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DIAGNOSTIC EVALUATION OF ADENOSINE DEAMINASE ASSAY TEST IN THE DIAGNOSIS OF EXTRAPULMONARY TUBERCULOSIS AT TERTIARY HEALTH CARE CENTRE, BHAVNAGAR.

Clinical Microbiology

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ABSTRACT

Aim: To evaluate the sensitivity and specificity of Adenosine Deaminase (ADA) to diagnose extrapulmonary tuberculosis. Study design: This is a Retrospective study. **Place and duration of study:** This study was conducted in the Serology section of the Microbiology Laboratory at Sir Takhtsinhji Hospital, Bhavnagar from July 2021 to December 2021. **Methodology:** Out of 75 samples of suspected Extrapulmonary Tuberculosis, 17 Cerebrospinal fluid, 38 Ascitic fluid, and 20 Pleural fluid samples in a sterile vial for Adenosine deaminase Assay were received and labelled with a laboratory identification number and tested on the same day. Adenosine deaminase activity in all the collected samples was determined using a Diazyme Adenosine Deaminase assay kit that determined ADA level by kinetic enzymatic method on a Semi-automated analyzer (Erba Chem-5 plus v2). Adenosine deaminase Assay which is based on enzymatic deamination of adenosine to inosine. **Result:** Out of 17 cases of suspected Tubercular Meningitis cases, ADA was raised in 9 cases. In contrast, out of 38 suspected Tubercular Peritonitis cases and 20 suspected Tubercular Pleural Effusion cases, ADA was raised in 9 and 11 cases, respectively. Sensitivity of ADA was 100%, 85.71% and 90.90% and specificity of ADA was 88.88%, 90.30%, and 88.88% for the detection of Tubercular meningitis, Tubercular peritonitis, and Tubercular pleural effusion respectively. The overall sensitivity of ADA in this study was 92.30%, and specificity 89.79% in extra-pulmonary disease. **Conclusion:** Adenosine deaminase test is a simple to perform, inexpensive, less time-consuming, specific, and sensitive method to help the physician to make an early diagnosis of Extra-pulmonary tuberculosis, where an expensive laboratory diagnostic setting is not required.

KEYWORDS

INTRODUCTION

One of the most prevalent infectious diseases in the world is tuberculosis (TB). Almost one-third of the world's population is thought to have been infected with Mycobacterium tuberculosis bacillus, with new infections occurring at a rate of about one per second and one of them dies every 10 seconds. Tuberculosis in human beings is usually caused by Mycobacterium tuberculosis which is a part of complex organisms including Mycobacterium Bovis and Mycobacterium Africanum.[1] Tuberculosis is a leading cause of morbidity and mortality in developing countries Especially in low socio-economic status. According to the World Health Organization (WHO) (2020), there are around 10 million TB cases globally. Of them, 1/4th of the cases is reported from India accounting for approximately 2.6 million cases. [2,3] In India, extra-pulmonary tuberculosis (EPTB) burden 15 to 20 percent of all types of TB, in comparison to 25 percent in France and 50 percent in Canada, partly due to the dual infection of TB with human immunodeficiency virus (HIV). [4]

EPTB is not uncommon. Pulmonary tuberculosis is the most common clinical presentation; however, it may affect many other parts of the body. The most common sites affected in EPTB are lymph nodes which are followed by bone and joints, pleura, urogenital tract, and meninges.[5] which may manifest as lymph adenopathy, osteo-articular tuberculosis, tubercular pleural effusion, tuberculous meningitis, and tuberculosis peritonitis. Antitubercular Drugs can be fully cured EPTB, but the major problem is the late diagnosis of the disease due to the most of tests developed for the diagnosis of EPTB are not sensitive and may not be affordable for routine use which leads to delays in the initiation of anti-tubercular medication in suspected EPTB. A reliable and rapid diagnostic test could be of help in the diagnosis of EPTB. [6,7]

Diagnosis of EPTB is a challenge because it presents with varied features which may mimic malignancy. For diagnosis of EPTB, direct or indirect methods are available. Direct methods include the microscopic demonstration of TB bacilli, culture, and molecular methods. Indirect methods involve the detection of humoral or cell-mediated immune response of the host to mycobacterium antigen or detection of biomarkers like Adenosine Deaminase (ADA), Interferon (IFN), etc. [8]

Culturing of Mycobacterium tuberculosis is the gold standard method for the diagnosis of EPTB. But it has been certain disadvantages such as high cost, poor resource settings, and delay in getting the result i.e., 6-8 weeks on solid media like Lowenstein-Jensen media and 3 - 4 weeks on liquid media like Middlebrook 7H9 broth because of Mycobacterium spp. grows very slowly. [9] Determination of susceptibility to drugs can add another three to six weeks to the process which leads to a delay in the initiation of anti-Tubercular medication in suspected Tuberculosis patients. Meanwhile, the disease may progress and results in a poor prognosis.

