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Cerebrospinal Fluid Adenosine De Aminase and Lactate Levels as a Rapid Diagnostic Marker for Tuberculous Meningitis among Adults

Archa Anna Anil[1],
[1]Senior Resident, Department of General Medicine,
Sri Ramachandra Institute of Higher Education and Research, Chennai

Mohini Singh[2*],
[2]Associate Professor, Department of General Medicine,
Sri Ramachandra Institute of Higher Education and Research, Chennai

S.R. Ramakrishnan[3],
[3]Professor, Department of General Medicine,
Sri Ramachandra Institute of Higher Education and Research, Chennai

N. Rajeev Roy[4]
[4]Research Scholar, ICMR MD-PhD, Department of Community Medicine,
Sri Ramachandra Institute of Higher Education and Research, Chennai

ABSTRACT

Tuberculous Meningitis (TBM) with a high mortality requires early diagnosis and prompt treatment. Current advances have brought out expensive investigations which help in early diagnosis of TBM. This study intends to bring out the diagnostic role of CerebroSpinal Fluid (CSF) Adenosine Deaminase (ADA) and lactate in differentiating TBM from non-TBM **Objectives:** To determine the role of CSF Adenosine Deaminase and lactate levels as a rapid diagnostic test for differentiating pyogenic and non-pyogenic meningitis in adults. **Methodology:** Cross sectional study involving 58 participants, with participants having presumptive Tb or TB by biochemical assays categorised as group A and the remaining participants as group B. Cut-off values for CSF ADA and Lactate were calculated using ROC, statistical analysis done and 95% confidence interval limits was calculated. **Results:** CSF ADA levels above 6.05IU/L had 9.24times chances of predicting TBM and those with CSF lactate levels above 47.0mg/dl had 18.0 times greater chance of being diagnosed with TBM. **Conclusion:** Widespread use of CSF ADA and Lactate, which are less expensive and undemanding especially in TBM helps in early diagnosis and prompt initiation of treatment.

Keywords: Meningitis, Tuberculosis, Cerebrospinal Fluid Analysis, Adenosine De Aminase, Lactate

INTRODUCTION

An inflammatory response of the pia, arachnoid and sub arachnoid space CSF resulting either from bacterial or non-bacterial infection. Bacterial meningitis includes pyogenic and tuberculous meningitis while non-bacterial meningitis includes viral meningitis.

A preventable and curable disease namely Tuberculosis is responsible for claiming 4000 lives per year with millions falling sick due to the disease. WHO in their Global TB report estimates the burden of Tb at 10 million in the year 2019 with 1.4 million people having lost their lives. In 2019, eight countries accounted for two thirds of new cases of TB among which India was the leading contributor[1]. The incidence of various forms of extrapulmonary tuberculosis depends on the prevalence of tuberculosis infection and CNS TB accounts for only 10% of all those cases[2].

In patients with extrapulmonary tuberculosis, though central nervous system tuberculosis is seen only among 5-10%, the outcomes in terms of mortality and morbidity are much higher than with any other forms of tuberculosis[3].Tuberculous meningitisis essentially one of the most severe form of tuberculosis which was first described by Willis as early as 17th century[4]

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and still poses a significant public health issue all over the world.Factors such as deficiencies in the knowledge of the pathogenesis, lack of reliable and affordable diagnosticslead to diagnostic delay in tuberculous meningitis which can be an impediment to favourable long-term clinical outcomes.

Adenosine Deaminase (ADA) enzyme catalyses the conversion of deoxyadenosine to deoxyinosine and adenosine to inosine in the purine salvage pathway. Ten times higher levels are seen in T-lymphocytes and their concentration decreases with increase in differentiation of lymphocytes[5]. A marker of T lymphocyte activation, ADA is released by Tubercle bacilli following activation of cell medicated immune response. Several studies have used ADA levels to differentiate between tubercular meningitis from bacterial meningitis[6-8].The cut-off values for ADA are different as suggested by various studies for TBM thereby suggesting a lack of standardization[8-10]. The sensitivity ranges from 40% to 100%, and the specificity between 70% to 100% with several factors influencing the outcomes such as the selected cut-off values and local prevalence of tuberculosis[10-12].

Another diagnostic marker which is a cost effective, easy to estimate and which could aid in easy estimation of TBM. Conditions which decrease oxygen supply to the brain resulting from anaerobic metabolism tends to increase CSF lactate levels[13]. Studies on the predictive value of CSF lactate levels have focussed on bacterial meningitis and used it as a differentiating factor for pyogenic and non-pyogenic meningitis[13,14].
Very few studies are available on CSF ADA and Lactate levels in TBM, which are less expensive and easier to evaluate even is low-cost settings where the possibility of performing a Polymerase Chain Reaction is difficult. This intent of this study is to assess the diagnostic accuracy of both CSF ADA and lactate levels in the diagnosis of TBM.

