IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861. Volume 4, Issue 1 (Jan.- Feb. 2013), PP 21-24
www.iosrjournals.org

Cerebrospinal fluid Adenosine deaminase: its evaluation as a marker for diagnosing tuberculous meningitis in paediatric patients.

Gupta B K[1], ParulGoel[2], HarenBaruah[3], PreetiShukla[4], Jas Kiran[5],
[1]*M.B;B.S; D.T.C.D.; Ph D, Professor, Department of Biochemistry, Subharti Medical College, Swami VivakanandSubharti University, Meerut- 250005, India*
[2,3]*M.D; Assistant Professor, Department of Biochemistry, Subharti Medical College, Swami VivakanandSubharti University, Meerut- 250005, India*
[4]*Research Scholar, Department of Biochemistry, Subharti Medical College, Swami VivakanandSubharti University, Meerut- 250005, India*
[5]*Post Graduate Student, Department of Biochemistry, Subharti Medical College, Swami VivakanandSubharti University, Meerut- 250005, India*

Abstract: *Five hundred thousand patients of tuberculosis die every year in India. Delay in diagnosis and in initiating treatment results in poor prognosis. This disease is affecting the children more and more. Meningeal tuberculosis has acquired an endemic shape with about 12% incidence. The aim of the present study is to evaluate Cerebrospinal fluid Adenosine deaminase: as a marker for diagnosing tuberculous meningitis*

Eighty paediatric patients of age group 6-24 months having symptoms and signs of meningitis were divided into two groups; tuberculous and non-tuberculous, as per the accepted criteria. The CSF was drawn and ADA levels estimated in all patients.

Out of 38 tuberculous patients, 36 had CSF ADA at or above the cutoff value, while 02 had below. Out of 42 non-tuberculous patients, 04 patients had at or above while 38 were below the cutoff value. Results of our study indicate that ADA levels in CSF are not only of considerable value in the diagnosis of TBM; CSF ADA level 10 IU/L as a cutoff value exhibited 94.73% sensitivity;90.47% specificity; 90.00% positive predictive value and 95.00% negative predictive value.

Cerebrospinal fluid Adenosine deaminase estimation is less expensive, rapid and fairly specific method for the diagnosis of tuberculous etiology in TBM; it may prove to be diagnostic marker for diagnosing tuberculous meningitis in paediatric patients.

KeyWords*: Adenosine deaminase (ADA), Cell-mediated immunity (CMI), Cerebrospinal fluid (CSF), Tuberculous meningitis (TBM),*

I. Introduction

Tuberculosis kills five hundred thousand patients every year in India; out of which 8.3 % are children[1]. Delay in the diagnosis and so in initiation of specific treatment, results in poor prognosis and 25% of such patients are cured with residual permanent sequelae[2]; the multidrug resistance in tuberculosis and acquired immuno-deficiency syndrome (AIDS) has added further importance to this disease in last two decades[3].

Tuberculous meningitis (TBM) is affecting the children more and more and has acquired an endemic shape[4]; more so in poor socio-economic group. Incidence of TBM is 7-12% in developing countries. The diagnosis of active TB can often be challenging, with results remaining inconclusive. Available methods of diagnosis of TBM were evaluated [5] and all of them were shown to have low sensitivity and specificity. Direct evidence of acid fast bacilli (AFB) is available only in small percentage of cases.

Routine CSF laboratory findings like cytological and biochemical analysis are usually not helpful in differentiating tuberculous etiology in meningitis from other causes and show a considerable overlap for the support of diagnosis. This advocates for a new tool for the diagnosis, preferably sensitive and rapid.

New diagnostic tool for the diagnosis of TB like interferon gamma assays (IGRAs) can measure the presence of an adaptive immune response to M. tuberculosis antigens, butit is only an indirect measure of M. tuberculosis exposure. Nucleic acid-based amplification (NAA) tests have emerged as potentially important tools for diagnosing TB though no commercial test is licensed for use in non-respiratory specimens like CSF. The reliability of PCR depends on the amplification of DNA with primers specific for different target sequences in the mycobacterial genome, and on optimal DNA isolation and PCR procedures. The high cost and specialized laboratories needed, make it difficult to include PCR as a routine test in resourcelimited areas, and it would be difficult to maintain quality control of the results for thistechnically very demanding test.

Adenosine deaminase levels (ADA) has been considered by several researchers to differentiate tubercular disease from non-tubercular [6, 7, 8, 19, 20 & 21]. ADA is released by T cells during cell mediated immune response (CMI) to the tubercle bacilli. Literature survey reveals that some reports are in favor while some are against the role of CSF ADA in the diagnosis of TBM; we planned the present study not only to estimate ADA levels in CSF but also to evaluate its role as a marker for diagnosing tuberculous meningitis.

