MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.36478/makrjms.2022.4.99.102

Study on Levels of Serum Adenosine Deaminse Levels in Patients with Pulmonary Tuberculosis

[1]P.N. Kiran Kumar and [2]C. Ramanjula Reddy

[1,2]Department of TBCD, Fatima Institute of Medical Sciences, Kadapa, A.P., India

OPEN ACCESS

Key Words
Adenosine deaminse, pulmonary tuberculosis, microscopy

Corresponding Author
C. Ramanjula Reddy,
Department of TBCD, Fatima Institute of Medical Sciences, Kadapa, A.P., India

Author Designation
[1]Associate Professor
[2]Assistant Professor

Received: 22 September 2022
Accepted: 28 November 2022
Published: 26 December 2022

Citation: P.N. Kiran Kumar and C. Ramanjula Reddy, 2022. Study on Levels of Serum Adenosine Deaminse Levels in Patients with Pulmonary Tuberculosis. Res. J. Med. Sci., 16: 99-102, doi: 10.36478/makrjms.2022.4.99.102

ABSTRACT

The study's objectives were to grade the patients based on their sputum smear grading and estimate the predictive value of Tuberculosis using serum adenosine deaminse (ADA). The present study was a hospital-based cross-sectional study on 40 patients who tested positive on sputum smear microscopy. The positive samples are graded into 1+, 2+ and 3+ on microscopy based on the severity. Serum ADA was sent for each patient and their values were correlated with the Sputum microscopy grading. The Majority (80%) of subjects in the present study were male. The most common symptom with which the subjects presented was cough. This was followed by shortness of breath, fever, weight loss and hemoptysis. Smoking was noted in the Majority (70%) of the study subjects. The Majority (85%) of the subjects were newly diagnosed cases of pulmonary Tuberculosis. The remaining were reinfection cases. The most common sputum smear grading was Grade-1 (40%), followed by Grade-2 (35%) and Grade-3 (25%). Serum ADA Levels were highest in Grade-3 subjects, followed by Grade-2 and Grade-1. This difference was found to be statistically significant. The diagnostic utility of serum ADA for diagnosis of Grade-1 smear-positive tuberculosis was not found to be adequate (AUC=0.19). The diagnostic utility of serum ADA for diagnosis of Grade-2 smear-positive tuberculosis was not found to be adequate (AUC=0.49). The diagnostic utility of serum ADA for diagnosing Grade-3 smear-positive tuberculosis was good (AUC=0.89). As the AUC is highest for 3+grading patients, this is significant only in 3+pulmonary Tuberculosis. This indicates that the bacterial load is important for the raised ADA levels. Finally indicates that serum ADA may be useful in Multi bacillary Tuberculosis rather than Pauci bacillary Pulmonary Tuberculosis.