Immunological methods like Tuberculin skin test (Mantoux test) or Interferon-gamma release assay (IGRA) are done to rule out Tuberculosis infection but negative results cannot exclude the possibility of extrapulmonary forms of Tuberculosis, since it has been reported that up to 68% of cases may present negative results. Real-Time Polymerase Chain Reaction based method like the Cartridge-based Nucleic Acid Amplification Test (CBNAAT) or Gene-Xpert Mycobacterium tuberculosis/rifampicin (MTB/RIF) has a high diagnostic yield and give rapid result within 2 hours and it is also helpful in detecting rifampicin resistance. [10] But it is expensive and is not found to be more sensitive to pleural fluid.

In a resource-poor country like India, it is necessary to develop a test that is rapid, cost-effective, and easy to perform which may reduce morbidity and mortality associated with EPTB. Many studies have proven the high sensitivity and specificity of biochemical parameters like Adenosine Deaminase (ADA) level in body fluids especially pleural fluid for early diagnosis of extrapulmonary TB and smear-negative TB. [11,12]

ADA is an essential enzyme involved in the purine salvage pathway. This enzyme is produced in all cells, but the highest levels of adenosine deaminase occur in immune system cells called T-lymphocytes, which develop in lymphoid tissues. ADA is required for differentiation as well as the proliferation of these cells. [13] It increases in biological fluids in the course of infectious disease caused by micro-organisms infecting the macrophages. It has been shown that mycobacterial antigen stimulates the production of ADA in lymphocytes and monocytes. ADA production increase by T-lymphocytes because of the stimulation by a mycobacterial antigen. [14] In EPTB, it can be accessed easily in body fluids such as Cerebrospinal fluid (CSF), Pleural fluid, and Ascitic fluid. So, ADA has been a surrogate marker for EPTB. ADA has been developed and widely used for the diagnosis of TB because it is a single test that is at the same time inexpensive and easy to perform. [15,16] ADA can be used as a prognostic test for all forms of TB.

The present study was planned to investigate the diagnostic value of ADA in CSF, Pleural fluid, and Ascitic fluid in presumptive cases of Extrapulmonary Tuberculosis like Tubercular pleural effusion [TPE], Tubercular meningitis [TBM], and Tubercular peritonitis [TP]. In this study, our main goal is to evaluate the sensitivity and specificity of ADA in Extra-pulmonary tuberculosis patients.

METHOD:

This retrospective study was conducted in the Serology section of the Microbiology Laboratory at Sir Takhtsinhji Hospital, Bhavnagar, Gujarat, India from July 2021 to December 2021. The present study was conducted on presumptive cases of Extrapulmonary Tuberculosis (EPTB) like Tubercular meningitis [TBM], Tubercular pleural effusion [TPE], and Tubercular peritonitis [TP] according to a detailed history, clinical symptoms, physical examination, sputum smear examination for AFB, radiological investigation, biochemical finding such as elevated protein level, decreased glucose level, lymphocytic predominance.

Out of the total 75 samples of suspected EPTB patients, 17 Cerebrospinal fluids, 20 Pleural fluids, and 38 Ascitic fluids in sterile vials for ADA was received and labelled with a laboratory identification number and tested on the same day.

In this study, ADA in all the collected samples was determined using a Diazyme Adenosine Deaminase assay kit (Diazyme Laboratories, 12889 Gregg Court Poway, CA 92064, USA) that determine ADA level by a kinetic enzymatic method which is one of the modified *GIUSTI* method performed in Semi-automated analyzer (Erba Chem-5 plus v2).[17] The enzymatic deamination of adenosine to inosine, which is then transformed to hypoxanthine by the enzyme purine nucleoside phosphorylase, is the foundation of ADA. Xanthine oxidase then transforms hypoxanthine into uric acid and hydrogen peroxide (H2O2). H2O2 is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase to form quinone dye that is kinetically observed.