II. Material And Methods

After obtaining the informed consent from the guardian of the patient, routine procedures of history taking, clinical examination and routine investigations were carried out. A total of 80 patients form paediatric population falling in the age group of six to twenty four months having symptoms and signs of meningitis, admitted in the Pediatrics ward from Jan 09 to November 12 at Subharti Medical College and associated ChhatrapatiShivajiHospitals, Meerut, U P, India were included in the present study.

Presence of first or a minimum of two of the following accepted criteria was adopted to label a case as tuberculous: -

1. Bacteriological proof of the presence of Mycobacterium tuberculosis; either positive direct smear or AFB culture in sputum and/or CSF.
2. Tissue biopsy showing caseating granulomas.
3. Radiological findings consistent with TB.
4. Clinical presentation consistent with TB
5. Definite clinical and radiological improvement in two months after specific anti-tubercular treatment.
6. History of contact with current disease and positive reaction (>10 mm induration) to 5 tuberculin unit (TU) purified protein derivative (PPD).

Amongst a total of 80 patients, 56 children were male and 24 were female. Out of these, 38 patients having fulfilled the criteria were labeled as tuberculous, while the other 42 were labeled as non-tuberculous. Amongst tuberculous group (n=38) only four patients had evidence of presence of Mycobacterium (10.53%); two in CSF smear, one in sputum smear and one in CSF culture positive.

Lumbar puncture was undertaken in each case and atleast 2 ml of clear CSF was collected in a sterile tube without anticoagulant for total ADA quantification. The enzyme was stable for 24 hours at 25^0 C, for 7 days at 4^0 C and for 3 months at -20^0 C [9, 10]. Hemorrhagic CSF was excluded from the study. This CSF was subjected to biochemical and microscopic examination. Total ADA activity was estimated in all these patients by the method reported by Guisti[9] by estimating the rate of hydrolysis of adenosine to ammonia and inosine. The ammonia so formed on reaction with Phenol and hypochloride gave a blue colored indophenol complex which was measured spectophotometrically. The results were expressed as IU/L. One international unit of total ADA is defined as the amount of enzyme required to release 1μmol ammonia per minute from adenosine under standard assay conditions. The cutoff reference range of 10 IU/L for CSF ADA was taken as positive for TB, as is recommended by the manufacturer of the chemicals used. The values of CSF- ADA in tuberculous (n=38) and non-tuberculous (n=42) are expressed in terms of Mean ± S D. **Z-test (double sample)** was applied to test the significant difference between the two groups for their ADA levels. Further, sensitivity, specificity, positive predictive value and negative predictive value were also calculated.

III. Observations And Results

CSF ADA at or above the cutoff value was observed in 36 patients in tubercular group (n=38), while 02 had ADA below cutoff value. In non-tubercular group (n=42), 04 patients were found to have CSF ADA at or above the cutoff value while 38 had values below the cutoff (Table 1).

In tuberculous group ADA activity in CSF ranged between 9.2 to 110 U/L with a median of 22, mean ± S D as 27.1684 ± 22.4563while in non-tuberculous group ADA activity ranged between 2 to 10.5 U/L with a median of 6, mean ± S D as 6.0619 ± 2.5399.

On comparison of CSF ADA in the two groups, Z value is 6.0345 and p value is .000097, the difference was found to be highly significant ($p<.001$) (Table 1).The screening for TBM by estimating CSF ADA activity was evaluated according to the standard formulae. The sensitivity of the test was found to be 94.73%, specificity 90.47%, positive predictive valve 90.00 % while negative predictive value was 95.00% (Table 2).

IV. Discussion

Demonstration of AFB in direct smear, culture, cytochemistry, and CT scan are the various means to confirm the etiology of TB. The visualization of AFB in direct smear or in cultures of CSF is present in very low percentage of patients and in majority of patients it is negative and so the diagnosis is usually difficult [11]. In the present study we found the evidence of AFB only in 10.53% of the tubercular cases. Newer methods such

as those involving the amplification of bacterial DNA by the PCR and comparable systems, are not available for widespread use in the developing countries.

The ADA had been considered as a marker of cell-mediated immunity and its activity has been observed in various infections including TBM[12]. Both humoral and cell-mediated immunity play an important role in TBM infection. It has been suggested that ADA activity in CSF may help to differentiate TBM from non-TB infectious meningitis. Not only this, CSF - ADA has been reported to be useful in differentiating TBM from normal subjects and patients with other neurological disorders [12, 22]. CSF – ADA estimation is also a useful method to differentiate TBM from aseptic meningitis [13]. Other researchers have also observed the usefulness of CSF-ADA activity in the diagnosis of TBM [14, 15, & 22].

