ORIGINAL RESEARCH PAPER

Volume-7 | Issue-4 | April-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CSF– ADENOSINE DEAMINASE ACTIVITY AS A BIOCHEMICAL MARKER OF TB MENINGITIS DIAGNOSIS AND ITS CORRELATION WITH CSF–PROTEIN CONCENTRATION – A CROSS-SECTIONAL STUDY IN A SUPER-SPECIALITY HOSPITAL

Biochemistry

Santa Saha-Roy* Associate Professor, Dept. of Biochemistry, B.S. Medical College, Bankura. *Corresponding Autrhor

Gargi Sen Assistant Professor, Department of Biochemistry, B.I.N., Kolkata.

Sumanta Banerjee PGT, Department of Biochemistry, B.S. Medical College, Bankura.

Avijit Hazra Professor, Department of Community Medicine, IPGME&R, Kolkata.

Swati Bhattacharyya Professor, Department of Biochemistry, N.B. Medical College, Darjeeling.

Harendra Nath Das Ex-Professor, Department of Biochemistry, B.I.N., Kolkata.

ABSTRACT

Adenosine deaminase activity (ADA) and CSF- protein was estimated in cerebrospinal fluid (CSF) of 1072 suspected and diagnosed tubercular meningitis (TBM) patients. Clinically suspected TBM cases were 903 and 169 patients were diagnosed by combination of direct staining, culture method and clinical evaluation.

Sensitivity and specificity of ADA levels in CSF to diagnose tubercular meningitis were 86.4% and 70% respectively at > 5.6 IU/L cut off of ADA levels in CSF. Sensitivity and specificity of protein levels in CSF were 82.8% and 80.7%, respectively at > 100 mg/dL cut off of protein levels in CSF. The CSF-ADA level in TBM cases had significant positive correlation with CSF protein ($p < 0.001$). CSF -glucose level was done as routine test and CSF -ADA was found negatively correlated with CSF glucose ($p = 0.03$).

Thus, determination of ADA activity and CSF- protein are reliable and valuable adjunct in diagnosing tuberculous meningitis.

KEYWORDS

CSF - Cerebrospinal Fluid, ADA - Adenosine Deaminase, TBM - Tubercular Meningitis, CSF - Protein.

INTRODUCTION

Tuberculous meningitis (TBM) is one of the dangerous and endemic communicable diseases of extra pulmonary tuberculosis (EPTB) among socioeconomically suppressed communities. Acquired immuno-deficiency syndrome (AIDS) and multidrug resistant TB (MDR-TB) increases the complexity and morbidity of the disease.[1]

Isolation and identification of *Mycobacterium tuberculosis* in the cerebrospinal fluid (CSF) by direct staining or culture is not mandate to diagnose the disease, as the diagnostic yield of CSF smears and cultures has been very low.[2] Acid-fast bacilli (AFB) are found in CSF in a very few cases of TBM. Therefore, the diagnosis of TBM depends on the clinical manifestations of meningeal irritation with lymphocytic predominance and low glucose levels in CSF. Viral or fungal meningitis may mimic as tubercular meningitis. Now-a-days several rapid tests based on CSF study have been developed for diagnosis of TBM. These tests are called 'Indirect tests' like adenosine deaminase (ADA), the radioactive bromide partition test and antibodies to the mycobacterial antigen which are usually measuring a product of the host response to this infection,[3],[4]. There are some direct tests also such as measuring 3- (2'-Ethylhexyl) indoline, a product of the infecting organism; detection of tuberculostearic acid, a component of the cell wall of *M. tuberculosis*; mycobacterial antigens and fragments of mycobacterial DNA by polymerase chain reaction (PCR).[5] Direct methods needs a specialized molecular set up and expertization. Estimation of CSF-ADA activity in the diagnosis of tubercular (TB) meningitis yields good results with low monetary cost. [6],[7] ADA is widely distributed in mammalian tissue particularly in T-lymphocytes.[8],[9]

In the above background the present study was planned to measure ADA activity in all CSF samples, sent for biochemical investigations for suspected cases of tubercular meningitis with an aim to diagnose TBM with the help of this simple, cost effective, non-invasive and fairly rapid test and to correlate values of CSF-protein concentration with that of ADA activity and to see if there is any cut-off value for CSF-ADA level or protein level to predict TB meningitis.

