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Original Research Article

The predictive role of combined adenosine deaminase activity in Serum and body fluids to diagnose extrapulmonary tuberculosis

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ABSTRACT

Background: EPTB comprises 10-15% of all TB cases in developing countries. Diagnosis of TB from body fluids like pleural, peritoneal and cerebrospinal fluid (CSF) is challenging as all these fluid samples possess very few bacilli.
Aim: To determine the role of adenosine deaminase (ADA) assay for reliable prediction of EPTB from different body fluids, particularly in low-resource areas with high disease prevalence.
Materials and Methods: This prospective study was out in a rural medical college hospital. An enzymatic deamination method in a kinetic manner was used to monitor the ADA activity. The study processed 100 serum samples from 50 Suspected TB patients and 50 from the control group and 100 samples of body fluids from 50 Suspected TB patients and 50 control samples. Data were recorded in MS Excel sheets, and statistical analysis was performed using MS Office software.
Results: Out of 50 serum samples from the suspected TB patient and control groups, 48 (96%) and 17 (34%) were positive for ADA, respectively. Out of 50 samples of body fluids obtained from both suspected TB patients and the control group, 16 (32%) and 3 (06%) were positive for ADA, respectively. Serum ADA positivity was significantly high in suspected TB patients as compared to the control group
Conclusions: In our study, observations suggest that serum and serosal fluid Adenosine deaminase (ADA) measurement has good prediction potential for EPTB. Hence, it can be used as a supportive surrogate marker for challenging to diagnose extrapulmonary TB. ADA activity in body fluids is also a sensitive biomarker, especially when combined with serum ADA levels and may become a routine investigation for early detection of extrapulmonary TB.
Key Messages: Serum and serosal fluid Adenosine deaminase (ADA) measurements have good prediction potential for PTB & EPTB.

1. Introduction

Tuberculosis (TB) is a protean disease caused by Mycobacterium tuberculosis (M. tuberculosis). Usually, TB patients present with pulmonary manifestations. Nevertheless, extrapulmonary tuberculosis (EPTB) is not uncommon in TB-endemic countries like India and other south-east Asian countries. EPTB comprises 10-15% of all TB cases in developing countries.[1,2] Diagnosis of TB from body fluids like pleural, peritoneal and cerebrospinal fluid (CSF) is challenging as all these fluid samples possess very few bacilli. Conventional methods like microscopy and culture are widely used for diagnosis, but the sensitivity of AFB (Acid-fast bacilli) smear is only 5-20%, and culture takes about three to six weeks which may prolong the initiation of treatment.[3–6]

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Owing to this fact, newer tests have been developed like antigen-antibody detection, antibody in lymphocyte supernatant (ALS) assay, cellular IFNγ release assays (IGRAs) and T-Spot.[7,8] However, the reliability of these tests in diagnosing active TB disease is not proven yet. With the advent of molecular technology, polymerase chain reaction (PCR) has been developed to detect M. tuberculosis rapidly. PCR can reliably detect a very low concentration of organisms in extrapulmonary samples.[9–11] However, the requirement of dedicated laboratory areas, rigorous quality control, and the high test cost limits its routine use in resource-poor countries.

In this regard, our study aimed to study the role of adenosine deaminase (ADA) assay for reliable prediction of EPTB from different body fluids, particularly in resource-poor areas and where the disease is prevalent.[12–15]

2. Materials and Methods

The study was conducted from October 2018 to November 2019 in the Microbiology department of a tertiary care rural Hospital, utilizing serum samples and body fluids like Pleural fluid, ascitic fluid, CSF, Urine and pericardial fluid received in the microbiology laboratory from all clinically suspected extrapulmonary tuberculosis patients. Serum samples and body fluids like Pleural fluid, ascitic fluid, CSF, Urine and pericardial fluid were collected from randomly selected patients suffering from other confirmed non-tuberculosis diseases like cancer, cirrhosis, post pneumonic effusions, pyogenic meningitis, and peritonitis were used as controls.

