

Utility of ADA (Adenosine Deaminase) enzyme assay in diagnosis of tuberculous meningitis

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Abstract

Introduction: Tuberculous meningitis (TBM) remains one of the leading causes of morbidity and mortality worldwide, including India. Treatment and prevention of TBM is depended on early and quality assured diagnosis. Earlier studies have showed that there is wide delay in diagnosing TBM. ADA is essential for proliferation and differentiation of lymphoid cells, especially T cells. ADA activity increases during activation of the cell, for energy demand to detoxify the toxic metabolites generated. This enzyme increases in TB patients because of delayed hypersensitivity reaction to mycobacterial antigen.

Objective: To evaluate the diagnostic utility of the ADA assay for the diagnosis of tuberculous meningitis and to compare ADA results with results of Ziehl Neelsen stain, routine biochemical tests and cytology.

Materials and Method: Total 86 Cerebrospinal fluid (17 cases of TBM and 69 cases of non-TBM) were studied for ADA levels, ZN stain, routine biochemical tests and cytological evaluation.

Results: Mean level of ADA in TBM is 11.81 U/L and in non-TBM 1.65 U/L. Comparison between different tests for TBM shows: ZN stain is having high specificity of 100% but its sensitivity is 5.88%. Sensitivity of cytological analysis is 88.83% and specificity is 26.87%. Specificity of sugar test is only 34%. Protein estimation is also very less specific, 61%. ADA test is having both sensitivity (64.70%) and specificity (97%) higher than other tests.

Conclusion: As an alternative to subjecting CSF of suspected tuberculous meningitis to various tests (cytology, protein and sugar estimation and ZN stain), subjecting sample for single test, ADA test (very easy, sensitive, specific, cheap and less time consuming: one and half hour) is better option with good results.

Introduction

Tuberculosis remains one of the leading causes of morbidity and mortality worldwide accounting for approximately 10.4 million new cases and 1.4 million deaths annually. India is one of the six countries accounting for the 60% of the new cases. Although the number of TB death fell by 22% between 2000 to 2015, TB remained one of the top causes of death worldwide in 2015.⁽¹⁾

Care of patients with tuberculosis starts with a quality assured diagnosis. Early diagnosis and initiation of optimal treatment would not only enable the cure of an individual patient but also prevent the transmission of infection as well as the chances of emergence of drug-resistant strains.

Earlier studies showed extensive delay in TB diagnosis specific for TBM. Although different diagnostic methods are available, they all have some drawbacks. ZN stain for the acid-fast bacilli is the quick screening method for pulmonary TB diagnosis, so it is commonly performed rapid and specific test with sensitivity of 30 to 40%, its sensitivity decreases in case of all extra pulmonary TB, including TBM. Mycobacterium culture and sensitivity, which is the gold standard for TB diagnosis, but as the bacilli are slow growing, it takes 2 to 8 weeks to grow. Tuberculin skin test is false positive in one third of cases especially in vaccinated. Many genotypic tests based on nucleic acid amplification have been designed including Cobas amplicor test, Xpert gene, GenProbe amplified

Mycobacterium tuberculosis direct test, abott LCx test, Roche Amplicor MTB test and BD ProbeTec test. The polymerase chain reaction (PCR) test diagnosing TBM is costly and it requires skilled technician and many expensive equipments and well designed infrastructure. As a result, there is a great demand for searching a new diagnostic test for TBM, which should be quick and accurate.⁽²⁾

Adenosine Deaminase (ADA) enzyme is an enzyme, which catalyses the hydrolytic cleavage of adenosine and 2-deoxyadenosine and convert them into inosine and 2'-deoxyinosine respectively thus contributes in purine metabolism. ADA is necessary for proliferation and differentiation of lymphoid cells, especially T cells and helps in the maturation of monocytes to macrophages. ADA activity increases during cellular activation for energy demand to detoxify the toxic metabolites. Increase in ADA enzyme in TBM patients is possibly due to delayed hypersensitivity reaction to mycobacterial antigen. ADA is an index for cellular immunity. Previous studies have proved its value in TB diagnosis, even for assessing TBM.⁽³⁾ Advantage of ADA test are that it is an (1) inexpensive, (2) non-invasive, (3) less time consuming as well as (4) reliable marker.⁽⁴⁾

Hence the present study was conducted to evaluate the diagnostic utility of the ADA assay for the diagnosis of tuberculous meningitis and to compare ADA test results with results of ZN stain, biochemical tests and cytology.