MATERIALS AND METHODS

This prospective study was conducted in the department of Biochemistry, Bangur Institute of Neurosciences (BIN), Kolkata and Medical College Kolkata in collaboration with department of Neuromedicine, and department of Pathology, BIN, Kolkata, during the period of 2009 to 2013. CSF samples were collected through lumbar puncture from 1072 patients of suspected and diagnosed TB meningitis from indoor patients of the department of Neuromedicine, BIN and Medical College Kolkata. Ethical permission was taken from the concerned ethical committee.

Exclusion criteria: Patients having kidney disorders, diabetes mellitus, infectious diseases like hepatitis, infectious mononucleosis, typhoid, and malignant tumours are excluded from the study.

Parameters done: CSF was analysed for conventional tests such as protein and glucose concentration, WBC count, gram stain, Ziehl-Neelsen stain and culture to identify TB bacilli and test for ADA activity.

CSF Microprotein (mg/dL) was estimated by Pyrogallol Red Method.[10] Protein in an acidic medium, combines with Pyrogallol Red and Molybdate to form a blue purple coloured complex. Intensity of the colour formed is directly proportional to the amount of proteins present in the CSF sample.

CSF- ADA activity (U/L) was measured by chemical method with reagent ADA-MTB (Tulip diagnostics).[11] ADA hydrolyses adenosine to ammonia and inosine. The ammonia formed reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenols complex with sodium nitropruside acting as a catalyst. Intensity of the blue coloured indophenols complex formed is directly proportional to the amount of ADA present in the CSF sample.

CSF- Glucose was estimated by GOD-POD method. [12]

Estimation of **Plasma Glucose, Serum Urea, Creatinine** as well as **LFT** was done to assess exclusion criteria.

Statistical analysis

Data have been summarized as mean and standard deviation for numerical variables (along with median and interquartile) and counts and percentages for categorical variables. Normality was tested by Kolmogorov-Smirnov test for goodness-of-fit to a normal distribution. Associations between CSF- ADA levels and numerical variables have been quantified by calculating Spearman's rank correlation coefficient (rho), after constructing the necessary scatter plots. Receiver Operator Characteristics (ROC) curve analysis was undertaken to see if there is any cut-off for CSF- ADA level or protein level (mg/dL) to predict TB meningitis. $p < 0.05$ was taken to be statistically significant. MedCalc

version 11.6 [Mariakerke, Belgium: MedCalc Software 2011] software was used for the analysis.

RESULTS AND DISCUSSION

The present study included participants in the age group of 16 – 53 years (Mean ± SD = 34.9 ± 18.404 years; Median 35 with IQR 20- 49). The value of CSF- ADA was 6.04 ± 7.84 IU/L (Mean ± SD) with median 3.8 (IQR= 1.36- 8.1); value of CSF- protein was 106.65 ± 126.06 mg/dL (Mean ± SD) with median 66.2 (IQR= 42.4- 118.1) and CSF- glucose was 70.62 ± 25.0 mg/dL (Mean ± SD) with median 59.0 (IQR= 50- 69), respectively. All parameters had shown the p value < 0.001.

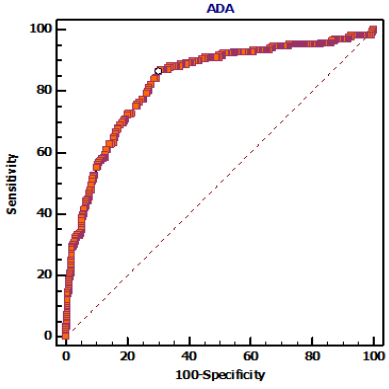

Figure 1: Receiver Operator Characteristics (ROC) curve analysis to see if there is any cut-off for CSF -ADA level (IU/L) to predict TB meningitis

ROC curve (fig. 1) suggested that CSF-ADA > 5.67 IU/L indicated the possibility of TBM with 86.4% (95% CI= 80.3 - 91.2) sensitivity and 70.0% specificity (95% CI= 66.9 - 73.0).

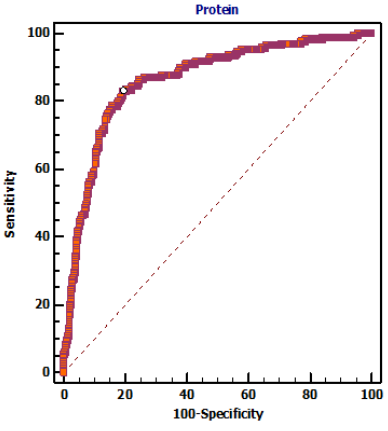

Figure 2: Receiver Operator Characteristics (ROC) curve analysis to see if there is any cut-off for CSF protein level (mg/dL) to predict TB meningitis

ROC curve (fig. 2) suggested that CSF-Protein > 100 mg/dL indicated the possibility of TBM with 82.8% (95% CI= 76.3 - 88.2) sensitivity and 80.7% specificity (95% CI= 78.0 - 83.3).

