Original Research Article

Cerebrospinal fluid adenosine deaminase level as a diagnostic marker in adult tuberculous meningitis: a study conducted in a tertiary care hospital of Eastern India

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ABSTRACT

Background: Tubercular meningitis is one of the highly prevalent form of meningitis in the world and is a significant cause of morbidity and mortality in developing countries like India. Lack of early and timely diagnosis and subsequent initiation of treatment makes the fatality rate even higher. Cerebrospinal fluid (CSF) analysis is most important aspect of lab diagnosis in tuberculous meningitis (TBM) worldwide. The objective of this study was to study the cerebrospinal fluid CSF adenosine deaminase (ADA) levels in TBM and non-TBM meningitis cases and to determine its diagnostic significance as a biochemical marker of TBM infection.
Methods: The study population comprised three different patient groups. TBM (n=36), pyogenic meningitis (n=17) and aseptic meningitis group (n=12). Total 75 subjects were enrolled consecutively in the study and CSF specimens were collected from them. ADA and other cytological and biochemical estimation were carried out using standard protocol.
Results: ADA level in TBM in compare to non-TBM was more and mean ADA level of TBM, AM and PM are 26.423±3.8, 2.602±0.5 and 6.29±0.3 respectively. There are highly significant differences between the TBM and non-TBM groups and also in compare with individual groups.
Conclusions: CSF ADA levels are elevated in the TBM cases as compared to the non-TBM - meningitis cases. Results are statistically significant. It is a simple and inexpensive diagnostic adjunctive test in the rapid and early diagnosis of TBM.

Keywords: Tuberculosis, Meningitis, ADA

INTRODUCTION

Tuberculosis remains the single largest infectious disease-causing high mortality leading to more than 3 million deaths annually, about five deaths every minute. India is the highest TB burden country with world health organization (WHO) statistics for 2011 giving an estimated incidence figure of 2.2 million cases of TB for India out of a global incidence of 8.7 million cases.[1] In India, an estimated 3 lac deaths occur from tuberculosis every year and nearly 900 people die of tuberculosis that is approximately more than three deaths every five minutes. An estimated 15% of all tubercular infections are extra-pulmonary consisting of TB lymphadenitis, genitourinary TB, central nervous system TB and others. Extra pulmonary involvement can occur in isolation or along with a pulmonary TB in the patients with disseminated tuberculosis. Central nervous system tuberculosis is the most severe form of extra-pulmonary tuberculosis. It includes tubercular meningitis (TBM)

which occurs in 4.5% of all cases[2]. The diagnosis of TBM is difficult because TB meningitis presents with nonspecific symptoms and signs. Bacterial yield is poor.[3] Other sophisticated investigation and imaging are expensive and resource based. Early diagnosis and treatment of the disease is very important as the disease can result in morbidity and mortality if left delay or untreated. However, the other factor that makes diagnosis difficult is the small number of bacilli in the CSF due inadequate treatment which reduce the sensitivity of conventional bacteriology.[4] In recent years, new diagnostic assays, in particular molecular techniques like GeneXpert MTB/RIF/CBNAAT have been developed, and these could contribute to the diagnosis of extra pulmonary forms of tuberculosis.

Adenosine deaminase (ADA) is an enzyme widely distributed in tissues and body fluid used in the diagnosis of TB in pleural, meningeal and pericardial fluids.[5] ADA is an enzyme which is required for lymphocyte proliferation and differentiation, and the principal biological activity is detected in T-lymphocytes.[6] Although the measurement of ADA has become popular in various institutions across the world, there is no consensus regarding its current usefulness in clinical practice.

This cross-sectional study was performed to determine the usefulness of ADA measurement in the diagnosis of TBM and differentiate TBM patients from the one suffering from meningitis of different etiology.

METHODS

Total 75 adult patients (of age 12 years and above) of meningitis of varied etiology admitted in the medical ward and emergency over a period of two years (2016-2018) in Burdwan Medical College and Hospital were recruited as participants. The diagnosis of meningitis was established by detailed clinical history, neurological examination and laboratory findings.

Patients with focal neuro-deficit, taping site infection, brain tumour, hemi-cranial headache, cranial nerve palsies, bleeding disorders, cardiovascular instability, raised intracranial pressure (except raised fontanels), and papilledema were excluded. Based on diagnosis patients were segregated into three groups, group A contains patients with TBM (n=36), group B contains patients with pyogenic meningitis (PM) (n=17), and group C had patients with aseptic meningitis (AM) (n=22).