Materials and Method

In this study, a total number of 86 cases were studied. Samples were collected from the department of TB & chest department, Medicine department, Surgery department and Obstetrics and Gynaecology department of PDU Medical College and Hospital, Rajkot from June, 2012 to July, 2013.

Patients were divided in two groups; one is tubercular meningitis group and second is non-tubercular meningitis group. Complete history with detail examination of patient done and recorded in the Proforma, which included age, sex, registration number, type & duration of disease, treatment history along with any predisposing factor like smoking, past history or family contact were noted.

The study was divided into following parts:

1. Collection and transportation of sample.
2. Sample subjected to various tests.
3. Analysis of test results and comparison between different tests

Sample Collection and Transportation: In case of meningitis CSF is collected by lumbar puncture, each patient's sample was collected in three vials. In one vial app 1 ml of CSF collected for ZN stain, gram stain and ADA testing. In second vial app 1 ml collected for cytological examination and in third vial app 1 ml collected for biochemical tests. Samples were transported aseptically to the laboratory in sterile, leak proof containers as early as possible to prevent contamination.

Processing of the Samples: First vial of CSF were first tested for ADA test and then centrifuged and used for ZN and gram staining. 2nd vial, without centrifugation, were used for cytological analysis and 3rd vial without centrifugation, were used for biochemical tests.

A. ADA analysis

Principle of the test: If adenosine deaminase enzyme is present in the specimen, it will hydrolyze adenosine to ammonia and inosine. Ammonia formed reacts with phenol and hypochlorite in an alkaline medium to form blue indophenol complex with sodium nitropruside acting as catalyst. Intensity of blue coloured indophenols complex formed is directly proportional to the amount of ADA present in the sample.

Adenosine + H₂O $\xrightarrow{\hspace{1cm}}$ Ammonia + Inosine
 Ammonia + Phenol + Hypochlorite $\xrightarrow{\hspace{1cm}}$ Blue
 Indophenol complex

Procedure: The ADA was analysed using a commercial colorimetric assay kit according to manufacturer's instructions. For each batch control, blank(B) and standard(S) were put and for each sample is tested by putting test (T1, T2, T3,...Tn) and standard blank (SB1, SB2, SB3.....SB n). Specimens without substrate were run in parallel (SB), to control for the ammonium present in the patient's specimen before addition of exogenous adenosine. The amount of ammonium ion released by the ADA reaction was

determined as absorbance (optical density, OD) at 620 nm wavelength.

Calculation: The activity in the patient's specimen was calculated with the formula: Activity in specimen = (OD specimen – OD specimen blank) / (OD standard – OD reagent blank) x 50 with the result expressed in U/L. In CSF, the ADA value of > 10 U/l is positive and < 10 U/l is considered as negative.

B. ZN Stain: CSF is centrifuged at 3000 rpm for 15min. Drop of concentrated CSF was placed on a clean microscope slide and spread evenly to make a smear. The smear was then air dried. After that a drop of methanol is used for fixation of the smear. Methanol is allowed to evaporate and slide was stained by standard ZN stain method. Slide was examined under oil immersion objective. Results were noted as POSITIVE (acid fast bacilli seen) or NEGATIVE: (acid fast bacilli not seen).

C. Cytology and Biochemical tests: Cytological analysis done by wet preparation and methylene blue preparation and presence of approximate number of leucocytes and percentage of various types of leucocytes were noted.

D. Biochemical analysis: Among biochemical tests sugar estimation is done by colorimetric method and protein estimation is done by turbidometric method.

Analysis of test results and comparison between different Tests: The results of the ADA assay and other tests against clinical confirmation of MTB were entered into 2X2 tables, where in the sensitivity was calculated by using the formula: TP/ (TP+FN), specificity by using the formula: TN/ (FP+TN); where TP = the true positives; FN= false negatives; FP= false positives and TN= true negatives. Finally comparison between different tests was done.

Results and Discussion

Out of 17 cases of Tuberculous meningitis, 12 were male and 5 were female, male to female ratio of 2.4:1 suggests that TBM is more common in male.

Mean level of ADA in tubercular meningitis is 11.81 U/L and in non-TBM is 1.65 U/L.