Table 1: Quantification of association as Spearman's rank correlation coefficient (rho)

S. No.	Parameters	rho	p value
1	CSF- ADA with age	- 0.018	0.560
2	CSF- ADA with CSF- protein	0.29	< 0.001
3	CSF- ADA with CSF- glucose	- 0.068	0.030

CSF -ADA was found positively correlated with CSF protein (p < 0.001) and negatively correlated with CSF glucose (p 0.03). CSF ADA has no correlation with age of the patient (Table-1).

DISCUSSION

Tuberculous meningitis (TBM) remains a major global health problem.[13],1[4] Routine CSF laboratory parameters may not be helpful to differentiate TBM from other meningities like partially treated pyomeningitis and aseptic meningitis.

CSF- ADA estimation is a useful method to diagnose TBM and can differentiate TBM from normal subject or patients with other neurological disorders[15]. Many researchers have reported the usefulness of CSF-ADA activity in the diagnosis of TBM.[16],[17],[18]

ADA is an enzyme in purine catabolic pathway converting adenosine to ionosine and ammonia. It plays an important role in differentiating lymphoid cells. It is present in abundance in active lymphocyte whose number is inversely proportional to the degree of differentiation. Its level in lymphocytes is about ten times higher than in RBC. The ADA activity increases during mitogenic and antigenic response of T lymphocytes. T lymphocyte blastogenesis can be inhibited by ADA inhibitors. A deficiency of ADA is associated with severe defect in cell mediated immunity as well as humoral immune deficiency, predisposing the patients to opportunistic infection. ADA is released by T lymphocytes during cell mediated immune response, particularly during T cell activation.[19]

ADA is now recognised as a marker of cell mediated immune response as well as an index for differentiation of TB and non- TB infection. The source of raised ADA in CSF of TBM patients may be damaged blood brain barrier permitting ADA to enter into CSF blood or adjacent cerebral tissue or as a result of lymphocytic proliferation indicating local immune response. [20] Few studies have been made to use CSF-ADA activity as a diagnostic tool of TBM considering that both cell mediated and humoral immunity may play an important role in TBM. Rajesh Baheti et al. showed that CSF - ADA level 6.5 IU/L as a cut-off value exhibited a sensitivity of 95.83%, specificity of 92.85% for the diagnosis of tuberculous meningitis.[18]

In the present study CSF -ADA level 5.67 IU/L as a cut-off value exhibited a sensitivity of 86.4%, specificity of 70% for the diagnosis of tuberculous meningitis. Generally routine CSF laboratory parameter may be helpful in the diagnosis of bacterial, cryptococcal and eosinophilic meningitis. In clinical practice there are diagnostic difficulties in differentiating tubercular meningitis from other lymphocytic CSF conditions like aseptic meningitis.

The positive correlation between CSF- ADA activity and CSF- protein concentration (p < 0.001) in this study is corroborated with other studies. Mishra et al. found that ADA level had significant correlation with CSF protein concentration (p < 0.02).[21] In this study CSF-Protein level > 100 mg/dL as a cut off value indicates presence of TB meningitis with a sensitivity of 82.8%, specificity of 80.7%. As indicated in our study, subjects having CSF-protein below the cut off value do not show CSF-ADA activity suggestive of the TBM.

CONCLUSION

The assay of CSF-ADA activity and CSF-protein was found to be simple, less expensive, useful and rapid diagnostic tests for the early recognition of TBM. Moreover CSF-protein level may be helpful in determining the requirement of additional CSF-ADA assay.

ACKNOWLEDGEMENT

We are thankful to the Director, BIN and the Principal, Medical College Kolkata for their support to perform this study. We are also grateful to the participants of the study for their co-operation.