INTRODUCTION

Tuberculosis(TB) is a common communicable disease[1]. It is a significant cause of ill health worldwide, affecting most commonly (90%) adults[1]. A bacillus named Mycobacterium Tuberculosis (MTB) causes Tuberculosis[1]. It is an acid-fast bacillus stained with the Ziehl-Neelsen method. From person to person, the most common route of transmission is by droplet infection[1]. It can affect any organ, most commonly affecting lungs (pulmonary TB)[1]. It most commonly affects men than women[1]. TB ranks one among the top ten causes of death worldwide[1]. It is above HIV/AIDS as the leading cause of death from a single infectious agent[1]. About 10 million people are affected worldwide from TB in 2019, according to WHO 2020 global TB report[1]. Out of these men are 5.6 million, women 3.2 million and children are 1.2 million[1]. In the deaths, a total of 1.4 million estimates died of TB in 2019[1]. Only 30 countries hold a caseload of 87%[1] of these high burden countries., only eight countries have almost about two-thirds cases of which India is in the lead[1]. Globally an incidence of 2% is declining between 2015-2019[1]. There is a cumulative decline of 9%, but this is only halfway to the **"END TB STRATEGY"** milestone, aiming to reduce 20% cases[1]. The theme of **WORLD TB DAY 2020 is "IT'S TIME"**[1]. With an estimated incidence of about 26.9 lakh cases, India holds the highest-burden in 2019[2]. A person with active TB can affect 5-15 people in a year through close contact[1]. The high incidence explains the need for rapid and accurate TB diagnosis early in the cases and deaths. TB is curable and preventable with proper and appropriate management. Though there are several methods to diagnose TB, direct observation of acid-fast bacilli under a microscope is considered the standard form practiced worldwide[3]. It is not only specific but also a rapid and inexpensive test[4]. The major disadvantage is that it needs at least 10,000 bacilli per ml of Sputum to test positive. It also has several inter-observer variations[5]. The role of chest X-ray in the diagnosis of pulmonary Tuberculosis is very minimal[6]. It is because several non-tubercular pulmonary lesion mimics TB on a chest X-ray. So this is considered nonspecific in the diagnosis[7]. The culture of MTB is considered the gold standard for the diagnosis[8]. But it takes at least 6-8 weeks to confirm the diagnosis, which is the major drawback[8]. It ultimately results in a delayed diagnosis. WHO recommends using rapid molecular tests as initial diagnostic methods for detecting TB like XPERT MTB, XPERT ULTRA, TRUENAAT ASSAY. Though this helps in rapid and almost accurate diagnosis, the high cost makes it unavailable at all centers where the facilities are minimal. There is also a need for rapid, accurate and inexpensive test for diagnosing TB. With timely diagnosis and the use of appropriate drugs, TB can be cured easily[9]. But the primary difficulty in this is the lack of effective and simple procedures with high sensitivity and specificity[9]. Nowadays, estimation of ADA has got importance. It is already being used almost widely for diagnosing pleural effusions. Assessment of serum ADA has gained prominence in diagnosing PTB. ADA is a biochemical test that is an indicator of active cellular immunity[10]. The levels of this enzyme were thought to rise in TB patients as this affects cellular immunity. This study evaluates serum ADA levels in micro biologically proven cases of pulmonary Tuberculosis and its correlation with the sputum microscopy grading.

Aim of Study: To study the levels of serum ADA in pulmonary Tuberculosis.

MATERIALS AND METHODS

Hospital-Based Cross-sectional Analytical Study conducted at Department of Respiratory Medicine in 40 Microscopically proved cases of pulmonary Tuberculosis cases.

Sampling Technique: Selecting patients who test positive on sputum microscopy after they met inclusion and exclusion criteria.

Inclusion Criteria:

- Microscopically proven cases of Tuberculosis.
- Age above 18 years.
- Patient willing to participate in the study.
- Symptoms suggestive of Tuberculosis.
- chest x-ray suggestive of Tuberculosis.

Exclusion Criteria:

- Sputum negative pulmonary Tuberculosis.
- Patients who are already on ATT.
- Cases of EPTB.
- Patients with malignancies who also suffered from PTB.

Study Procedure:

- A detailed history from each patient was taken and recorded.
- A complete physical examination was done and all the vitals were recorded for every patient in the study.
- Blood investigations like complete blood picture, complete urine examination, viral screening (HIV, HBsAg, HCV), Random blood sugar were sent.
- A chest X-ray was done for every patient.
- Two samples of Sputum were collected from every patient (one sample of early morning sputum and the other was a spot sputum sample).
- They were sent for examination under microscopy after fluorescent staining under RNTCP.
- Based on the bacterial load in the smear, they were graded into 1+, 2+,3+.

- 5ml of blood was collected from every positive patient and was sent for serum ADA.
- The serum ADA levels were then compared with the sputum smear grading.

Fluorescent Staining Method:

- An unscratched new clean slide is labeled on one side with the laboratory number using a diamond-tipped stylus.
- The mucopurulent portion is used for the smear preparation.
- An appropriate portion of the specimen is transferred using a broomstick or nichrome wire loop of 5mm dm to the slide.
- over an area of approximately 2 by 3mm smear the specimen
- Make it thin enough to be able to read through it.
- For each specimen, use a fresh slide.
- Allow it to air dry for 15min. Do not use heat for drying.
- The smear is fixed to the slide by passing it over the flame 3-5 times for 3-4 sec each.
- Dispose of the broomstick or flame wire loop thoroughly using the side burner before re-use after making smear.
- Materials required for staining are Auramine-Phenol solution, 1% Acid alcohol, 0.1% Potassium permanganate solution.
- The slides are placed on a staining rack, with the smeared side facing up.
- Flood the slides with freshly filtered Auramine-Phenol and let it stand for 7-10 min.
- Wash well with running water and control the flow of water to prevent washing away the smear.
- Decolorize by covering entirely with acid alcohol for 2min, twice. 15) Wash well with running water, as before to wash away acid alcohol.
- Counterstain with potassium permanganate for 30min.
- Wash as before with water and slope the slides to air dry.
- The bacilli appear as slender bright yellow fluorescent rods against a dark background.
- Grade positive smears to four degrees of positivity using 20x, 25x objective and 10x eyepiece.
- The serum ADA levels were compared with the sputum microscopy grading.

