

Differential Diagnosis of Cerebral Malaria by CSF ADA, Serum ADS and its Ratio

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ABSTRACT

A case control study was conducted comparing CSF ADA, Serum ADA and its ratio for differentiating cerebral malaria from viral encephalitis in the Department of Pediatrics, M.L.B. Medical College, Jhansi during January 2010 – October 2011. Patients admitted with history of fever, convulsion and altered sensorium were enrolled in the study and further divided in three groups comprising of Cerebral Malaria, Viral Encephalitis and Control Group. Lumber puncture were performed in all three groups and ADA estimation was done. The statistical analysis was performed by using chi square test to find out the significance of difference. For determination of cutoff value for parameters, coordinates of ROC curve indicating the greatest sensitivity and specificity were chosen. Fifty two patients of cerebral malaria, 56 patients of viral encephalitis and 54 controls were recruited for the study over 2 year period. Patients with cerebral malaria had significantly higher serum ADA, CSF ADA but lower CSF/ serum ADA compared to control ($p < 0.01$) as well as to viral encephalitis ($p < 0.01$). Serum ADA of >78.9 , CSF ADA of >6.31 and CSF/Serum ADA ratio of 0.093 were selected as the cutoff value with highest sensitivity and specificity for differentiation between two conditions. ADA estimation in CSF and Serum is a rapid, simple, and reliably specific test to differentiate cerebral malaria from viral encephalitis. Therefore, this test can be used to avoid diagnostic dilemma. ADA estimation in cerebral malaria patients should find place in routine laboratory methodology.

KEY WORDS: adenosine deaminase, cerebral malaria, cerebrospinal fluid, serum, viral encephalitis.

INTRODUCTION:

Acute Encephalitis Syndrome (AES) is major public health challenge at present. About 25% of affected children die and about 30-40% among survivors suffer from physical and mental impairment. In 2011, AES was reported from 135 Districts of 17 States. Till November India recorded 6297 AES cases and 861 deaths, while 70-75% of disease burden was seen in Uttar Pradesh^[1]. Malaria is a disease of global importance that results in 300-500 million cases annually. 40% of world population live in malaria endemic area and 1.5-2 million death, mostly children (<5 years) occur annually. Cerebral malaria is most common complication of P.falciparum infection, which may results in severe complications including long term n

eurological impairment, multiorgan failure high mortality rates in absence of prompt and appropriate treatment^[2]. In the Bundelkhand region where plasmodium falciparum malaria is endemic, viral encephalitis and cerebral malaria are also reported. It is very difficult to differentiate cerebral malaria from presumed viral encephalitis on clinical ground as well as by CSF studies. Rapid virological diagnostic methods to diagnose viral encephalitis are very expensive and not easily available in most of the developing countries like India^[3].

With the benefit of acyclovir in herpes encephalitis for prevention of death or neurological sequelae^[4] and the need to recognize the presence of possible arboviral encephalitis with its public health implications^[5], it has become more important to differentiate viral encephalitis from cerebral malaria to initiate prompt and appropriate therapy to reduce morbidity as well as mortality.

Chief physiological function of ADA is related to lymphocytic proliferation and differentiation. As a marker of cellular immunity, enzyme activity is found to be elevated in those

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diseases in which there is a cell mediated immune response^[6,7]. Previous investigators have found that CSF ADA may be useful in discriminating infectious disease of CNS such as meningitis, encephalitis and cerebral malaria^{8,9}. In another study, it was found that CSF ADA alone was not useful in differentiating cerebral malaria from viral encephalitis. However the CSF / Serum ADA ratio was lower in patient with cerebral malaria due to high level of ADA in peripheral blood. This ratio may be discriminating factor between cerebral malaria and viral encephalitis^[3].

In view of the above said published observations, the present study has been done to evaluate CSF ADA, Serum ADA and their ratio as discriminating factor between cerebral malaria and viral encephalitis.

MATERIALS AND METHODS:

This study was conducted in the Department of Pediatrics, MLB Medical College, Jhansi in active collaboration of Department of Pathology, MLB Medical College, Jhansi during January 2010 to October 2011.