METHODOLOGY

A cross sectional study of 58 patients who presented to the departments of General Medicine and Neurology from September 2017 to October 2018 with clinical features of meningitis at Sri Ramachandra Institute of Higher Education and Research. Patients of either sex above the age of 18 with clinical features of meningitis like fever, headache, altered sensorium, neck stiffness, photophobia or projectile vomiting were included in the study.

Exclusion criteria: 1. Patients with contraindications for lumbar puncture 2. Patients who had trauma, surgery or cerebrovascular accidents within 3 months 3. Patients who received antibiotics prior to obtaining the Cerebrospinal fluid (CSF) The study was approved by the institutional ethics committee and written informed consent was obtained from the study participants or from the attender (in case of loss of consciousness) prior to the start of the study. Detailed history, relevant symptoms and and other comorbidities were recorded. All participants were subjected to lumbar puncture under aseptic precautions and appropriate investigations (CSF – total and differential counts, protein, glucose, lactate, chloride, adenosine deaminase (ADA), procalcitonin, GeneXpert and CSF culture), were carried out. Based on the clinical criteria for TBM (Table 1) the population was divided into two groups

- Group A included participants with Clinical Criteria for TBM score 4 and above
- Group B included the rest of the participants
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IBM SPSS software version 16.0 was used for the statistical analysis. Descriptive statistics was employed in the form of mean and standard deviation for quantitative variables and, frequency and proportion for categorical variables. Chi square test/ Fisher’s exact test was

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used to test statistical significance and p value < 0.05 within 95% confidence limits was considered statistically significant.

RESULTS

This study included 58 subjects between the age group of 19 to 89 years with 41.7% of study subjects of the TBM group were in the age group of 56 – 70 years. Sex distribution was equal between the groups (Table 2). Based on the CSF biochemical parameters 12 patients were diagnosed with definitive TB / probable TB and remaining 46 were included in group B as non-tuberculous meningitis. Mortality and recovery were equal in TBM while a higher percentage of recovery was seen in group B. There was significant difference among mean values of parameters like WBC, proteins, ADA and Lactate which was statistically significant ($p < 0.0001$). However mean difference among other parameters like glucose and procalcitonin was not statistically significant (Table 3).

CSF ADA level of 6.05IU/L was taken as a reference based on Receiver Operating Curves (area under curve 0.924, sensitivity 91.7 specificity 63.0, $p< 0.001$)Among study participants, 91.66% of those with TBM and 54.35% of non TBM had elevated levels. The risk of being diagnosed as TBM was about 9.24 times when ADA levels were elevated above6.05IU/L (Table 4).

CSF Lactate level of 47 mg/dl was taken as a reference based on Receiver Operating Curves (area under curve 0.894, sensitivity 83.3, specificity 79.3, $p < 0.001$) Among study participants, 83.3% of those with TBM and 21.7% of non TBM had elevated CSF lactate levels. The risk of being diagnosed as TBM was about 18.0 times when CSF lactate levels were elevated above the reference level of 47 mg/dl (Table 5). The availability of extensive studies on other CSF parameters like WBC, proteins and glucose to differentiate TBM from non-TBM has made us conduct this study focussing on ADA and Lactate levels. Between the two parameters, CSF lactate levels have a higher association in detecting TBM compared to ADA levels. Hence a combination of both the parameters would help in early clinical diagnosis of TBM.

DISCUSSION

Higher incidence of tuberculosis and non-availability of well-equipped laboratory services has been the most common reasons for the increased prevalence of TBM especially in the low and middle-income countries. In the past several studies have highlighted the usefulness of CSF-ADA and Lactate in differentiating TBM from non-TBM. In this study, an attempt is made to determine and validate lower cut-off value of CSF-ADA and lactate for differentiating TBM from non-TBM in a middle-income country setting like India.

In the present study, mean CSF ADA values of 17.98 ± 8.51 of group A was higher than 4.62 ± 2.72 of group B and this difference was statistically significant($p<0.0001$). In a similar study by Raviraj et al, the mean ADA levels among TBM group was 10.97 ± 4.43 and that of non-TBM group was 5.09 ± 1.53 and the difference was statistically significant[16].

In the present study with cut-off values for CSF ADA of 6.05 IU/L when applied gave a positive likelihood ratio 2.11 and negative likelihood ratio of 0.15.In a study by Raviraj et al, the positive likelihood ratio was 5.44 and the negative likelihood ratio was 0.17 when a cut off of 6.65 IU/L was applied[16]. In contrast, a study by Chander A et al, a higher cut off of 10 IU/L was used which yielded a higher positive likelihood ratio of 9.03% and a similar negative likelihood ration of 0.19%[18] These studies (Table 6) suggest that despite a lower cut

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off value for CSF ADA in TBM, the positive and negative likelihood ratios were comparable to other studies.