We have observed a statistically highly significant difference in the CSF - ADA levels of meningitis due to tuberculosis and non-tuberculous etiology ($P < .001$) (Table 1), which indicates that ADA levels in CSF are of considerable value in diagnosis of TBM and in differentiating this disease from other etiologies. At 10 IU/L cut-off value of CSF ADA our study exhibited sensitivity of 94.73%, specificity of 90.47%, positive predictive value 90.00% and overall accuracy 95.00% (Table 2) for the diagnosis of tuberculous meningitis.

The levels of ADA in CSF of adult patients of TBM have been evaluated in earlier studies [6-8]. Elevated levels of ADA in CSF are not specific to meningeal inflammatory disease but it can be a test for confirming its etiology with good predictive value. Raised ADA levels have also been noted in other conditions particularly in certain intracranial tumors [16].

` In earlier study the mean ADA levels in CSF of TBM cases of pediatric age groups has been reported to be ranging from 11.6-13.7 [6]. The level of 15.7-21.3 has been observed in adult TBM patients [7, 8]. These results show that levels of ADA vary in different age groups in TBM. This might be due to difference in immunological reactivity to tubercular antigen in children as compared to adults.

Literature reports (Kashyap et al) that when the cutoff value 11.39 IU/L was taken; the researcher obtained sensitivity of 82% and specificity as 83% in TBM cases [17]. Rana et al has taken 10 IU/L as cutoff value for diagnosis of TBM and found sensitivity as 66.6% and specificity 90% [18]. Baheti et al found that CSF ADA may differentiate tuberculous from non-tuberculous meningitis even at a cut-off level of 6.5 IU/L [19].

Gupta et al [20] observed that ADA levels in nontuberculous disease rarely exceeded the cut-off value; set for tuberculous disease. They [21] have further observed that ADA estimation is not only a fairly sensitive and specific test (more than 90 %), helpful in differentiating tubercular from non-tubercular etiology; both in pulmonary and extra-pulmonary disease but is also simple, inexpensive and rapid. For these reasons this test may help in early diagnosis, improve the prognosis and reduce spread of disease and sequelae.

In the present study, median ADA levels in CSF were significantly higher in TBM patients as compared to those with other etiologies. Ribera et al. have also demonstrated similar finding but his study was in TBM patients of adult age group [8].

Although Tuon et al [22]in their meta-analysis concluded that, ADA cannot distinguish between bacterial meningitis and TBM, but these observations are based on studies having heterogeneity, publication bias and nonstandardized method for ADA estimation and so further studies are needed to obtain reliable observations.

Gupta et al [23] have further found the sensitivity of this test to be 94.73%; specificity 90.47%, positive predictive value is 90.00 % and negative predictive value 95.00% and they concluded that ADA estimation in CSF is not only simple, inexpensive and rapid but also fairly specific method for making a diagnosis of tuberculous etiology in children with meningitis.

The authors are of the opinion that estimation of CSF- ADA is less expensive, easy to perform, provides instant results and the present study exhibited a high specificity and sensitivity with fairly good negative and positive predictive values; therefore estimation of ADA in CSF may be established as a marker for diagnosis of tuberculous etiology in paediatric patients of meningitis

V. Conclusion

Cerebrospinal fluid Adenosine deaminase estimation is less expensive, rapid and fairly specific method for the diagnosis of tuberculous etiology in TBM; especially when there is a dilemma of differentiating the tuberculous etiology from non-tuberculous and for this reason ADA analysis may find a place as a marker for diagnosing tuberculous meningitis . CSF- ADA is not only helpful in the diagnosis of TBM but can also be useful to differentiate it from other causes with fairly good accuracy; and which is simple, quick to perform and cost effective test.

Table 1: Distribution of the cases according to accepted criteria and CSF ADA levels:

Group	Total No of cases	ADA levels in U/L	No of cases	Mean ± SD	Z-value	P-value
Tuberculous	38	ADA ≥ 10 ADA < 10	36 02	27.1684 ± 22.4563	**6.0345**	**.000097** (p<.001)*
Non-tuberculous	42	ADA ≥ 10 ADA < 10	04 38	6.0619 ± 2.5399		
Total	80		80			

*** (p<.001) shows highly significant difference between tuberculous and non-tuberculous groups.**

Table 2: Distribution of the cases according to true and false positivity and negativity with statistical accuracy:

Cell entries	Number of cases	Statistical indices of diagnostic accuracy	Percentage
True Positive	36	Sensitivity	94.73%
False Negative	02	Specificity	90.47%
True Negative	38	Positive predictive valve	90.00 %
False Positive	04	Negative predictive value	95.00%

Reference

[1] Garcia-Monco, J.C. (1999) CNS Tuberculosis. In: Neurologic Clinics. Marra, C.M. (ed.). 17(4), 737-760.