Children of 6 months – 5 years admitted in hospital with history of fever, convulsion and altered sensorium were studied. The detailed history, complete physical and systemic examination and routine investigations were performed. After ethical approval and informed consent by parents or guardians, patients fulfilling inclusion criteria were enrolled in study and further divided in three groups A, B, C and rest were excluded.

Study group A (Cerebral Malaria): As per WHO guidelines for diagnosis of cerebral malaria^[10,11], the following criteria were considered:

- 1) Deep unarousable coma – Motor response to noxious stimuli is non localizing or absent.
- 2) Exclusion of other encephalopathies – Coma should persist for more than 30 minutes after generalized convulsion to exclude transient postictal coma, hypoglycemia, meningoencephalitis, head injury and cerebrovascular accident should be excluded as the cause of Coma.
- 3) Confirmation of Plasmodium infection – asexual form of *P. falciparum*/ *vivax* must be demonstrated in peripheral blood smear.

Study group B (Presumed viral encephalitis)

The definition of presumed encephalitis is a reduced level of consciousness and fever that could

not be explained by metabolic abnormality, dehydration or shock, negative CSF staining, microscopic examination, culture and normal CSF sugar (60% of plasma) and negative slide for malaria parasite and complete blood count within the normal limits^[12].

Control group C (Febrile convulsion):

Control subjects recruited from the population of patients who have lumbar punctures to exclude meningitis in the context of reduced consciousness following febrile convulsion and who recovered from the postictal state within 30 minutes without further sign of central nervous system illness or septicemia.

Exclusion criteria:

Exclusion criteria included: CSF showing predominantly polymorphs and decrease sugar or gram staining/AFB staining or culture positive for organisms; CSF showing lymphocytic pleocytosis with markedly elevated protein and decrease sugar; *P. falciparum* positive comatose patient of less than 30 min duration after convulsion; CT scan finding of hydrocephalus with basal exudates or meningeal enhancement of tuberculoma or ICSOL or Hemorrhage; *P. falciparum* negative with deranged RFT, Blood sugar, serum calcium and LFT; Hypertensive patients will be excluded; History or evidence of poisoning or exogenous intoxication.

Sample analysis:

CSF was obtained by lumbar puncture as soon as possible after admission, if there were no contraindications, and after informed consent obtained from their parents. CSF cell count, glucose, protein and were determined by standard methods as in previous studies^[13]. ADA levels were determined using the Berthelot reaction through the ammonia released when adenosine is broken down to inosine. After incubation of plasma or CSF with a buffered solution of adenosine, the ammonia is reacted with a Berthelot reagent to form a blue colour, which is proportional to the amount of enzyme activity^[14].

Statistical calculations

A total of 162 children were enrolled in the study, of which 52 patients of cerebral malaria, 56 cases of presumed viral encephalitis and 54 cases of febrile convulsion. The statistical analysis was performed by using Chi square test to find out the significance of difference. Statistical calculations

were performed with SPSS version 12.0 and Medcalc data analysis software. For determination of a cutoff value for a CSF parameter for possible discrimination between presumed viral encephalitis and cerebral malaria, the coordinate of a receiver operating characteristic (ROC) curve indicating the greatest sensitivity and specificity was chosen. The ROC curve is a computer generated curve of data from the malaria and viral encephalitis patients with sensitivity on the vertical axis plotted against 1-specificity (T-true negative rate) on the horizontal axis^[15,16]. Accuracy of test is measured by area under ROC curve an area of 0.9 – 1.0 represents perfect test while 0.5 – 0.6 represents failed test^[17]. A p value <0.05 was taken as indicator for a statistically significant difference.

RESULTS:

Table I reflects that patient with cerebral malaria had higher CSF ADA than that with cases of viral encephalitis and febrile convulsion. When these values were compared statistically significant difference was seen with viral encephalitis (p value 0.0078) and also with febrile convulsion with p value <0.0001).