2.1. Ethical consideration

Ethical clearance from the Institutional Ethical Review Committee was taken and informed consent from the patients was waivered by an ethical committee before commencing the study.

2.2. Specimen preparation

2.2.1. Serum/ CSF/ Body Fluid

No special preparation of the patient is required prior to sample collection by approved techniques.

ADA is reported to be stable in Serum for three days at 2-8°C and in biological fluids for two days at 2-8°C, as after this, ammonia may be released in the samples even without any microbial contamination. Hence all samples were processed on the same collection day to prevent false negative results.

2.3. Estimation of ADA levels

2.3.1. Principle

ADA Assay is based on the enzymatic deamination of adenosine to Inosine. Purine nucleoside phosphorylase (PNP) enzyme later converts it to Hypoxanthine. Furthermore, Hypoxanthine gets converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD). In the presence of peroxidase (POD), H_2O_2 is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methyl aniline (EHSPT) and 4-amino antipyrine (4-AA) to generate quinone dye which is thereby monitored in a kinetic manner.

Below, the entire enzymatic reaction scheme is shown.

$$Adenosine + H_2O \xrightarrow{ADA} Inosine + NH_3$$

$$Inosine + Pi \xrightarrow{PNP} Hypoxanthine + Ribose-1-phosphate$$

$$Hypoxanthine + 2H_2O + 2O_2 \xrightarrow{XOD} Uric\ acid + 2H_2O_2$$

$$H_2O_2 + 4-AA + EHSPT \xrightarrow{POD\ dry} 2H_2O + Quinone\ (\lambda\ max\ 556nm)$$

One unit of ADA is defined as the amount of ADA that generates one μmole of Inosine from adenosine per min at 37°C.

2.3.2. Reagent

Microxpress ADA-MTB is a reagent for laboratory use only.

ADA-MTB comprises of:

1. L1 - ADA-MTB Reagent - Buffer Reagent, ready to use.
2. L2 - ADA-MTB Reagent - Adenosine Reagent, ready to use.
3. L3 - ADA-MTB Reagent - Phenol Reagent.
4. L4 - ADA-MTB Reagent - Hypochlorite Reagent.
5. L5 - ADA-MTB Standard - ADA Standard, ready to use.

Specimen preparation: No special preparation of the patient is required prior to sample collection by approved techniques

ADA is reported to be stable in Serum for three days at 2-8°C and in biological fluids for two days at 2-8°C, as after this, ammonia may be released in the samples even without microbial contamination.

2.4. Test procedure

1. Pipette into clean, dry test tubes labelled Blank (B), Standard (S), Sample Blank (SB) and Test (T), as shown in Table 1.
2. Mix well and incubate at 37°C for precisely 60 minutes, and then follow Table 2.
3. Mix well and incubate at 37°C for 15 minutes or at RT for 30 minutes.
4. Measure the absorbance of the Blank (Abs. B), Standard (Abs. S), Sample Blank (Abs. SB) and Test (Abs. T) against distilled water.

Table 1: Additionsequence of reagents

Addition sequence	B (ml)	S (ml)	SB (ml)	T (ml)
Buffer reagent	0.20	0.20	-	-
Adenosine Reagent	-	-	0.20	0.20
Deionized water	0.02	-	-	-
Standard	-	0.02	-	-
Sample	-	-	-	0.02

Table 2: Addition sequence of reagents

	B (ml)	S (ml)	SB (ml)	T (ml)
Working Phenol Reagent	1.00	1.00	1.00	1.00
Sample	-	-	0.02	-
Working Hypochlorite Reagent	1.00	1.00	1.00	1.00

2.5. Calculations

$$Total\ ADA\ activity\ in\ U/L = \frac{Abs.T - Abs.SB}{Abs.S - Abs.B} x\ 50$$

Table 3: Reference values:

Sample	Interpretation	Result
Serum, Plasma, Pleural, Pericardial and Ascitic Fluids	Normal	<30U/L
	Suspect	30U/L to 40U/L
	Strong Suspect	>40U/L - 60U/L
	Positive	>60U/L
CSF	Normal	<10U/L
	Positive	>10U/L

2.6. Details of data tabulation and statistical analysis employed

Data was recorded in MS Excel sheets, and statistical analysis was done using MS Office software.