The quantity of ADA needed to produce one mole of inosine per minute from adenosine at 37 °C is referred to as one unit of ADA.The result is expressed as units per litre per minute (U/L/min). The reference values of ADA for the diagnosis of Tuberculosis in Cerebrospinal fluid samples are 10 U/L and in Pleural fluid & Ascitic fluid are 60 U/L.

RESULT:

Table 1: shows Adenosine Deaminase (ADA) positivity in various body fluids to detect extrapulmonary tuberculosis.

Test	Cerebrospinal Fluid	Ascitic Fluid	Pleural Fluid	Total Sample
ADA Positive	9	9	11	29
ADA Negative	8	29	9	46
Total Sample	17	38	20	75

Table 2: shows Adenosine Deaminase (ADA) positivity in reference to Cartridge-based Nucleic Acid Amplification Test (CBNAAT) in Cerebrospinal fluid.

ADA Test Cut off	CBNAAT Positive	CBNAAT Negative	Total Sample
>10U/L (ADA Positive)	8	1	9
<10U/L (ADA Negative)	0	8	8
Total Sample	8	9	17

In this study out of 9 ADA positive patients, 8 patients were found to be CBNAAT positive while 1 patient was found to be CBNAAT negative. Out of 8 ADA negative patients, all 8 patients were found to be CBNAAT negative.

Table 3: shows Adenosine Deaminase (ADA) positivity in reference to Cartridge-based Nucleic Acid Amplification Test (CBNAAT) in Ascitic fluid.

ADA Test Cut off	CBNAAT Positive	CBNAAT Negative	Total Sample
>60U/L (ADA Positive)	6	3	9
<60U/L (ADA Negative)	1	28	29
Total Sample	7	31	38

In this study out of 9 ADA positive patients, 6 patients were found to be CBNAAT positive while 3 patients were found to be CBNAAT negative. Out of 29 ADA negative patients, 1 patient was found to be CBNAAT positive while 28 patients were found to be CBNAAT negative.

Table 4: shows Adenosine Deaminase (ADA) positivity in reference to Cartridge-based Nucleic Acid Amplification Test (CBNAAT) in Pleural fluid.

ADA Test Cut off	CBNAAT Positive	CBNAAT Negative	Total Sample
>60U/L (ADA Positive)	10	1	11
<60U/L (ADA Negative)	1	8	9
Total Sample	11	9	20

In this study out of 11 ADA positive patients, 10 patients were found to be CBNAAT positive while 1 patient was found to be CBNAAT negative. Out of 9 ADA negative patients, 1 patient was found to be CBNAAT positive while 8 patients were found to be CBNAAT negative.

Table 5: shows sensitivity and specificity of Adenosine Deaminase (ADA) and Cartridge-based Nucleic Acid Amplification Test (CBNAAT) in various body fluids to diagnose extrapulmonary tuberculosis.

Extra pulmonary Tuberculosis	Test	Sensitivity	Specificity
Tubercular meningitis	ADA	100%	88.88%
	CBNAAT	88.88%	100%
Tubercular peritonitis	ADA	85.71%	90.30%
	CBNAAT	66.66%	96.55%
Tubercular pleural effusion	ADA	90.90%	88.88%
	CBNAAT	90.90%	88.88%
Overall	ADA	92.30%	89.79%
	CBNAAT	82.75%	95.65%

DISCUSSION:

Most developing countries have a higher prevalence and incidence of TB and lack well-equipped laboratory services for the proper diagnosis of TB. Assessment of various body fluids depending upon the involvement of the site for detection of EPTB was identified as an important tool for predicting EPTB. The body fluid may show atypical features but the absence of these features does not rule out EPTB. Definitive diagnosis of TB includes a demonstration of the presence of Mycobacterium tuberculosis by microbiological, cytological, or histopathological methods. These methods of TB diagnostics have significant limitations for the diagnosis of EPTB.

Molecular methods like cartridge-based nucleic acid amplification test (CBNAAT) and chip-based NAAT (TRUENAT) are available for the rapid detection of EPTB but it requires costly instrumentation and trained staff.

