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Cerebrospinal fluid adenosine deaminase levels as a diagnostic marker in tuberculous meningitis in adult Nepalese patients

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PEER REVIEW

Peer reviewer

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Comments

This is a good study designed to investigate the diagnostic role of CSF ADA levels in TBM cases and to differentiate it from non-TBM –viral meningitis cases. A simple, rapid and inexpensive test is critically important and an urgency in clinical practice especially in developing countries to diagnose a case of TBM. The results of this study, confirms that CSF ADA levels are significantly elevated in TBM cases as compared to viral meningitis cases with a good sensitivity and a high specificity.

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ABSTRACT

Objective: To study the cerebrospinal fluid (CSF) adenosine deaminase (ADA) levels in tuberculous meningitis (TBM) and non-TBM –viral meningitis cases and to determine its diagnostic significance as a biochemical marker of TBM infection. **Methods:** The study population comprised two different patient groups. TBM – group I – 28 cases and non-TBM–viral meningitis – 22 cases. These were enrolled consecutively in the study and CSF specimens were collected from them. ADA estimation was carried out by spectrophotometry. **Results:** ADA levels (mean±SD) in the TBM and non-TBM groups were 16.46±6.24 U/L and 5.13±2.96 U/L, respectively (highly significant $P<0.001$). Using a CSF ADA cut off reference value of >10 IU/L, the test showed a good sensitivity of 82.14% (95% CI 64.41–92.12) and a high specificity of 90.91% (95% CI 72.19–97.47). Positive and negative predictive value and positive and negative likelihood ratios and accuracy of the test in TBM cases were 92% (95% CI 75.03–97.77), 80% (95% CI 60.86–91.13), 9.03 (95% CI 2.38–34.25), 0.19 (95% CI 0.09–0.44) and 86%, respectively. **Conclusion:** CSF ADA levels are elevated in the TBM cases as compared to the non-TBM – viral meningitis cases with a good sensitivity and a high specificity. It is a simple and inexpensive diagnostic adjunctive test in the rapid and early diagnosis of TBM.

KEYWORDS

Adenosine deaminase, Diagnostic marker, Tuberculous meningitis, Sensitivity, Specificity

1. Introduction

Tuberculous meningitis (TBM) is the most common and the most serious form of extrapulmonary tuberculosis (TB). TBM is an endemic disease among socioeconomically disadvantaged communities in both developing and developed countries. The importance of this disease has increased since there is emergence of acquired immunodeficiency syndrome (AIDS) and multidrug resistant TB[1]. TBM is the most dangerous form of extrapulmonary TB occurring in 7% to 12% of TB patients in developing countries with a high rate of mortality and sequelae due to delay in diagnosis and proper treatment[2,3]. The prevalence

of disease is supposed to be high in Nepal, however, there is no concrete authentic data in this regard[4].

Classical methods of tuberculosis diagnostics have limited application for the diagnosis of TBM. Ziehl–Neelsen (ZN) stain and culture for TB bacilli still remain the basic methods for confirming the laboratory diagnosis of TBM. But sensitivity of these methods as applied to cerebrospinal fluid (CSF) is low and it takes a very long time such as 4–6 weeks to produce results by culture methods[5]. The characteristic CSF cytological and biochemical changes are also variable and may even be absent[1]. Rapid diagnosis of TBM is very important but current rapid diagnostic methods such as polymerase chain reaction (PCR) or gas liquid

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chromatography (GLC) are expensive and not commonly available[6]. Mortality and sequelae rates may be reduced considerably with early diagnosis and treatment. Therefore studies are in progress to develop faster and more sensitive diagnostic methods[7]. Recently, biochemical markers of tuberculosis infection, such as levels of adenosine deaminase (ADA) concentration has shown promising results, in the rapid and early diagnosis of tuberculosis[8].

Adenosine deaminase [EC 3.5.4.4] is an enzyme involved in purine catabolism and whose principal biologic activity is detected in T lymphocytes[6]. An increase in ADA levels is observed in TB as well as bacterial infections in which the cellular immunity response is actively involved[7]. There are also studies reporting an increase in CSF ADA levels in TBM[9–12]. Sensitivities and specificities of ADA levels in CSF were found to be in the range 44%–100% and 71%–100% respectively[13]. One study had indicated that the measurement of ADA level alone is not useful as a rapid diagnostic test for TBM[6]. Literature survey revealed only one study being reported from Nepal, that had analyzed CSF ADA role in TBM[14]. The aim of the present study was to evaluate the role of CSF ADA as a biochemical diagnostic marker in TBM in adults in Nepalese population.

2. Materials and Methods

2.1. Setting

This prospective study was conducted from January 2009 to June 2010 at a large centrally located tertiary care hospital in Kathmandu, Nepal. The study protocol was approved by the institute ethical review committee and informed consent was obtained from the patients prior to inclusion in the study.

2.2. Patients

A total of consecutive 50 cases of clinically suspected of meningitis admitted in the medical ward of the hospital were selected. Out of these 50 cases of meningitis–28 cases were of TBM and 22 cases of non–TBM–viral meningitis.