3. Results

We included 50 suspected EPTB patient's Serum samples and body fluids as cases and compared them with 50 randomly selected patient's Serum samples and body fluids suffering from other confirmed non-tuberculosis diseases

The age wise distribution of 100 samples was 51-60yrs (27%), 31-40yrs(20%), 41-50yrs (18%), 20-30yrs (13%) and 61-70yrs (13%) and 71-90yrs (9%). Concerning gender, 75% of the patients in this study were males and 25% were females.

ADA activity in both cases and control was compared, and it was found that 48(96%) serum samples and 16 (32%) body fluid samples obtained from the suspected EPTB patient group were positive for ADA. In comparison, 17 (34%) serum samples and 3(06%) body fluid samples in the control group showed ADA activity.

Out of 50 serum samples in the suspected EPTB patient group, 48 (96%) were positive, and out of 50 serum samples in the control group, 17 (34%) were positive for ADA enzyme. Out of 50 body fluid samples in the suspected EPTB patient group, 16 (32%) were positive, and out of 50 body fluids in the control group, 3 (06%) were positive for ADA enzyme.

In the suspected TB patient group, combined ADA activity in both Serum and body fluid samples was analyzed. It was found that 14 (28%) were positive for ADA in the same patient's serum and body fluids. However, 34(68%) of the sample were positive in Serum and no ADA activity in body fluids. No samples were negative for ADA activity in both Serum and body fluid. In the Control group, 30 (60%) samples were negative for ADA activity in both Serum and body fluid. However, 17(34%) samples were positive for only Serum ADA. 3(6%) samples showed ADA activity only in body fluids, as shown in Figure 1.

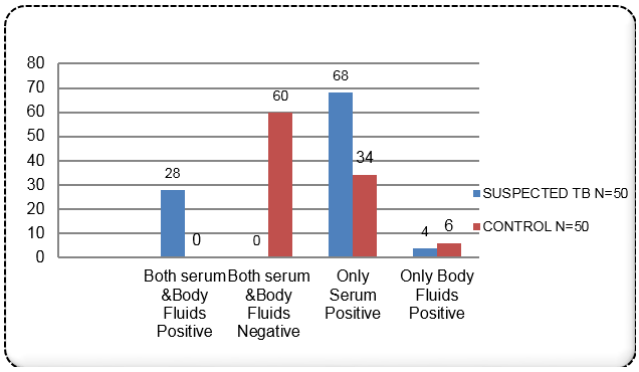

Fig. 1: Combined ADA Activity in both serum and body fluid

In the suspected TB group, mean ADA values in Serum were 34.1 and 86.7 in body fluids, whereas in the control group, mean ADA serum levels were 17.3 and 42 in body fluids, as shown in Figure 2.

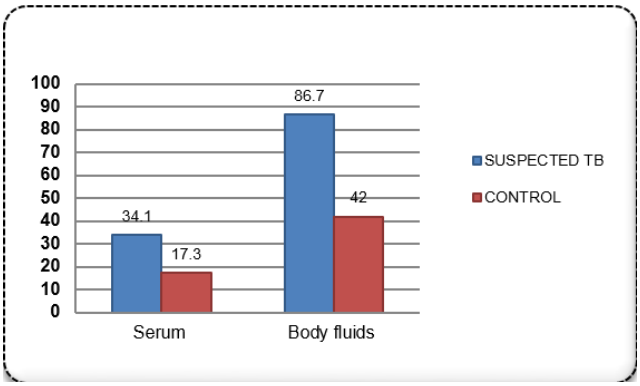

Fig. 2: Mean ADA levels in serum and Body fluids in both study groups

Table 4: Number of samples positive for adenosine deaminase enzyme

	Suspected EPTB N=50		Control N=50	
	Serum N(%)	**Body Fluid N(%)**	**Serum N(%)**	**Body Fluid N(%)**
Positive	48(96)	16(32)	17 (34)	3(6)
Negative	2(4)	34(68)	33(66)	47(94)