RESULTS AND DISCUSSIONS

In the present study, a majority were men (80%). In the present study, a majority of subjects reported a history of cough (80%). Majority of the subjects (42.5%) presented with Grade 2 Shortness of Breath. The Majority of them (95%) reported a history of fever. The Majority of the study subjects (95%) reported weight loss. The Majority of the study subjects (72.5%) did not have a history of hemoptysis. Scanty hemoptysis was reported by 12.5%, mild and moderate by 7.5% each. A majority of them (70%) had a history of smoking. Only 15% of the study subjects reported a history of pulmonary Tuberculosis. Most of the patients (40%) belong to the 1+category. This was followed by the 2+ category (35%) and 3+ category (25%). The Majority of them (85%) were new cases. Only 15% were reinfections.

Table 1: Correlation Between Sputum Smear Categories and Serum ADA Levels

Sputum smear grade	Mean	SD
1+Sputum Smear	21.5	6.9
2+Sputum Smear	34.2	22.3
3+Sputum Smear	76.2	48.8

The mean ADA levels were highest for Cat 3, followed by Cat 2 and then Cat 1. The difference noted between the three groups was statistically significant. ROC curve -diagnostic yield of ADA for Pulmonary Tuberculosis with Sputum Smear Category 1+: The AUC is 0.19 (95%CI 0.05-0.32., p-0.01). The cutoff value of ADA for 1+ sputum smear was 17.5, with a sensitivity of 68% and specificity of 12.5%. Due to poor AUC, sensitivity, and specificity, ADA cannot be taken as a diagnostic marker for 1+category sputum smear. ROC curve -diagnostic yield of ADA for Pulmonary Tuberculosis with Sputum Smear Category 2+: The AUC is 0.49 (95%CI 0.31-0.68., p-0.98). The cutoff value of ADA for 2+sputum smear was 21.5, with a sensitivity of 78% and specificity of 32%. Due to poor AUC, sensitivity, and specificity, ADA cannot be taken as a diagnostic marker for 2+category sputum smear. ROC curve -diagnostic yield of ADA for Pulmonary Tuberculosis with Sputum Smear Category 3+: The AUC is 0.89 (95%CI 0.77-1.0., p<0.01). The cutoff value of ADA for 3+ sputum smear was 39, with a sensitivity of 90% and specificity of 87%. Due to doog AUC, sensitivity and specificity, ADA can be considered a diagnostic marker for the 3+category sputum smear.

In the present study, the Majority of the participants were male (80%). Only 20% of the study subjects were female. The gender difference in the prevalence of Tuberculosis has been an area of interest worldwide. A similar high male preponderance was noted by Saini[11](66%). The study subject's mean age was 52 years, of which the majority of them belonged to 50-60 years. In the present study, the cough was reported in 87.5% of subjects. This was the most common symptom. Shortness of breath Grade 2 was seen in 42.5% and Grade 3 was seen in 27.5%. Fever was reported in 95% of the subjects. Hemoptysis was seen in 27.5%. Alaarag[12] reported that the most prevalent complaint was cough, as it was found in all the patients, followed by expectoration in 83.3% of patients, dyspnea in 80% patients, chest pain in 43.3%, toxic manifestations in 38.3% patients and hemoptysis in 28.3%patients. Of the total subjects, 85% were new cases diagnosed with Tuberculosis for the first time.