2.2.1. Diagnostic criteria

All the cases were thoroughly examined clinically and investigated. Diagnosis of TBM was based on the clinical criteria, lymphocytic pleocytosis in the CSF with raised protein level and a low glucose level (<40% of matched blood glucose), negative bacterial and fungal cultures, a positive finding on ZN stain and / or a positive response to anti tubercular treatment for the duration of two months. Viral meningitis was diagnosed on the basis of acute onset of symptoms and signs of meningeal irritation, biochemical examination of CSF, negative results in gram stain, ZN stain and India Ink stain microscopic examinations of CSF, negative bacterial and fungal cultures and complete response to symptomatic treatment without antibacterials.

2.2.2. Exclusion criteria

Exclusion criteria for the study were the cases with age less than 14 years and cases who have undergone prior treatment outside the hospital and cases with CSF showing turbidity, hemorrhage and / or were xanthochromic.

2.3. Specimen collection

CSF specimens were obtained by lumbar puncture performed by a trained medical officer. A total of 3 ml of CSF was collected and distributed in three sterile vials and was subjected to various laboratory investigations involving biochemical, cytological and microbiological procedures.

2.4. ADA Assay

ADA estimation was carried out by spectrophotometry method based on the principle of Guisti and Galanti method of enzymatic analysis[15]. ADA MTB diagnostic kit from Microexpress—a division of Tulip Diagnostics Pvt. Ltd., India was used according to the manufacturer's instructions. A cut off reference value of >10 IU/L CSF ADA was considered to be positive as per the guidelines provided in the test kit literature.

2.5. Data analysis

The results were expressed as mean \pm SD. Statistical comparison was carried out by using the Student's t test. A two-tailed *P* value of <0.001 was taken as statistically significant. Diagnostic test 2 \times 2 contingency tables were made. Sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratio and diagnostic accuracy were calculated. All parameters were estimated with 95% confidence interval using the Stata 10.1 statistical software package (Stata Corp. College Station, Tx).

3. Results

Based on the diagnostic criteria, the patients were divided into two groups as TBM cases and non–TBM–viral meningitis cases. In TBM cases the age (mean \pm SD) and sex ratio (male:female) were 36.21 \pm 15.21 and 1.15:1, respectively and in non–TBM–viral meningitis cases the age (mean \pm SD) and sex ratio (male:female) were 38.59 \pm 18.30 and 1.44:1, respectively (Table 1).

Table 1

Age and Sex ratio of the different study population groups

Study Group	No. of patients	Age (years) (Mean \pm SD)	Sex Ratio (M:F)
TBM	28	36.21 \pm 15.21	1.15:1
Non–TBM –viral meningitis	22	38.59 \pm 18.30	1.44:1

The ADA levels (mean \pm SD) in TBM and non–TBM were 16.46 \pm 6.24 IU/L and 5.13 \pm 2.96 IU/L, respectively. The difference between the two groups of patients studied was found to be highly significant (Table 2).

Table 2

CSF ADA levels in different study populations groups.

Study Group	No. of patients	Mean \pm SD (U/L)	<i>P</i> value comparison TBM & Non–TBM–viral meningitis
TBM	28	16.46 \pm 6.24	<i>P</i> <0.001
Non–TBM–viral meningitis	22	5.13 \pm 2.96	

Out of 28 TBM patients, 23 were found to be having CSF ADA above the cutoff value while 5 patients had values below the cut off. Of the 22 non–TBM–viral meningitis

patients, two were found to be having CSF ADA values above the cut off, The CSF ADA test in TBM patients showed sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios and accuracy of 82.14%, 90.91%, 92.00%, 80.00%, 9.03%, 0.19% and 86.00% respectively in differentiating from non-TBM patients (Table 3).

4. Discussion

Differentiating TBM from non-TBM meningitis especially viral meningitis, by current laboratory methods is a major diagnostic challenge in clinical practice. There is considerable urgency in establishing the correct diagnosis in patients with TBM because specific therapy is most effective when instituted early in the course of illness. Rapid diagnostic tests with good sensitivity and specificity are required to aid the presumptive diagnosis of TBM^[16]. ADA had been of interest for many years in TB diagnosis^[17]. The determination of ADA activity in body fluids such as pleural fluid, peritoneal fluid and CSF has been reported to be a valuable marker in the diagnosis of extra-pulmonary TB^[13]; It is of great relevance to evaluate the efficiency of the determination of ADA activity, which can be used in countries where TB prevalence is high. In addition, the determination of ADA activity can, in many cases favour the diagnostic confirmation, replacing biopsy, laparoscopy and other tests that definitely confirm the diagnosis but are more sophisticated, expensive and in many health care facilities, unavailable^[18].