Table 5: Predictive value of ADA activity in only Serum, only Body fluid,d and Combined

Specimen type	**Serum**		**Body Fluids**		**Serum & Body Fluids**	
Result	**Test group (50)**	**Control group (50)**	**Test group (50)**	**Control group (50)**	**Test group (50)**	**Control group (50)**
ADA Positive	34	17	2	3	14	0
ADA Negative	16	33	48	47	0	30
PPV	66.67%		40.00%		100%	
NPV	67.35%		49.47%		100%	

3.1. Predictive value of the test

It was observed that both Positive Predictive value (PPV) and Negative Predictive value (NPV) for ADA detection were low when only either Serum (66.67% & 67.35%) or body fluid (40% & 49.47%) was considered for the diagnosis of EPTB. The PPV and NPV were 100% when a combined ADA activity in Serum and body fluids were considered to diagnose EPTB, as shown in Table 5.

4. Discussion

Tuberculosis is one of the most common infectious bacterial diseases and continues to threaten human health worldwide. Tuberculosis can be classified as pulmonary, extrapulmonary and disseminated tuberculosis. However, the mycobacterium culture of the serous fluid specimens has a relatively lower success rate; thus, its role in diagnosing extrapulmonary TB is still controversial. Due to the non-specific clinical manifestations and negative laboratory findings, it is challenging to diagnose extrapulmonary TB. Consequently, it is imperative to develop a reliable biomarker that can be used to rapidly and accurately diagnose extrapulmonary TB. The efficacy of ADA activity was studied as a biomarker for the diagnosis of extrapulmonary TB.

In our study of the group of suspected TB patients, we found that ADA activity was higher in Serum (96%) as well as body fluids (32%). Compared to the study group, the control group showed significantly low levels of ADA positivity in both Serum (32%) and body fluids (6%). These findings in our study are the first of their kind, as no literature is available for comparison.

Our present study observed that serum ADA positivity was significantly high 34(68%). ADA activity in body fluid alone was at least 2(4%). An additional eight samples were diagnosed when both Serum and ADA were considered for diagnosis. The predictive value of the test also rose to 100% when combined ADA activity was considered for the diagnosis of EPTB. These observations are the first time observed in our study

Mean serum ADA levels were significantly higher in suspected TB patients (34.1) than in our study's control group (17.3). These observations are in concordance with other studies done by Prashant C et al., 2017 [16] (37.12 and 15.7 respectively) and Abhijit Ninghot et al., 2019 [17] (43.67 and 14.97 respectively)

Mean body fluid ADA levels were significantly higher in suspected TB patients (86.) than in the control group (42) in our study. Similar observations were made in other studies done by Sibel Yurt et al. 2014 [18] (87.6 and 40.11 respectively) and Poonam Nanwani et al. 2018 [19] (85.97 and 39.33 respectively)

5. Conclusion

In the present scenario of the increasing incidence of tuberculosis worldwide, particularly in developing countries, has created a need for cheaper, less time-consuming and more effective diagnostic techniques. Though X-ray and acid-fast staining are the two most common tuberculosis diagnosis methods in the developing world, these are less effective in diagnosing paucibacillary and extrapulmonary tuberculosis. Our observations suggest that serum and serosal fluid Adenosine deaminase (ADA) measurement has good prediction potential for EPTB. Hence it can be used as a supportive surrogate marker for challenging to diagnose extrapulmonary TB. ADA activity in body fluids is also a sensitive biomarker, especially when combined with serum ADA levels, and may become a routine investigation for early detection of extrapulmonary TB.

6. Limitations of the study

ADA may substantially reduce invasive tests like biopsy and culture; however, further studies are required at different locations and over different populations to generalize this study's results.

7. Source of Funding

None.

8. Conflicts of Interest

None.

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