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International Journal of Clinical Biochemistry and Research

Journal homepage: https://www.ijcbr.in/

Original Research Article

Role of serum adenosine deaminase (ADA) in the diagnosis of tuberculosis & other respiratory diseases

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ARTICLE INFO

Article history:
Received 04-09-2023
Accepted 15-09-2023
Available online 25-10-2023

Keywords:
Serum adenosine deaminase
Pulmonary tuberculosis
Extrapulmonary tuberculosis
Respiratory infections
Diagnosis

ABSTRACT

Background & Objectives: The WHO Tuberculosis (TB) statistics for India in 2021 gave an estimated incidence figure of 25,90,000 cases, i.e., about 40% of Indian population is infected with TB. There are different investigative methods available for TB diagnosis like ZN-staining of *M.tb*, which lacks sensitivity & another method of Mycobacterial culture takes around 6-8 weeks to isolate *M.tb* in culture which results in delayed diagnosis & treatment and meanwhile further progression of disease. Other sensitive methods like PCR & CBNAAT are costly & they require skilled personnel & lots of equipment. Therefore, there is a need of simple, cost-effective, rapid & reliable test which can be easily carried out in the clinical laboratories of resource limited countries. In some previous studies, the level of ADA in effusion fluids was used for the diagnosis of TB, but it is not always possible to access effusion fluid & it requires skilled personnel. Thus, the aim of the present study is to evaluate the usefulness of measuring the serum level of ADA as noninvasive biochemical marker in early diagnosis of TB.

Materials and Methods: The present cross-sectional study was conducted on 200 serum ADA samples. Patient samples were divided into four groups based on their diagnosis, i.e., 50 patients with pulmonary TB, 50 patients with extra-pulmonary TB, 50 patients with respiratory infections other than TB & 50 healthy people not having TB.

Results: The ADA level for each group was presented as mean + SD & compared by applying post hoc Bonferroni test which showed that the pulmonary TB group was significantly different from the other 3 groups with $p<0.001$. According to ROC curve analysis, the best cutoff point was 21.8 IU/L at which sensitivity & specificity were 88% & 87% respectively.

Conclusion: Serum ADA activity with high sensitivity & specificity percentage can be used as a reliable marker in the diagnosis of TB & to differentiate TB from other respiratory illness.

1. Introduction

Tuberculosis (TB), an infectious disease caused by Mycobacterium tuberculosis (M.tb) bacteria, generally affects the lungs, but it can also affect other parts of the body.[1] The World Health Organization (WHO) TB statistics for India in year 2021 gave an estimated incidence figure of 25,90,000 cases. This is a rate of 188 per 1,00,000 population, i.e., about 40% of Indian population is infected with TB bacteria & the vast majority of whom have latent TB rather than TB disease.[2]

There are different investigative methods available for diagnosis of TB.[3–6] Conventional smear microscopy with Ziehl-Neelsen (ZN) stain lacks sensitivity as it has very

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https://doi.org/10.18231/j.ijcbr.2023.042

low detection rate around 16-50% & large portion remains negative in spite of clinical & radiological signs suggestive of TB.[7] Mycobacterial culture being gold standard method for diagnosis of TB possesses major limitation of longer incubation period that is around 6-8 weeks to get isolate in culture to which antibiotic susceptibility testing can further add 3-6 weeks. Meanwhile the disease may progress which cause delay in an appropriate treatment & increase hospitalization.[8] Recent advances in molecular techniques like polymerase chain reaction (PCR) & cartridge based nucleic acid amplification test (CB-NAAT) have shortened the time needed to diagnose TB, leading to improved case detection & management but these methods require skilled personnel & expensive instruments. This limits their usefulness & access in areas with insufficient resources.[9,10]

Therefore, there is a need of simple, cost effective, rapid & reliable test which can be easily execute in the clinical laboratories of resource limited areas of developing countries like India.

