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 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).\[3\] 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.\[4\] ADA is widely distributed in mammalian tissue particularly in T-lymphocytes.\[5\]

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.\[6\] 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).\[7\] ADA hydrolyses adenosine to ammonia and inosine. The ammonia formed reacts with a phenol reagent ADA-MTB (Tulip diagnostics). ADA hydrolysates adenosine to ammonia and inosine. The ammonia formed reacts with a phenol reagent ADA-MTB (Tulip diagnostics). ADA was quantified by calculating the product of the dye complex with sodium nitropruside acting as a catalyst. Intensity of the blue coloured indophens complex formed is directly proportional to the amount of ADA present in the CSF sample.

CSF-Glucose was estimated by GOD-POD method.\[8\]

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

KEYWORDS
CSF - Cerebrospinal Fluid, ADA - Adenosine Deaminase, TBM - Tubercular Meningitis, CSF - Protein.
Tuberculous meningitis (TBM) remains a major global health problem. Routine CSF laboratory parameters may not be helpful to differentiate TBM from other meningitis 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. Many researchers have reported the usefulness of CSF-ADA activity in the diagnosis of TBM. ADA is an enzyme in purine catabolic pathway converting adenosine to inosine 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.

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 lymphocyte proliferation indicating local immune response. 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.

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). 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.

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REFERENCES

2. Chotmongkol V, Jitpimolmard S, Thavornpitak Y. (1996), “Corticosteroid in cerebral tissue or as a result of lymphocytic proliferation indicating local immune response.” 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.