In the present study, out of 17 CSF samples, 9(64.28%) samples were showing Adenosine deaminase activity and 8 samples were not showing Adenosine deaminase activity. Out of 38 ascitic fluids, 9(23.68%) samples were ADA positive and 29 samples were ADA negative while out of 20 samples of pleural fluid, 11(55%) samples were ADA positive and 9 samples were ADA negative. **(Table-1)**

In the present study **(Table-5),** for diagnosis of tuberculosis meningitis [TBM] the cut-off level of ADA was >10 U/L with a sensitivity of

100%, and specificity of 88.88%. The positive predictive value of the ADA test is 88.88% and the negative predictive value is 100%. The study by Rajendra prasad et al calculated 100% sensitivity and 97.87% specificity of the ADA test for the diagnosis of TBM.[18] Kashyap et al take the cut-off value of 11.39 U/L and obtained a sensitivity of 82% and specificity of 83% in TBM cases. [19] Rana et al take 10 U/L as the cut-off value for the diagnosis of TBM and found a sensitivity of 66.6% and a specificity of 90%.[20]

The cut-off level of ADA for the detection of tubercular peritonitis [TP] was >60 U/L with a sensitivity of 85.71%, and specificity of 90.32%. **(Table-5)** The positive predictive value of the ADA test is 66.66% and the negative predictive value is 96.55%. The study by Hortiwakul et al calculated 84.8% sensitivity and 82.6 % specificity of the ADA test at the cut-off value of 22 U/L [21], by Dwivedi et al. suggested sensitivity of 100% and specificity of 96.6% at an ADA cut off level >33 U/L. [22] and Gupta et al. suggested a sensitivity of 95% and specificity of 94.1% at an ADA level >30 U/L. [23] for the diagnosis of TP.

For diagnosis of tubercular pleural effusion [TBPE], the cut-off level of ADA was >60 U/L with a sensitivity of 90.90%, and a specificity of 88.88%. **(Table-5)** The positive predictive value of the ADA test is 90.90% and the negative predictive value is 88.88%. The study by Burgess et al calculated 90% sensitivity and 89% specificity of the ADA test at the cut-off value of 50 U/L [24], by Strankinga et al. suggested a sensitivity of 100% and specificity of 87% at the cut-off value of 53 U/L. [25] and Farhana et al suggested a sensitivity of 95 % and specificity of 83.3% at a cut-off value of 40 U/L [26] for the diagnosis of TBPE.

In extra-pulmonary disease, the overall sensitivity of ADA in this study was 92.30%, and specificity 89.79%. **(Table-5)** In Stevanovic G et al study, in extra-pulmonary disease, overall sensitivity was found to be 94.29%, and specificity 92.16%. [27] Many studies have confirmed the high sensitivity and specificity of ADA (sensitivity 92% and specificity 89%) for early diagnosis of EPTB, such as tuberculous pleuritis, ascites, and meningitis. [28]

In this study for Diagnosis of TBM, TP, and TBPE, the sensitivity of CBNAAT is 88.88%, 66.66%, and 90.90% respectively and the specificity of CBNAAT is 100%, 96.55%, and 88.88% respectively. Where in this study we found that the overall sensitivity and specificity of CBNAAT are 82.75% and 95.65% respectively. **(Table-5)**

This study suggested that the sensitivity of ADA was higher as compared to CBNAAT whereas the specificity of CBNAAT was higher for the detection of TBM and TP. The sensitivity and specificity of ADA and CBNAAT were the same for the detection of TBPE. Overall, the sensitivity of ADA was higher but the specificity of CBNAAT was higher for all the EPTB.

CONCLUSION:

This study concludes that Adenosine deaminase assay (ADA) levels were significantly higher among Extrapulmonary Tuberculosis (EPTB) cases, especially in Tubercular pleural effusion, tubercular meningitis, and tubercular ascites. ADA assay has high sensitivity so it can be performed in healthcare centres where expensive laboratory diagnostic facilities for culture and PCR are not available.

In case of negative AFB smear from the body specimens, the ADA test is simple to perform, inexpensive, less time consuming, specific and sensitive method to help the physician make an early diagnosis of EPTB. Prompt treatment of TB is crucial, especially in India, where it is a high-burden TB area so, early diagnosis is essential to start Antitubercular treatment for an effective Tuberculosis control program to decrease morbidity and mortality due to EPTB.

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Nil

Conflict of Interest:
The authors declare no conflict of interest.

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