CSF samples of the subjects were collected by standard lumbar puncture. All samples were stored at 4°C and estimated within 24 hours. All CSF samples were undergone biochemical and cytological analysis that includes estimation of protein, sugar chloride levels. ADA levels were estimated in all samples of CSF using the GALANTI and GIUSTI methods.

Statistical analysis

Data was entered on Microsoft excel. Continuous variables were summarized as mean and standard deviation. Categorical variables were summarized as percentages. One-way analysis of variance (one-way ANOVA) was used to analyze mean differences among different diagnosis groups. To compare the mean ADA activity between the TBM, non-TBM infectious meningitis and non-infectious neurological disorders groups, the Kruskal-Wallis test (non-parametric analysis of variance ANOVA) with the Dunnett post-test was used. A p value <0.05 was considered significant.

RESULTS

In our study the febrile patients with neurological sign and symptoms, total 75 patients selected. Clinical presentation of patients with meningitis are fever (85.33%), headache (89.33%), nausea and vomiting (70.66%), meningeal sign (60.00%), Kernig's sign (89.33%), neck rigidity (84.00%), altered sensorium (54.66%), focal neurological deficit (57.33%), seizures (24.00%), hepatomegaly (46.66%), splenomegaly (30.66%) and adenopathy (34.66%).
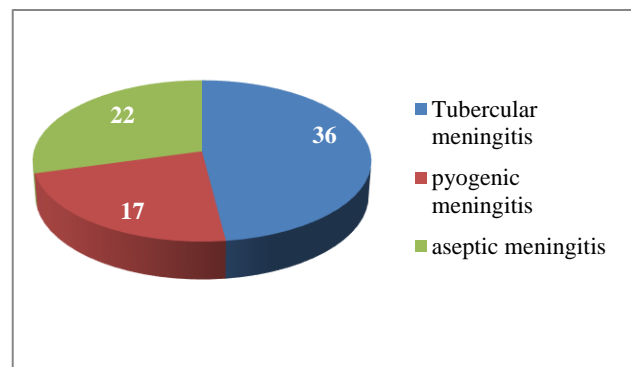

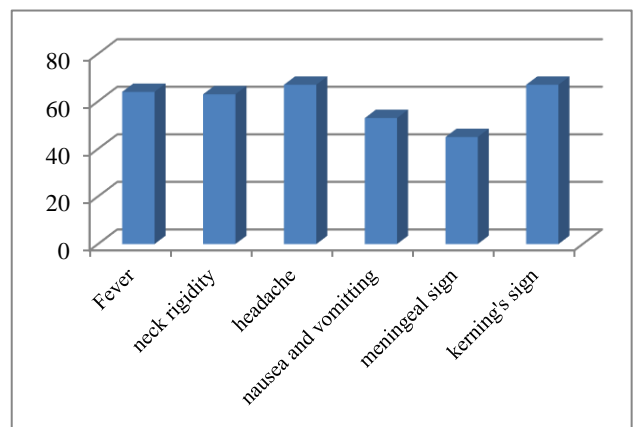**Figure 1: Distribution of the cases of different types of meningitis (n=75).**

Figure 2: Clinical presentation of patients with meningitis (n=75).

Table 1: Nutritional status in BMI of meningitis (n=75).

Nutritional status in BMI	TBM N (%)	PM N (%)	AM N (%)
<16 (very severely under weight)	04 (11.11)	0	0
16-16.9 (severely under weight)	06 (16.66)	01 (05.88)	0
17-18.4 (under weight)	15 (41.66)	06 (35.29)	08 (36.36)
18.5-24.9 (normal)	11 (30.55)	10 (58.82)	14 (63.63)
Total (n=75)	36 (48)	17 (22.66)	22 (29.33)

Neck rigidity and Kernig's sign were present in majority of cases (Table 2) (Figure 2). Among all the participants 36 (48%) patients were in the TBM group, 22 (29.33%) patients were in the aseptic group and rest i.e., 17 (22.66%) were within pyogenic meningitis (Figure 1).

Table 2: Clinical presentation of patients with meningitis (n=75).