The current study with cut off values of 2.61 mmol/l for CSF lactate had 83.3% of those with TBM and 21.7% of those with non TBM above the cut off levels. Also, the mean difference between the two groups in terms of CSF lactate levels was also higher for TBM with statistical significance ($p<0.0001$). Previous literature studies have demonstrated a cut off value of 4.2 mmol/l as a predictor of bacterial meningitis. However in a study by KarthikaRemash et al shows that CSF lactate levels of 3mmol/l could be a reliable predictor for diagnosing bacterial meningitis from non-bacterialmeningitis[14].Studies by Curtis et al in 109 patients with proven bacterial meningitis showed the mean lactate concentration to be above 2.8 mmol/l in all the patients[19,20]. This study attempts to highlight the importance of CSF Lactate in the diagnosis of TBM while most other studies have taken TBM as a part of bacterial meningitis. As the clinical features and lab parameters are similar in TBM and viral meningitis, CSF lactate could come handy especially in the absence of a conclusive CSF gram stain and culture for initiating early treatment and improved outcomes in TBM.

CONCLUSION

Despite the fact that CSF lactate could be elevated due to other conditions which cause CNS hypoxia and infarction, till a cost-effective diagnostic modality for TBM is found, CSF ADA and Lactate could provide an answer to saving millions of lives. A lower cut off value of 6.05 IU/L for CSF ADA together with 2.61mmol/l cut off for CSF lactate could be used in addition to other routinely used CSF parameters like mononuclear lymphocytosis, low glucose and high protein, as a rapid and economical diagnostic modality to differentiate TBM.

LIMITATIONS AND FUTURE STUDIES

The small sample size and the use of GeneXpert as a diagnostic tool for TBM are the major limitations of this study

CONFLICTS OF INTEREST

There is no conflict of interest.

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Table 1: Clinical criteria for TBM[15]

Variable		Score
Age in years	≥ 36	+2
	< 36	0
Blood WCC (1000/ml)	≥ 15000	+4
	< 15000	0
History of Illness	≥ 6 days	-5
	< 6 days	0
CSF total WCC (1000/ml)	≥ 750	+3
	< 750	0
CSF Neutrophils	≥ 90%	+4
	< 90 %	0

Table 2: Demographic details of study population

Parameter	Group An (%)	Group Bn (%)
Age Group		
< 25 years	1(8.3)	5 (10.9)
26 – 40 years	3 (25.0)	15 (32.6)
41 – 55 years	2 (16.7)	10 (21.7)
56 – 70 years	5 (41.7)	12 (26.1)
> 70 years	1 (8.3)	4 (8.7)
Gender		
Male	8 (66.7)	31 (67.4)
Female	4 (33.3)	15 (32.6)
Clinical Symptoms (fever, headache, altered sensorium and nuchal rigidity)		
All 4 symptoms present	1 (8.3)	0
3 symptoms present	1 (8.3)	12 (26.1)
2 symptoms present	8 (66.7)	25 (54.3)
1 symptom present	2 (16.7)	9 (19.6)
Mortality patterns		
Recovered	6 (50.0)	35 (76.1)
Death	6 (50.0)	11 (23.9)

Table 3.Descriptive Statistics of CSF biochemical parameters among study participants

Parameter	Group A	Group B	p value
WBC	95.50 (± 12.17)	204.33 (± 63.86)	< 0.0001
Protein (mg.dl)	341.90 (± 16.22)	125.77 (± 12.20)	< 0.0001
Glucose (mg/dl)	44.83 (± 26.16)	61.63 (± 30.56)	0.087
Chloride (mg/dl)	114.08 (± 8.34)	119.83 (± 7.92)	0.031
ADA (IU /L)	17.98 (± 8.51)	4.62 (± 2.72)	< 0.0001
Lactate (mmol /L)	3.55 (± 0.82)	2.02 (± 0.94)	< 0.0001

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Procalcitonin (IU/L)	0.368 (± 0.09)	0.326 (± 0.10)	0.192

Table 4: Distribution of CSF ADA levels

Parameter	Group A	Group B	Total	Chi Square (p value)*	Odds Ratio (CI)
CSF ADA levels >6.05mg/dl	11 (91.66)	20 (54.35)	31	5.63 (< 0.05)	9.24 (1.01 – 77.57)
CSF ADA levels <6.05mg/dl	1 (8.33)	26 (45.65)	27		

*t test with 95% confidence levels, Fisher's exact test

Table 5: Distribution of CSF Lactate levels

Parameter	Group A	Group B	Total	Chi Square (p value)	Odds Ratio (CI)
CSF Lactate levels >2.61 mmol/l	10 (83.3)	10 (21.7)	20	15.98 (< 0.0001)	18.0 (3.38 – 95.81)
CSF Lactate levels <2.61 mmol/l	2(16.7)	36 (78.3)	38		

*t test with 95% confidence levels, Fisher's exact test

Table 6: Cut off values for CSF ADA by different authors

Study	Sample Size	ADA reference level (IU/L)	Sensitivity (%)	Specificity (%)	Year
Present study	58	6.05	91.7	63.0	2020
Raviraj et al[16]	85	6.65	85.3	84.3	2017
Kashyap et al[9]	177	11.39	82	83	2006
Gautam N et al[17]	45	6.97	85	88	2007