Table 1 : CSF Adenosine Deaminase (ADA) of cases

CSD ADA (IU/L)	Cerebral malaria n (%)	Viral encephalitis n (%)	Febrile convulsion n (%)
0-5	14 (26.92%)	23 (41.07%)	41 (75.93%)
5-10	32 (61.54%)	32 (57.14%)	10 (18.52%)
>10	6 (11.54%)	1 (1.79%)	3 (5.56%)

From table II, it is evident that serum ADA in cerebral malaria was higher than viral encephalitis and febrile convulsion. When these values were compared statistically highly significant difference was seen with p value (<0.0001 and <0.0001) respectively.

Table III reveals that in our study, ratio of CSF and serum ADA in cerebral malaria lower than viral encephalitis and febrile convulsion. When these values were compared statistically significant difference was seen with (p value 0.0001) and (p value 0.00182) respectively.

Table IV reveals cut off value of parameters we use to differentiate cerebral malaria from viral encephalitis. The best cut off value was taken from the coordinates of ROC curve. It also shows sensitivity and specificity with 95% confidence limit along with positive predictive value and area under ROC curve.

For CSF ADA best cut off is (>6.31) i.e. above 6.31 IU/L cerebral malaria is more likely than viral encephalitis with sensitivity 59.62% and specificity 77.27%. This table also reveals area under ROC curve. For serum ADA area under curve 0.915 which signifies that serum ADA is perfect test to differentiate cerebral malaria from viral encephalitis. However CSF ADA had fair ability (area under curve is 0.7) but ratio had become poor test (area under curve is 0.68) in differentiating cerebral malaria from viral encephalitis.

Figure 1 shows that for Serum ADA best cut off is (>78.9) i.e. above 78.9 IU/L cerebral malaria is more likely than viral encephalitis with sensitivity 96.15% and specificity 88.45%.

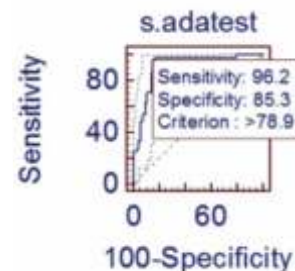


Figure 1: ROC curve of Serum ADA Test

Area under ROC curve	=	0.915
Standard error	=	0.0239
95% confidence interval	=	0.861-0.953
Z statistics	=	17.355
Significance level (p)	=	<0.0001

Figure 2 shows that for CSF/Serum ADA ratio best cut off is (<0.093) i.e. below which cerebral malaria is more likely than viral encephalitis with sensitivity 90.38% and specificity 48.18%.

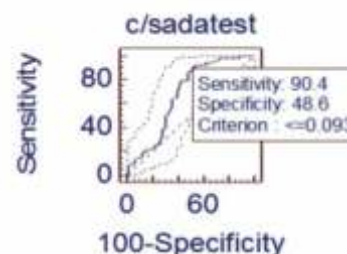


Figure 2: ROC curve of CSF/Serum ADA Ratio Test

Area under ROC curve	=	0.680
Standard error	=	0.0418
95% confidence interval	=	0.602-0.751
Z statistics	=	4.302
Significance level (p)	=	<0.0001

Table 2: Serum Adenosine Deaminase (ADA) of cases.

Serum ADA (IU/L)	Cerebral malaria n (%)	Viral encephalitis n (%)	Febrile convulsion n (%)
<30	0	7 (12.5%)	8 (14.81%)
30-60	1 (1.92%)	30 (53.57%)	38 (70.37%)
60-90	11 (21.15%)	9 (16.07%)	3 (5.55%)
90-120	27 (51.92%)	10 (17.86%)	5 ((9.26%)
>120	13 (25%)	0(%)	0(%)

Table 3: Ratio of CSF/ Serum Adenosine Deaminase (ADA) of cases.

Ratio of CSF/ Serum ADA	Cerebral malaria n (%)	Viral encephalitis n (%)	Febrile convulsion n(%)
<0.05	16 (30.77%)	14 (25%)	16 (29.63%)
0.05 -0.1	32 (61.54%)	11 (19.64%)	19 (35.19%)
0.1 -0.15	3 (5.77%)	16 (28.57%)	14 (25.93%)
0.15 -0.2	1 (1.92%)	9 (16.09%)	1 (1.85%)
>0.2	0 (0%)	6 (10.7%)	4 (7.41%)

Table 4: Sensitivity and specificity of various parameters.