Clinical symptoms and signs	Number	Percentage (%)
Fever	64	85.33
Headache	67	89.33
Nausea and vomiting	53	70.66
Meningial sign	45	60.00
Kernig's sign	67	89.33
Neck rigidity	63	84.00
Altered sensorium	41	54.66
Focal neurological deficit	43	57.33
Seizure (s)	18	24.00
Hepatomegaly	35	46.66
Splenomegaly	23	30.66
Adenopathy	26	34.66

Nutritional statuses in BMI of Meningitis were calculated. Nutritional status was under weight and severely underweight nearly 70%. On the other hand, in other meningitis groups more than 50% were within normal BMI (Table 1).

Out of 75 patients, mean age in years were 36.21 ± 15.21, 38.59 ± 18 and 3032.43 ± 12.43 and sex ratio (male: female) were 1.25:1, 1.43:1 and 1.75:1 in tubercular meningitis, pyogenic meningitis and aseptic meningitis respectively (Table 3).

Age distributions of different meningitis were different. In TBM, 41-60 years age groups were commonly presented (52.77%). On the other hand, younger i.e. less than 20 years were commonly presented in other meningitis groups (Table 4). ESR of meningitis patients were tabulated and it was seen that nearly 90% patients of TBM ESR had more than 40 in respect to other it is reverse (Table 5). In calculation of CSF sugar level according to the type of meningitis, less than 40 mg/dl in more than 59% TBM. But 80% in PM and 100% in AM had more than 40 mg/dl (Table 6). Most of TBM patients CSF protein was more than 60 mg/dl. In non-TBM it was not increased. In aseptic meningitis it was often raised more than 60 mg/dl (Table 7).

ADA level in TBM in compare to non-TBM was more and mean ADA level of TBM, AM and PM are 26.423 ± 3.8, 2.602 ± 0.5 and 6.29 ± 0.3 respectively. There are highly significant differences between the TBM and non-TBM groups and also in compare with individual groups (Table 8 and 9).

Table 3: Age and sex ratio of the different study population groups (n=75).

Study group		No. of patients (n=75)	Age (years) (mean±SD)	Sex ratio (M: F)
TBM	Tubercular meningitis	36	36.21 ± 15.21	1.25:1
Non-TBM	Pyogenic meningitis	17	38.59 ± 18.30	1.43:1
	Aseptic meningitis	22	32.43 ± 12.43	1.75:1

Table 4: Age distribution of meningitis (n=75).

Age in years	TBM N (%)	PM N (%)	AM N (%)
<20	04 (11.11)	07 (41.17)	08 (36.36)
21-40	11 (30.55)	03 (17.64)	06 (27.28)
41-60	19 (52.77)	03 (17.64)	07 (31.81)
>60	02 (05.55)	04 (23.52)	01 (04.54)
Total	36 (48)	17 (22.66)	22 (29.33)

Table 5: ESR of meningitis (n=75).

ESR in mm	TBM	PM	AM
	N (%)	N (%)	N (%)
<40	04 (11.11)	17 (100)	21 (95.45)
41-60	23 (63.88)	0	01 (4.55)
>60	09(25)	0	0
Total	36 (48)	17 (22.66)	22 (29.33)

Table 6: CSF protein level according to the type of meningitis (n=75).

CSF protein (mg/dl)	No. of cases	TBM	PM	AM
	N (%)	N (%)	N (%)	N (%)
<60	21 (28)	0	13 (76.47)	19 (86.36)
61-80	37 (49.33)	11 (30.55)	2 (11.76)	3 (13.63)
81-120	19 (25.33)	17 (47.33)	2 (11.76)	0
>120	8 (10.66)	8 (22.22)	0	0
Total	75	36	17	22

Table 7: CSF sugar level according to the type of meningitis.

CSF sugar (mg/dl)	No. of cases	TBM	PM	AM
	N (%)	N (%)	N (%)	N (%)
<40	31 (41.33)	29 (80.55)	02 (11.76)	0
>40	44 (58.66)	07 (19.44)	15 (88.23)	22 (100)
Total	75	36	17	22

Table 8: Distribution of the cases according to set criteria and CSF ADA levels (n=75).

