ± 2.7 with p value of < 0.001 .

In non-tuberculous pleural effusions, adenosine deaminase activity was low and never exceeded the cut of limit for tuberculous pleural effusion (>40 IU/L) in this study. The levels of adenosine deaminase are in accordance with the above-mentioned studies.

In another study done only on non-tuberculous patients by Y C Gary Lee et al¹³ were 16.6 ± 7.2 in post CABG effusions, 15.3 ± 11.2 in malignant effusions and 15.4 ± 13.1 in miscellaneous effusion.

5. Conclusion

In a developing country of high tuberculosis incidence like India, a diagnosis of Tuberculosis should always be considered when there is failure to respond to the conventional antimicrobial (not anti-tubercular) therapy or the patient presents with unusual manifestation.

In this study, adenosine deaminase levels in non-tuberculous exudative pleural effusions never exceeded the cut-off value set for tuberculous disease by other studies. The serum and pleural fluid adenosine deaminase levels were significantly higher in tuberculous exudative pleural effusions when compared with non-tuberculous exudative pleural effusions. Adenosine deaminase level more than the cut off value of 40 U/L practically confirms the tubercular etiology in exudative pleural effusion cases.

As adenosine deaminase makes up part of the combination in all the studies reviewed, pleural fluid adenosine deaminase estimation should be done routinely, particularly if the diagnosis of tuberculosis is in doubt, sputum AFB negative pulmonary tuberculosis effusions cases and to differentiate pulmonary tuberculosis effusions from non-tubercular pulmonary diseases effusions.

The present study concludes that a simple, less expensive, highly sensitive and specific test like pleural fluid adenosine deaminase estimation should be employed routinely to differentiate between tubercular and non-tubercular etiology in patients of pleural effusion particularly if diagnosis of tuberculosis is suspected and in places where prevalence of this disease is still high like in our country.

6. Acknowledgements

Authors acknowledge the subjects whose samples were studied and the staff of this college and hospital who gave us timely inputs and suggestions.

7. Source of funding

Nil

8. Conflict of interest

Nil

References

1. WHO – fact sheet on tuberculosis ;. Available from: <https://www.who.int/news-room/fact-sheets/detail/tuberculosis>.
2. Alladi Extrapulmonary tuberculosis *Indian J Med Res.* 2004;120:316–353.
3. Bloom BR, Atun R, Cohen T. Tuberculosis. In: Holmes KK, Bertozzi S, Bloom BR, editors. Major Infectious Diseases. 3rd edition. Washington (DC): The International Bank for Reconstruction and Development / The World Bank ; 2017,. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK525174/doi:10.1596/978-1-4648-0524-0/ch11>.
4. Tuberculosis (TB): Areas of work, TB detection and diagnosis ;. Available from: <https://www.who.int/tb/areas-of-work/laboratory/early-detection/en/>.
5. Afrasiabian S, Mohsenpour B, Bagheri KH, Sigari N, Aftabi K. Diagnostic value of serum adenosine deaminase level in pulmonary tuberculosis. *J Res Med Sci.* 2013;18:252–254.
6. Swami KK, Choudhary VS, Shekhawa J, Choudhary PR. Adenosine deaminase activity in pulmonary tuberculosis. *Indian J Appl Basic Med Sci.* 2011;17:1–3.
7. Lamsal M, Gautam N, Majhi S, Baral N, Bhattacharya SK. Diagnostic utility of adenosine deaminase (ADA) activity in pleural fluid and serum of tuberculous and non-tuberculous respiratory disease patients. *Southeast Asian J Trop Med Public Health.* 2007;38(2):363–369.
8. Gupta, Kumar B. Role of Adenosine Deaminase Estimation in Differentiation of Tuberculous and Non-tuberculous Exudative Pleural Effusions. *J Clin Med Res.* 2010;2(2):79–84.
9. Soe A, Shwe WH, Moe S. A study on tuberculous pleural effusion. *Int J Collaborative Res Intern Med Public Health.* 2010;2(3):32–48.
10. Mathur PC, Tiwari KK, Trikha S, Tiwari D. Diagnostic value of Adenosine Deaminase (ADA) Activity in Tubercular Serositis. *Indian J Tuberc.* 2006;53:92–95.
11. Jadhav AA, Jain A. Sputum adenosine deaminase and alkaline phosphatase activity in pulmonary tuberculosis. *Arch Physiol Biochem.* 2012;18(1):6–9.
12. Verma M, Narang S, Moonat A, Verma A. Study of adenosine deaminase activity in pulmonary tuberculosis and other common respiratory diseases. *Indian J Clin Biochem IJCB.* 2004;19(1):129–131.
13. Lee YG, Rogers JT, Rodriguez RM, Miller KD, Light RW. Adenosine deaminase levels in nontuberculous lymphocytic pleural effusions. *Chest J.* 2001;120(2):356–361.

Author biography

Shyam Prasad B R Associate Professor

G Mahesh Associate Professor

Cite this article: Prasad B R S, Mahesh G. Evaluation of adenosine deaminase in serum and exudative pleural fluid for diagnosis of HIV seronegative tuberculous pleural effusions. *Int J Clin Biochem Res* 2019;6(4):522-525.