Parameters	Cut off	Sensitivity ¹	95 %CI	Specificity ²	95 %CI	+PV ³	Area Under ROC Curve
CSF ADA	>6.31	59.62	47.0-74.7	77.27	68.3-84.7	55.4	0.7
Serum ADA	>78.9	96.15	86.8-99.5	88.45	77.5-91.5	75.8	0.915
CSF/Serum ADA	≤0.093	90.38	79.0-96.8	48.18	38.6-57.9	45.2	0.68

DISCUSSION:

Routine CSF parameters may not be helpful to differentiate cerebral malaria from viral encephalitis. Thus aim was to keep looking for simple and rapid test that can help to diagnose cerebral malaria from viral encephalitis. This is to our knowledge our study is most large scale study on ADA in cerebral malaria reported so far. We could not, however, exclude the possibility that patients labeled as having cerebral malaria had a combination of encephalitis and parasitaemia with *P. falciparum*, but the lack of white cells in their cerebrospinal fluid make this possibility seem very unlikely.

Patients with cerebral malaria had significantly higher mean value of CSF ADA (6.74±3.12) IU/L than viral encephalitis (5.35±2.19) IU/L and febrile convulsion (4.28±3.34) IU/L. Mishra OP et al^[8] had described CSF ADA levels in 10 patients with viral encephalitis, found mean ± SD of

(6.15±2.93 IU/L), which were comparable to the levels reported here (5.35±2.19 IU/L).

Patients with cerebral malaria had significantly higher serum ADA (114.475±58.82) levels than that of viral encephalitis (58.65±26.5) and febrile convulsion (49.93±22.6) p value<0.0001. Peter E. Daddona, et al.^[9] have demonstrated the absolute rise in Adenosine deaminase activity in adenosine deaminase deficient erythrocytes after *P. falciparum* infection (From <0.008 nmol/min/mg to 2 nmol/min/mg)^[19]. Ozcan E.et.al. had observed ADA activity elevated to more than toys the level of controls in study on patients with *P. vivax* malaria^[20].

The mean ratio of CSF/serum ADA in cerebral malaria (0.0616±0.0319) was significantly lower than that of viral encephalitis (0.11±0.07) and febrile convulsion (0.094±0.073). 31% of patient with cerebral malaria had CSF/serum ADA ratio between 0.05-0.1 as compared to 25% and 29% in encephalitis

and febrile convulsion respectively. Jakka, et al.^[3] had reported CSF/serum ADA ratio (0.31 ± 0.05) in cerebral malaria and (0.54 ± 0.11) in viral encephalitis. Lower ratio in our study was due to higher serum ADA levels as compared to Jakka, et al.

The sensitivity of the CSF ADA test to differentiate cerebral malaria from viral encephalitis and febrile convulsion was 59.62% and the specificity was 77.1%, when the cut off value of >6.31 IU/L was used. The sensitivity of serum ADA in differentiating cerebral malaria from viral encephalitis and febrile convulsion was 96.2% and the specificity was 85.3% when the cut off value of >78.9 IU/L was used.

The sensitivity of the CSF/serum ADA test in differentiating cerebral malaria from viral encephalitis and febrile convulsion was 90.4% and the specificity was 48.6%, when the cut off value of ≤ 0.093 was used. Jakka, et al.³, found that a CSF/serum ADA ratio of <0.38 was the best discrimination of cerebral malaria from presumed viral encephalitis with sensitivity 91% and specificity 100%. The difference of these results of Jakka, et al. with our study can be explained by the fact that we had higher serum ADA levels than that of Jakka, et al.

Conclusion

Serum ADA was found in this study as best discriminator between cerebral malaria and viral encephalitis. The ratio of CSF/serum ADA had high sensitivity but poor specificity in discriminating cerebral malaria from presumed viral encephalitis. ADA estimation in cerebrospinal fluid and serum is a simple, rapid and reliably specific test to differentiate cerebral malaria from viral encephalitis. For above reasons ADA estimation in cerebrospinal fluid and serum of cerebral malaria patients should find a place in routine laboratory methodology.

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