REFERENCES

1. Rana SV, Singhal RK, Singh K, Kumar L. (2004), "Adenosine deaminase levels in cerebrospinal fluid as a diagnostic test for tuberculous meningitis in children." Indian Journal of Clinical Biochemistry, 19 (2), 5- 9.
2. Chotmongkol V, Jitpimolmard S, Thavornpitak Y. (1996), "Corticosteroid in tuberculous meningitis." J Med Assoc Thai, 79, 83- 90.
3. Coovadia YM, Dawood A, Ellis ME, Coovadia HM, Daniel TM. (1986), "Evaluation of adenosine deaminase activity and antibody to Mycobacterium tuberculosis antigen 5 in cerebrospinal fluid and the radioactive bromide partition test for the early diagnosis of tuberculosis meningitis." Archives of Disease in Childhood, 61 (5), 428-435.
4. Sada E, Guillermo M, Palacios R, López-Vidal Y, Ponce S, León D. (1983), Detection of Mycobacterial antigens in cerebrospinal fluid of patients with tuberculous meningitis by enzyme-linked immunosorbent assay. The Lancet, 322 (8351), 651- 652.
5. Zuger A, Lowy FD. "Tuberculosis of the central nervous system." In: Scheld WM, Whitley RJ, Durack DT, eds. (1991), "Infections of the central nervous system." New York; Raven Press, 425- 456.
6. Ribera E, Martinez-Vazquez JM, Ocana I, Segura RM, Pascual C. (1987), "Activity of adenosine deaminase in cerebrospinal fluid for the diagnosis and follow- up of tuberculous meningitis in adults." J Infect Dis, 155, 603- 607.
7. Choi SH, Kim YS, Bae IG, et al. (2002), "The possible role of cerebrospinal fluid adenosine deaminase activity in the diagnosis of tuberculous meningitis in adults." Clin Neurol Neurosurg, 104, 10- 15.
8. Sullivan JL, Osborne WR, Wedgewood RJ. (1977), "Adenosine deaminase activity in lymphocytes." Br J Haematol, 37, 157- 158.

9. Kameoka J, Tanaka T, Nojima Y, Schlossman SF, Morimoto C. (1993), "Direct Association of Adenosine Deaminase with a T Cell Activation Antigen, CD26." Science, 261 (5120), 466- 469.
10. Tietz NW. (1986), "Textbook of Clinical Chemistry." W.B. Saunders; Philadelphia, 608.
11. Giusti G, Galanti B. "Adenosine deaminase." In: Bergmeyer HU, et al. (1974), "Methods of Enzymatic analysis." New York; Academic Press, 1092– 1099.
12. Lott JA, Turner K. (1969), "Evaluation of Trinder's Glucose Oxidase Method, Alternative Oxygen Acceptor." Ann. Clin. Biochem, 6, 24- 25.
13. Marais S, Pepper DJ, Marais BJ, Török ME. (2010), "HIV-associated tuberculous meningitis – diagnostic and therapeutic challenges." Tuberculosis, 90, 367- 374.
14. Fujiwara PI, Clevenbergh P, Dlodlo RA. (2005), "Management of adults living with HIV/AIDS in low-income, high-burden settings, with special reference to persons with tuberculosis." Int J Tuberc Lung Dis, 9, 946– 958.
15. Blake J, Berman P. (1982), "The use of adenosine deaminase assays in diagnosis of tuberculosis." S Afr Med Journ, 62, 19.
16. Prasad R, Kumar A, Khanna BK. (1991), "CSF - ADA for diagnosis of TBM." Ind J Tub, 38, 99- 102.
17. Mishra OP, Loiwal V, Ali Z. (1995), "CSF - ADA and C reactive protein in TBM and partially treated bacterial meningitis." Indian Ped, 32, 886- 889.
18. Baheti R, Laddha P, Gehlot RS. (2001), "CSF - Adenosine Deaminase (ADA) activity in various types of meningitis." Journal Indian Academy of Clinical Medicine, 2 (4), 285- 287.
19. Gupta BK, Bharat A, Bandyopadhyay D, Baruah H. (2010), "ADA levels of CSF of TB meningitis patient." J. Clin. Med. Res, 2 (5), 220- 224.
20. Valdes L, San Jose E, Alvarez D, Valle J M. (1996), "Adenosine deaminase (ADA) isoenzyme analysis in pleural effusions: diagnostic role, and relevance to the origin of increased ADA in tuberculous pleurisy." Eur Respir J, 9, 747- 751.
21. Mishra O P, Loiwal V, Ali Z, Nath G, Chandra L. (1996), "Cerebrospinal fluid deaminase activity for the diagnosis of tuberculous meningitis in children." J Trop Pediatr, 42 (3), 129- 132.