Some previous studies have used the level of adenosine deaminase (ADA) in effusion fluids to diagnose TB.[11] ADA (E.C. 3.5.4.4) is an enzyme of purine metabolism which catalyzes the irreversible deamination of adenosine into inosine.[12] ADA plays an important role in activation of lymphocytes.[13] Mycobacterial antigens infect macrophages which cause activation of T-cell lymphocytes resulting in increasing levels of ADA in TB.[14] ADA may be released from effusion fluids & be detected in serum of patients with active TB. As it is not always possible to access effusion fluid in pulmonary & extrapulmonary TB everywhere because it requires skilled personnel, therefore it would be helpful to take advantage of serum level of ADA to diagnose TB.

2. Objective

The aim of the present study is to evaluate the relevance of measuring serum ADA activity in TB patients as a non-invasive biochemical marker in the early diagnosis of disease.

3. Materials and Methods

The present cross-sectional study was completed over a period of 12 months, i.e., from May-2021 to April-2022 conducted on patients attending out-patient department & patients getting admitted in wards of pulmonary medicine department of one of tertiary health centers of central India. 200 age & sex matched randomly assigned subjects belonging to 18-50 years of age group diagnosed based on their CB-NAAT & histopathology report, were divided into four groups:

1. Group I= 50 patients newly diagnosed with pulmonary TB
2. Group II= 50 patients newly diagnosed with extrapulmonary TB
3. Group III= 50 patients with respiratory infections other than TB
4. Group IV= 50 healthy people not having TB.

3.1. Exclusion criteria

Patients having following ailments

1. HIV/AIDS
2. Liver Diseases
3. Typhoid Fever
4. Infectious Mononucleosis
5. Gout / Rheumatoid Arthritis
6. Renal Failure
7. Corticosteroid Therapy
8. Anti-TB treatment

After obtaining an informed consent, about 2ml of blood was drawn by venipuncture in plain tube from each patient following all aseptic techniques & immediately sent to laboratory. Then samples were centrifuged at 2,500 rpm for 10 minutes & the serum was separated into clean, dry & sterile vials. ADA activity was measured on the same day of collection of samples on Fully Automated Biochemistry Analyzer XL-640 manufactured by Erba Transasia Bio-Medicals Ltd.

3.2. Statistical analysis

All the results were expressed as mean + Standard Deviation (SD). Mean serum ADA level for each group was assessed by analysis of variance (ANOVA) test. Receiver operating characteristic (ROC) curve was used to determine a cut-off value for the ADA test. In addition, the diagnostic usefulness of ADA was assessed in terms of sensitivity & specificity. Area under curve was used to determine the discriminatory ability of the assay to distinguish between TB (pulmonary & extrapulmonary) from non-TB respiratory infections. Post Hoc Bonferroni test was used to determine the significant differences between the groups & p-value <0.001 was considered as statistically significant. All calculations were performed by the statistical package for social sciences (SPSS) software version 15.

4. Results

The mean levels of serum ADA were found to be significantly higher in the patients with active TB (groups I & II) in comparison to the ones who were suffering from any other respiratory illness (group III) & healthy subjects (group IV) (Table 2). Groups were compared by using post HOC Bonferroni test & test result showed highly significant difference between TB groups (group I & II) and other two groups, i.e., non-TB respiratory infections & Healthy

Table 1: Age & gender wise distribution of pulmonary TB (I), extrapulmonary TB (II), non-TB respiratory infections (III) & healthy subjects (IV)

Groups (N)	Gender		Age (Years)
	Male	Female	Mean + SD
I (50)	22	28	34.2 + 11.9
II (50)	22	28	44.1 + 13.2
III (50)	24	26	39.7 + 13.5
IV (50)	25	25	33.3 + 11.3
Total (200)	93	107	

Table 2: Levels of serum ADA in pulmonary TB (I), extrapulmonary TB (II), non-TB respiratory infections (III) & healthy subjects (IV)

Groups (N)	Sr ADA (IU/L) Mean + SD	95% confidence interval	
		Lower Bound	Upper Bound
I (50)	36.05+12.2**	32.57	39.51
II (50)	25.75+4.42**	24.4	27.01
III (50)	19.8 + 3.21	18.8	20.7
IV (50)	11.73+2.48	11.0	12.4