Table 1 shows results of ADA test; Out of 17 cases of tubercular meningitis, 11 specimens were positive in ADA test and 6 were negative in ADA test. So sensitivity of ADA test for TBM, calculated by formula, $a/(a+c)$, is 64.70%. Out of 69 cases of non-tubercular etiology, 2 specimens were positive in ADA test and 67 were negative in ADA test. So specificity of ADA test for TBM, calculated by formula, $b/(b+d)$, is 97.10%.

Table 2 shows results of ZN stain in meningitis; all 69(100%) CSF of non-tuberculous meningial diseases were AFB negative and 1 (5.88%) of the 17 CSF of tubercular meningitis was positive and 16 were negative. So sensitivity of ZN stain for tubercular meningitis is 5.88% and specificity is 100%.

Table 3 shows cytological report in meningitis; In TBM cases, lymphocytic predominance is seen in 15 cases and neutrophilic predominance is seen in 02. In non-tubercular pleural effusion group 50 cases were having lymphocytic predominance and 18 cases showed neutrophilic predominance. So sensitivity of cytological analysis for TBM is 88.23% and specificity is 26.47%.

Table 4 & 5 shows result of protein and sugar in CSF in meningitis; in tubercular group, protein is increased in 15 CSF and not increased in 02 pleural fluids. In non-tubercular group protein increased in 24 and not increased in 38. In tubercular group sugar is decreased in 01, normal in 12 and increased in 03. In non-tubercular group sugar is increased in 22, normal in 36 and decreased in 07. Which shows sensitivity and specificity of protein and sugar for tubercular meningitis is low. Protein estimation specific is 61.29% and Sugar test specificity 33.84%.

Fig. 1, shows comparison between sensitivity and specificity of different tests in CSF; ZN stain is having high specificity (100%) but its sensitivity is 5.88%, which is very low. Other tests including lymphocytic count and protein, sugar is not specific tests for tuberculosis. Lymphocytic predominance is seen in various viral diseases, malignancy, fungal diseases and many other conditions. ADA test is having sensitivity, 64.70% and specificity 97.10% in CSF, higher than other tests.

Table 1

ADA result	Tuberculous meningitis group (total 17)	Non-tuberculous meningitis group (total 69)
ADA positive (>10 U/L)	11 (a)	2 (b)
ADA negative (<10 U/L)	6 (c)	67 (d)

Table 2: Result of ZN stain in meningitis

ZN stain	Tubercular meningitis group (total 17)	Non-tubercular meningitis group (total 69)
AFB seen	01	00
AFB not seen	16	69

Table 3: Result of Cytological analysis in meningitis

Fluid Cytology	Tubercular meningitis group (total 17)	Non-tubercular meningitis group (total 68)
Lymphocytic predominance	15	50
Neutrophilic predominance	02	18

Table 4: CSF protein results in meningitis

CSF protein	Tubercular meningitis group (total 17)	Non-tubercular meningitis group (total 62)
> 45 mg/dl	15	24
< 45 mg/dl	02	38

Table 5: CSF sugar results in meningitis

CSF Sugar	Tubercular meningitis group (total 16)	Non-tubercular meningitis group (total 65)
< 40 mg/dl	01	07
40 to 70 mg/dl	12	36
> 70 mg/dl	03	22

Table 6: Various studies on ADA test for CSF and its results

Name of worker	Cut off	Sensitivity	Specificity
Kashyap et al ⁽⁹⁾	11.39 U/L	95%	95%
Fiju Chacko et al ⁽¹¹⁾	10 U/L	92.5%	97%
Ghosh GC et al ⁽¹³⁾	6 U/L	75%	96.8%
Gupta BK et al ⁽²⁾	10 U/L	94.73%	90.43%
Harsimran Kaur et al ⁽¹⁴⁾	9.5 U/L	83.3%	99.98%
Rana SV et al ⁽¹⁰⁾	10 U/L	90%	90%
Merrikhi A et al ⁽¹⁵⁾	6 U/L	90.9%	94%
Desai KJ et al ⁽¹⁶⁾	10 U/L	100%	93.75%
Prasad R et al ⁽¹⁷⁾	3.30 IU/L	100%	97.87%
Present study	10 U/L	64.70%	97.10%