[2] Mastroianni, C.M., Paolotti, F., Lichtrer, M.D., Agostino, C., Vullo, V. And Delia, S. (1997) Cerebrospinal fluid cytokines in patients with tuberculous meningitis, Clin Immunopathology 84, 171-176.

[3] G Thwaites, T T H Chau, N T H Mai, F Drobniewski, K McAdam, J Farrar. (2000) Neurological aspects of tropical disease:Tuberculous meningitis. *J NeurolNeurosurg Psychiatry;68:289-299*.

[4] Tandon, P.N. (1999) Neurotuberculosis: Clinical aspects. In: Neurology in Tripica. Chopra, J.S. and Sawhney, I.M.S. (eds.) Churchill Livingstone Ltd., 358-389.

[5] Bothamley, G. H. (1995). Serological diagnosis of tuberculosis. Eur. Respir. J. 8 (Suppl. 20):676–688.

[6] Malan, C., Donald, P.R., Golden, M. and Talard, J.J.F. (1984) Adenosine deaminase levels in cerebrospinal fluid in the diagnosis of tuberculous meningitis. J. Trop. Med. Hy. 87. 33-40.

[7] Piras MA, Gakis C (1973) Cerebrospinal fluid adenosine deaminase activity in tuberculous meningitis. Enzyme 14:311-317

[8] Ribera, E., Jose, M., Martinez-Vasquez, J.M., Inma, Ocana, Rosa, M, Segura, R.M. and Pascual, C. (1987) Activity of adenosine deaminase in cerebrospinal fluid for the diagnosis and follow-up of tuberculous meningitis in adults. J. Inf. Dis. 155, 603-607.

[9] Giust G. (1974) Adenosine Deaminase In: Methods of Enzymatic Analysis. Bergmeyer HU (Ed.), New York Academic Press, USA, 1092-1099.

[10] Neves DD, Silva Junior CT, Preza PCA, Morisson P (2004) Adenosine deaminase activity (ADA) measurement. Pulmão RJ. 13 (3): 182-189.

[11] Molavi, A. and Le Frock, J.L. (1985) Tuberculous Meningitis. Med. Clinics. North Am. 69, 315-331.

[12] Blake J, Berman P. (1982). The use of adenosine deaminase assays in diagnosis of tuberculosis. S Afr Med Journ. 62: 19.

[13] Malan C, Donald DR, Golden M. (1984). Adenosine deaminase levels in CSF in the diagnosis of TBM. J Trop Med Hyg. 87 (1): 33-40.

[14] Prasad R, Kumar A, Khanna BK *et al*.(1991) Adenosine deaminase activity in cerebrospinal fluid for diagnosis of tuberculous meningitis. *Indian J Tub*; 38: 99-102.

[15] O P Mishra; V Loiwal; Z Ali; G Nath; L Chandra; B K Das, (1995) Cerebrospinal fluid adenosine deaminase activity and C-reactive protein in tuberculous and partially treated bacterial meningitis. Indian pediatrics;32(8):886-9.

[16] Martinez- Vasquez, J.M., Ocana, I. and Ribera, E. (1986) Adenosine deaminase activity in diagnosis of tuberculous peritonitis. Gut. 27, 1049-1053.

[17] Rajpal S Kashyap, Rani P Kainthla, Anju V Mudaliar, Hemant J Purohit, Girdhar M Taori and Hatim F Daginawala, (2006). Cerebrospinal Fluid Research. 3:5doi: 10.1186/1743-8454-3-5

[18] Satya Vati Rana, Raj Kumar Singhal, Kartar Singh & Lata Kumar. (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.

[19] Rajesh Baheti, PurnimaLaddha, RS Gehlot, (2001). CSF - Adenosine Deaminase (ADA) Activity in Various Types of Meningitis, Journal, Indian Academy of Clinical Medicine, Vol. 2, No. 4. 285-87.

[20] Gupta B K, Bharat Vinay, BandyopadhyayDebapriya, (2010). Role of Adenosine deaminase estimation in differentiation of tuberculous and non-tuberculous exudative pleural effusions, J Clin Med Res. 2(2):79-84.

[21] Gupta B K, Bharat Vinay, BandyopadhyayDebapriya, (2010). Sensitivity, specificity, negative and positive predictive values of Adenosine deaminase in patients of Tubercular and Non-Tubercular Serosal Effusion in India, J Clin Med Res. 2(3):121-126.

[22] TuonF F, Higashino H R, Banks M I, Lopes F, Litvoc M N, Atomiya A N, et al,(2010). Adenosine deaminase and tuberculous meningitis - A systematic review with meta-analysis, scand J Infec Dis;42:198-207

[23] Gupta B K, Bharat Anchit, BandyopadhyayDebapriya, HarenBaruah, (2010). Adenosine deaminase levels in CSF of Tuberculous meningitis patients", J Clin Med Res; 2(5):220-224