**p-value <0.001 (highly statistically significant)

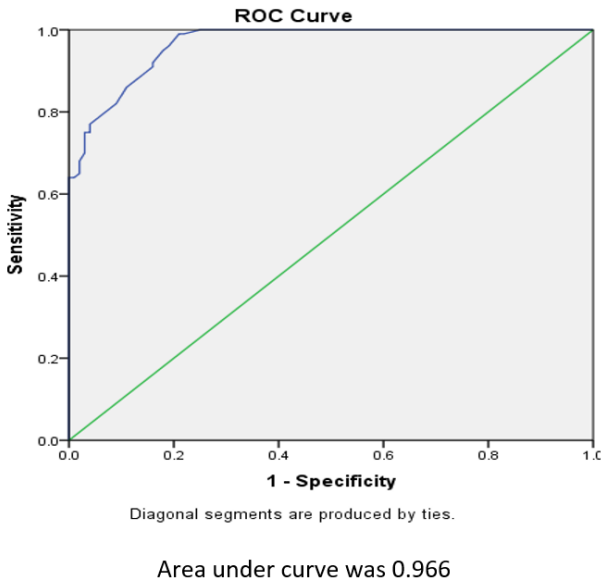

Fig. 1: Receiver operating characteristic curve for serum ADA in diagnosis of TB (Group I & II)

Subjects (group III & IV) (p-value <0.001). However, no significant relationship was observed between pulmonary & extrapulmonary TB groups (p-value = 0.222). Although mean ADA level of non-TB respiratory infection group (Group III) is higher than mean ADA value of healthy subjects (Group IV), the difference is not significant (p-value = 0.509).

The cutoff value of serum ADA activity for the diagnosis of TB established by the ROC curve method was 21.8

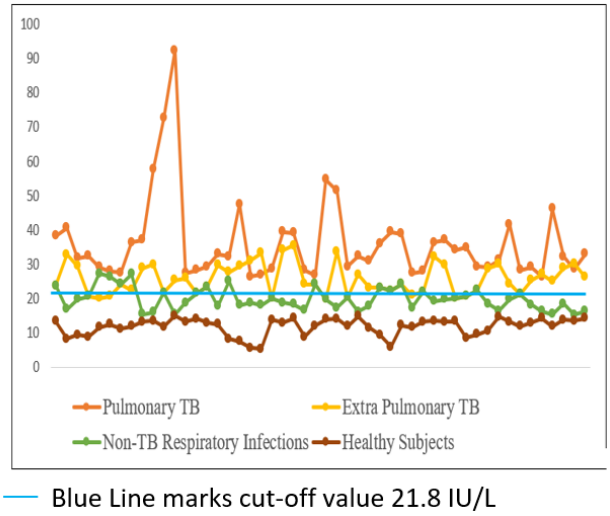

Fig. 2: Serum ADA values (Y-axis) of pulmonary TB (I), extrapulmonary TB (II), non-TB respiratory infections (III) & healthy subjects (IV)

IU/L (Figure 1). 88% of patients (88 out of 100) with active pulmonary & extrapulmonary TB had serum ADA values above cutoff level. 26% of patients (13 out of 50) with other respiratory illness had serum ADA values more than cutoff level while none of the healthy subjects showed serum ADA values above the cutoff level (Figure 2). The sensitivity of serum ADA for diagnosing TB was 88% & the specificity was 87%. The calculated positive predictive value (PPV) was 87.13% & negative predictive value (NPV) was 87.88%.

5. Discussion

Despite all the huge advances in the diagnosis & treatment of TB, the disease is still a major health problem in many parts of the world, especially in developing countries. Lack of timely TB diagnosis is the major cause of failure in managing the disease. Although the standard diagnosis of TB is based on isolation of M.tb in culture or direct observation of acid fast bacilli in ZN-stained sputum smear[15] yet, other diagnostic methods with shorter duration & acceptable sensitivity & specificity are necessary. Rapid diagnostic methods of TB by molecular techniques like PCR & CBNAAT[16–18] is not available in low-income areas due to the high cost of these methods.