The results of this study showed that the CSF ADA value (mean±SD) in TBM cases was 16.46±6.24 while in non-TBM cases, it was 5.13±2.96, respectively (highly significant $P<0.001$). At a cut off value of 10 IU/L, the sensitivity and specificity of the test were 82.14% and 90.91% respectively. The positive and negative predictive values were 92% and 80%, respectively while the positive and negative likelihood ratios and accuracy were 9.03, 0.19 and 86% respectively. A positive likelihood ratio of 9.03 suggest that TBM patients have an approximately 9-fold higher chances of being ADA assay positive as compared to patients without TBM. However, if the ADA assay result is negative, the probability that the patient has TBM is approximately 19%, which is not low enough to rule out TBM. These results suggest that a negative ADA assay result should not be used alone as a justification to exclude or discontinue anti-TB treatment. The choice of therapeutic strategy should be based on the results of microscopic examination of a smear or culture for *Mycobacterium tuberculosis*, as well as other clinical data, such as response to anti-TB treatment^[19].

Several studies had shown that ADA levels were found to be significantly high in TBM group as compared to bacterial and viral meningitis^[5,7]. Gupta *et al.* reported CSF ADA level 10 U/L as a cut off value showed 94.73 sensitivity and 90.47% specificity in differentiating TBM from non-TBM cases^[10]. Agarwal S, describes a sensitivity and specificity of 99.90 and 87.5% respectively using a cut off value of 10 IU/L^[12]. Gautam *et al.* demonstrated a sensitivity and specificity of CSF ADA activity as 85.0% and 88.0% respectively at a cut off value of 6.97 IU/L to diagnose TBM in CSF^[14]. In a study done in Gujarat, India the sensitivity and specificity was 98.46% and 97.65 respectively at a CSF ADA cut off value of 9 IU/L^[20]. Belagavi and Shalini, shown the sensitivity and

specificity was 73.9% and 92.6% respectively when a cut off value of ADA of 10 IU/L was used^[21]. Mehta *et al.* described a sensitivity and specificity of CSF ADA for TBM diagnosis to be 78% and 98% respectively at cut off value of 11 IU/L^[22]. The value of cut off has a great importance in the evaluation of the sensitivity and specificity of CSF ADA test. The amount of this cut off is controversial at the present time^[23]. The standardized cut off of ADA values for the diagnosis of TBM have not been established and the values used in various studies ranged from >5.0 to >15.0 IU/L making the practical use of this assay more difficult^[13,24]. Multicenter studies in different populations are needed to determine standard CSF ADA values^[11].

Due to the difficulty of establishing the diagnosis of TBM using clinical, radiological (magnetic resonance imaging or computed tomography), cytological, biochemical and even microbiological approaches, additional tests have been developed. The use of ADA as a diagnostic marker is increasing because it is simple and affordable^[5]. Currently, TBM and its early diagnosis is a global issue and is becoming more and more crucial. All relevant studies share the view that ADA is a useful test in early differential diagnosis of TBM^[11].

In conclusion, our results suggests that CSF ADA levels are elevated in TBM patients as compared to non-TBM (viral meningitis) patients and thus estimating CSF ADA is a useful differential diagnostic marker in these cases and can help the clinician make an early diagnosis of TBM. It is a simple, rapid and an inexpensive diagnostic tool that can be easily made available and performed with minimal training especially useful in resource-limited settings.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Tuberculous meningitis (TBM) is an endemic disease in developing countries. Multidrug resistance in tuberculosis and acquired immuno-deficiency syndrome (AIDS) further worsen the outcome of this disease. Delay in diagnosis and in the start of effective treatment results in poor prognosis and sequelae. Available methods of diagnosis of TBM were evaluated and all of them were found to have low sensitivity and specificity. Adenosine deaminase (ADA) is an enzyme in the purine pathway and an elevated ADA level in body fluids have been considered by several researchers to differentiate tuberculosis disease from non-tuberculosis disease.

Research frontiers

A reliable and rapid test which can be performed in any standard laboratory, could be of immense help in the diagnosis of TBM. Any test which facilitates a correct and simplified diagnosis of TBM should be very valuable. Estimating CSF ADA levels in TBM cases and its role in differentiation from non-TBM cases will provide one such alternative to us. Several studies are being carried out to establish its usefulness in different geographical areas.

Related reports

Several studies had shown that ADA levels were significantly elevated in TBM group as compared to non-TBM cases. The results of this study compare favorably with that reported by Gautam et al (2007) and Belagavi and Shalini (2011).

Innovations & breakthroughs

Reports and investigations about role of ADA levels in TBM are limited. This study has shown that CSF ADA levels are elevated in TBM cases as compared to non-TBM-viral meningitis cases with a good sensitivity and a high specificity.

Applications

This test is simple to perform and is also inexpensive that should be included in the diagnostic work-up of TBM cases on a routine basis. This is critically important and would greatly help in the rapid and early diagnosis of TBM in clinical practice.

Peer review

This is an excellent and a very significant study designed to investigate the diagnostic role of CSF ADA levels in TBM cases and to differentiate it from non-TBM –viral meningitis cases. A simple, rapid and inexpensive test is critically important and an urgency in clinical practice especially in developing countries to diagnose a case of TBM. The results of this study, confirms that CSF ADA levels are significantly elevated in TBM cases as compared to viral meningitis cases with a good sensitivity and a high specificity.

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