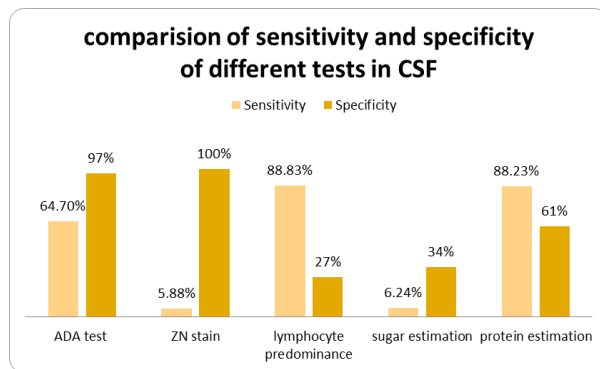


Fig. 1: Comparison between sensitivity and specificity of different tests in CSF

Discussion

In this study, mean level of ADA in tubercular meningitis is 11.81 U/L and in non-TBM is 1.65 U/L. Hence, for suspicious cases of TBM, increased levels of ADA could facilitate diagnosis, so irreversible brain damage may be prevented by early diagnosis. Symptoms, results of biochemical, microscopic and bacteriological examination are seldom conclusive. Thus, it is usual to begin specific therapy for tuberculosis on the basis of a presumptive clinical diagnosis. ADA test is very useful in this setting.

British Infection Society for the diagnosis and treatment of TB of the central nervous system in adults and children suggests that the activity of ADA is raised in the CSF of patients with tuberculous meningitis and has been evaluated as a diagnostic assay. The major problem was less specificity.⁽⁵⁾ They reported high CSF ADA activity in patients with lymphomas, malaria, brucellosis and pyogenic meningitis. Thus, they did not recommend CSF ADA test as a routine diagnostic test for TB of the central nervous system.

ADA test may be less useful in differentiating bacterial meningitis from tuberculous meningitis. Justification for this could be, ADA level in most assays detects total ADA which includes both ADA-1 and ADA-2. Consequently, fluid with high cellular content like in bacterial meningitis can have high total ADA level and ADA test may fail to differentiate tuberculous meningitis. In seropositive patients, ADA activity in the CSF had limited value for diagnosis of TB and tuberculous meningitis.

ADA test showed false-positive results in patients with neurological CMV (cytomegaloviral) disease, cryptococcal meningitis, lymphomatous and probable candidal meningitis.

Prevalence of tuberculous meningitis in India and other developing countries is high and positive predictive value for ADA test in diagnosis of tuberculous meningitis is very much higher than that of developed countries. The CSF ADA level may be useful in developing countries.

The mean ADA levels in CSF of TBM cases, in adult age group, ranges from 15.7 - 21.3 U/L, which has

been observed in few studies.^(7,8) And mean ADA level in pediatric age groups have been reported to be ranging between 11.6-13.7 U/L in one of the study.⁽⁶⁾ These results suggest that levels of ADA vary in accordance with age groups. All the patients who were selected for the study were between the age group of 6 to 24 months. So difference in result was may be due to difference in immunological reactivity to tubercular antigen in adults as compared to children.

Kashyap et al selected cutoff value of 11.39 U/L and has obtained sensitivity of 82% and specificity as 83% in TBM cases.⁽⁹⁾

Rana et al took 10 U/L as cutoff value for diagnosis of TBM and found sensitivity 66.6% and specificity 90%.⁽¹⁰⁾

Baheti et al selected 6.5 U/L as cut-off and found that CSF ADA may differentiate tuberculous from non-tuberculous meningitis.⁽¹¹⁾

Gupta et al, showed that ADA level in CSF is significantly high in TBM as compare to those with other diseases. In their study, they detected Sensitivity of ADA test 94.73% and specificity 90.47%.⁽²⁾

Table 6 shows various studies on ADA test for CSF and its results. Sensitivity of ADA test for CSF is low as compare to other studies, but specificity correlates well with other studies.

Conclusion

86 CSF were studied with the main aim to know the utility of ADA test in diagnosis of TBM. Mean level of ADA in TBM is significantly higher than in non-TBM. Sensitivity and specificity of ADA test for TBM is 64.70% and 97.10% respectively. Comparison of all the tests performed, findings are very low sensitivity of ZN stain and low specificity of cytological analysis, protein and sugar estimation. So as an alternative to subjecting CSF of suspected TBM to various tests (cytology, protein and sugar estimation and ZN stain), subjecting sample for single ADA test is better option.

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How to cite this article: Kothari K, Mistry MA, Goswami YS. Utility of ADA (Adenosine Deaminase) enzyme assay in diagnosis of tuberculous meningitis. *Indian J Microbiol Res* 2017;4(3):320-324.