There is need to practice such tests which determine the biological markers of TB infection such as ADA levels. ADA is an enzyme in the purine salvage pathway that catalyzes the conversion of adenosine & deoxyadenosine to inosine & deoxy-inosine with the release of ammonia.[19,20] Monocyte or macrophage activation by intracellular infections & inflammatory diseases leads to the release of ADA.[21,22] Therefore, its increased concentration

may be found in fluids & serum present in zones of TB infection, which can be used in diagnosis.[23] The importance of ADA activity measurement in pleural, peritoneal, synovial, cerebrospinal fluids has already been proven for the diagnosis of pulmonary & other extrapulmonary TB.[24,25] Piras MA et al. were first to report high ADA in tubercular pleural effusion.[26] However very limited studies are available for ADA levels in serum. The ease of venipuncture over access to other body fluids is going to be more helpful. Also, there is no such data available regarding any research on the diagnosis of TB using serum ADA level in central India.

In our study, mean serum ADA in patients with pulmonary & extrapulmonary TB is significantly higher than other patient group with respiratory illness (p-value <0.001). The results are concurrent with Agarwal MK et al.,[27] Afrasiabian S et al.,[28] Dilmac A et al.[29], Salmanzadeh S et al.,[30] Atta S et al.[31] and Al-sham-mary FJ.[32] This finding is in contrast with the study by Conde MB et al.[33] which shows that the mean serum ADA value among patients with active TB is similar to patients with other respiratory diseases (p-value =1.0).

Our results also showed that mean serum ADA in patients with pulmonary & extrapulmonary TB is clearly higher than that of healthy subject group (p-value <0.001). This finding is consistent with studies of Agarwal MK et al.,[27] Afrasiabian S et al.,[28] Badade ZG et al.,[34] Chander A et al.,[35] and Titarenko OT et al.[36] Bolursaz MR et al.[37] believed that although serum ADA level in pulmonary TB patients is higher than in normal individuals, ADA should not be considered as a suitable marker for differentiation between pulmonary TB & other pulmonary infections.

The current study showed high sensitivity (88%) & specificity (87%) of Serum ADA for diagnosing TB at cutoff value of 21.8 IU/L. From the above it is evident that estimation of serum ADA is reliable & useful test for diagnosis of TB. The area under ROC curve was 0.966 which shows that serum ADA can differentiate TB from other respiratory illnesses. A study by Agarwal MK et al.[27] shows 98% sensitivity & 100% specificity at serum ADA level of 33 IU/L. In Hassanein K et al.[38] study with an ADA level of 26.2 IU/L, sensitivity & specificity were 95% & 83.3% respectively. Another study by Afrasiabian S et al.[28] also showed high sensitivity (92.7%) & specificity (88.1%) for TB diagnosis at serum ADA cutoff value of 14 IU/L. This observation is in contrast with the study by Salmanzadeh et al.,[30] Conde MB et al.,[33] Farazi A et al.[39] that showed low sensitivity of serum ADA assay for differentiating TB from other respiratory illnesses & thus disapproved its utility for TB diagnosis.

Such differences are may be due to disease severity, immune status, genetic differences & accuracy of doing laboratory examination. There are some limitations of our study. This study involves small number of patients of single geographical area & study was the first of its kind in the region to evaluate the adequacy of serum ADA in diagnosis of TB & other respiratory illness. Therefore, conclusion based on one study should be accepted with caution. Some other diseases of cell mediated immunity like Typhoid fever, Infectious Mononucleosis, Rheumatoid arthritis, liver diseases are expected to be associated with high serum ADA levels.[32] We have excluded these diseases based on clinical presentation & not on the basis of specific diagnostic test. Therefore, further controlled studies with larger group are recommended.

6. Conclusion

According to this study, serum ADA level with high sensitivity & specificity may be proposed as reliable & useful index for diagnosis of TB, but the findings should be correlated with clinical presentation of disease. Serum ADA can also be used to differentiate TB from other respiratory illnesses. Thus, it can be included in routine diagnosis & prognosis of tuberculosis. However, further validation with larger studies must be done.

7. Source of Funding

None.

8. Conflict of Interest

None.

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Cite this article: Tadas AK, Meshram KW, Agarwal SB, Lalla P. Role of serum adenosine deaminase (ADA) in the diagnosis of tuberculosis & other respiratory diseases. *Int J Clin Biochem Res* 2023;10(3):242-246.