Group	No. of cases N (%)	ADA levels in U/l (mean±SD)	ADA levels in U/l	N (%)	Mean±SD	t cal	P value
Tubercular meningitis	36 (48%)	26.423±3.8	ADA≥10	34 (94.44)	28.342±1.03	4.1834	0.0006 (p<0.01)*
			ADA<10	02 (05.56)	09.563±2.32		
Non-TBM	39 (52%)	4.654±2.6	ADA≥10	03 (07.69)	6.061±9.34		
			ADA<10	36 (92.30)	3.954±4.21		
Total	75			75			

*A p value <0.05 was considered significant. There are highly significant differences between the TBM and non-TBM groups.

Table 9: Compare of ADA in TBM with others (n=75).

Group	t value	P value	Degrees of freedom DF
TBM vs PM	3.4912	<0.0001	44
TBM vs AM	3.7843	<0.0001	44
TBM vs non-TBM (both)	4.1834	<0.0006	56

P value <0.05 was considered significant.

DISCUSSION

Making a differential diagnosis between TBM and non-TBM is a critical clinical problem. Conventional methods like direct examination of CSF, are positive in only 5~20% of cases. The rate of positivity is about 40% in culture, which takes about 6 weeks.[7,8] Cerebrospinal levels of various biomarkers have been proposed to be helpful in the diagnosis of TBM, including ADA. TBM is one of the most serious forms of neuro-tuberculosis. India is among the nations with high incidence of TB. Usually there are 20% of extra pulmonary cases of whom 15% are neuro-tuberculosis.[9] TBM is associated with a high frequency of neurologic sequelae and mortality if not treated promptly. In recent times, even after the advent of molecular testing, diagnosis of tubercular meningitis (TBM) continues to be a clinical challenge in which disproportionate inflammatory exudates rather than numbers of circulating bacteria make bacteriological diagnosis difficult, and the available microbiological tests fail to attain the accuracy standards required.[10] As a result, most guidelines for the diagnosis and management of TBM depend on clinical setting, CSF analysis including ADA.

A total of seventy-five patients admitted during this period fulfilled the inclusion criteria. Out of 75 patients included in the study 22 (29.33%) cases were AM, 17 (22.66%) cases were PM and 36 (48%) cases were TBM.

The common clinical presentations of meningitis observed in our study was fever 74 (98.6%) followed by vomiting 62 (82.66%), headache 49 (65.33%), neck stiffness 45 (60.0%), Kerning's 25 (33.33%), altered sensorium 19 (25.33%), seizures 15 (20.0%), irritability 7 (9.33%) and Brudzinski's sign 5 (6.66). Similar clinical presentations were also noted by Rashid et al, Baheti et al but with closely different frequencies.[11,12]

We observed a significant high level of ADA 26.423±3.8 (9.50, 59.0) among the TBM cases. Its respective levels among AM was 2.602±0.5 (1.60, 8.2), PM was 6.29±0.3 (4.0, 9.7) and all the non-TBM was 4.654±2.6 (1.60, 9.7) U/l. Each of the differences was significant (p<0.05). In Rashid et al study, the mean level of ADA was 30.0±3.2 (20.0 and 54.0) among tubercular meningitis (TBM) cases which was close to our study.[11]

Rana et al in their analysis found mean±SE ADA levels in tubercular meningitis (TBM) of 18.22±3.35 (1.0-96.7), partially treated pyogenic meningitis (PTM) 6.28±0.91 (3.0-11.1), pyogenic meningitis (PM) 7.98±3.56 (0.3-29.0), Aseptic meningitis (AM) 3.43±0.86 (0.1-08.5), This difference of ADA values in CSF between TBM and other types of meningitis was statistically significant (p<0.01) which is comparable with our study.[13] The CSF-ADA value in TBM cases ranged from 9.1 to 48.0 IU/l with mean value of 26.423 IU/l and SD ±3.421 as shown in our study. Comparative study showed statistically significant difference in CSF-ADA level of TBM and other groups of meningitis (p<0.0006). This significantly high value of CSF-ADA had also been shown by various researchers like Kashyap et al.[14]

CONCLUSION

In conclusion, current evidence suggests a potential role of ADA assays in confirming a diagnosis of TBM. There is a great need to develop CSF biomarkers specific for TBM. Thus, it is evident that CSF ADA activity determination is a useful test for the early diagnosis of TBM. Since it is simple, relatively inexpensive and takes less time to perform, it can be included as rapid diagnostic test for TBM. The results of ADA assays should be interpreted with clinical findings and other examinations.

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