The remaining 15% were reinfection cases. The importance of reinfection as a cause for the recurrence of Tuberculosis is unclear and has potential public-health implications. According to the Drug Resistance survey, the prevalence of Multi-Drug Resistant Tuberculosis (MDR-TB) was 3.9% in new cases and 21% in re-treatment cases. This underlines the importance of the past disease/treatment status of the study subjects. In the present study, only Sputum smear-positive individuals were included. The Majority of the study subject's smears (40%) showed 1+. 2+ smears were seen in 35% and 3+smears were seen in 25% of the smears. In a similar study on serum ADA in pulmonary Tuberculosis, Saini *et al.,* reported that 26% of the smears were scanty+, 18% were 1+, 32% were 2+ and 24% were 3+, which are different from this study. The mean serum ADA estimated in this is 39.6 IU. Rao[13] also reported a similar mean ADA of 40.48 U/L. In the present study, ADA was a significant predictor of Tuberculosis only in sputum smears 3+ subjects. The ADA cutoff value to determine sputum smear grade 3+ Tuberculosis was found to be 39 IU/L. The sensitivity of this cutoff value was found to be 90%, and specificity was 87%. The serum ADA was not a reliable predictor for Tuberculosis with Sputum smears 1+ and 2+ grades. Atta *et al.,* reported that AUC was 0.89 for serum ADA and the cutoff value of serum ADA was 17.9 U/L and the sensitivity of this marker was 93.3 and the specificity was 51.0%.

CONCLUSION

Serum ADA levels were highest in sputum smear Grade-3 subjects. From the ROC curve serum, ADA is significant only for 3+pulmonary Tuberculosis. Serum ADA was a good diagnostic tool in Sputum Smear Grade-3 subjects. Serum ADA is a poor diagnostic tool in Sputum Smear Grade-1 and Sputum Smear grade-2 subjects. It indicates that the bacterial load is important for raised ADA levels. Thus it finally indicates serum ADA may be useful for Multi bacillary Tuberculosis rather than paucibacillary Pulmonary Tuberculosis.

REFERENCES

1. Genev, W.H.O., 2020. Global Tuberculosis Report 2020. World Health Organisation., Vol. 1 .
2. Salmanzadeh, S., H. Tavakkol, K. Bavieh and S.M. Alavi, 2015. Diagnostic Value of Serum Adenosine Deaminase (ADA) Level for Pulmonary Tuberculosis. Jundishapur J. Microbiol., Vol. 8 .10.5812/jjm.21760.
3. Tuberculosis, Surendra .K. Sharma., 2009. Laboratory Diagnosis. 2nd edition Edn., Pages: 162.
4. Surendra k. Sharma., 2020. Textbook of Tuberculosis and Nontuberculous Mycobacterial Diseases. 3rd edition Edn., Laboratory Diagnosis,, Pages: 106.
5. Mohankudo, Siladitya, Manjhi, Rekha, Dutta, Pravati, Pothal, Sudarsan, Jagaty, Suman, Chhotray and Pritam., 2019. Role of serum ADA in diagnosis of pulmonary tuberculosis. 0 2: 57-62.
6. Alladi, Mohan and S.K. Sharma., 2008. Fiberoptic Bronchoscopy in the diagnosis of sputum Smear-negative Pulmonary Tuberculosis: Current status. The Indian Journal of Chest Diseases and Allied Science., 50: 67-78.
7. Imtiaz, A., S. Sadia, M.D.A. Billal, H. Nazmul and A.B.M. Abdullah., 2020. Role of Serum ADA in Patients of Extra-Pulmonary Tuberculosis in a Tertiary Care Hospital of Bangladesh. Int J Med Res Prof., 6: 63-67.
8. Kuyucu, N., C. Karakurt, E. Bilaloglu, C. Karacan and T. Teziç., 1999. Adenosine deaminase in childhood pulmonary tuberculosis: Diagnostic value in serum. J. Trop. Pediatr.s, 45: 245-257.
9. Dilmaç, A., G.Ö. Üçoluk, F.U. G, A. Gözü, B. Akkalyoncu, T. Eryilmaz and B.S. G, 2002. The diagnostic value of adenosine deaminase activity in sputum in pulmonary tuberculosis. Respir. Med., 96: 632-634.
10. Alaarag, A.H, O.I. Mohammad and N.M. Farag., 2016. Diagnostic utility of serum adenosine deaminase level in the diagnosis of pulmonary tuberculosis. Egyptian Journal of Bronchology., 10: 133-139.
11. Rao, K.S., H.A. Kumar, B.M. Rudresh, T. Srinivas and K.H. Bhat., 2012. Evaluation of serum adenosine deaminase activity during the course of pulmonary tuberculosis treatment. Biomed Res., Vol. 23.