

# A study of Cerebrospinal Fluid Adenosine deaminase and C-reactive protein in Bacterial, Tubercular and Viral meningitis.

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## Research Article

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## ABSTRACT :

Cerebrospinal Fluid Adenosine deaminase (ADA) activity and C-reactive protein (CRP) measurement were done in 30 patients of tubercular meningitis (TBM), 36 patients of bacterial meningitis (BM) and 34 patients of Viral meningitis (VM), to evaluate, whether CSF CRP and ADA levels could be used to differentiate the bacterial, tubercular & viral meningitis. The mean CSF ADA activity was significantly raised in TBM as compared to BM and VM. ( $p < 0.001$ ) while mean CSF CRP activity was significantly raised in BM as compared to TBM and VM. ( $p < 0.001$ ). At cut of level 10 IU/L, the sensitivity and specificity of ADA for TBM was 90% and 97.14% respectively while at cut of level 15 mg/L, the sensitivity and specificity of the CRP for BM was 86.11% and 98.43% respectively. Since both the tests are simple and take lesser time to perform, they can be used to differentiate BM, TBM and VM.

## INTRODUCTION:

Infectious diseases remain a major cause of death and disability for millions of people around the world, despite decades of dramatic progress in their treatment and prevention. As vital tissues are involved, CNS infection can cause devastating sequelae and in some cases may result in both neurological and medical emergencies.<sup>1</sup>

Meningitis is an inflammation of the membranes that surround the brain and spinal cord. It is a common clinical problem during infancy and childhood. Delay in distinguishing between bacterial, tubercular & viral meningitis & treatment may have irrevoca-

ble consequences that lead to significant morbidity & mortality. The initiation of proper medication in meningitis patients can often be delayed because of a lack of confidence in the presently available laboratory tests.<sup>2,3</sup> Most of the tests developed for the early diagnosis of meningitis are not sensitive<sup>4</sup> and although some other tests are useful, they may not be affordable for routine use.<sup>5,6</sup>

So it is necessary to introduce simple, reliable and cost effective method for rapid diagnosis and differentiation of various types of meningitis.

In view of such observations, the present study was

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conducted to find out the usefulness of these two tests, CSF-ADA & CSF-CRP for the rapid diagnosis & differentiation of bacterial, tubercular & viral meningitis.

## METHODS

A present observational study was conducted in Department of Biochemistry, Dr. Shankarrao Chavan Government medical college, Nanded, on 100 meningitis children of age between 1 to 12 year. Depending on clinical features, examination, investigations like CSF Biochemistry, Cytology, Culture & others investigation, these cases were further divided in to 3 groups.

### Group I- Bacterial meningitis:

This group included 36 Patients with clinical and CSF laboratory findings consistent with BM. Clinical features being the acute onset of symptoms of meningitis, may be associated with sinusitis, otitis media, and signs of meningeal irritation. CSF analysis showing Pleocytosis of > 250 cells/mm<sup>3</sup> predominantly neutrophils, Proteins > 50mg/dl, Sugar < 40mg/dl. Gram stains and culture positivity. Neuroimaging showing evidence of diffuse meningeal enhancement, abscesses or parameningeal focus.

### Group II- Tubercular meningitis:

This group included 30 Patients with clinical and CSF laboratory findings consistent with TBM. Clinical features being the insidious in onset, may be associated with tuberculosis of other organs, signs of meningeal irritation. CSF analysis showing Pleocytosis of > 60 cells/mm<sup>3</sup> predominantly lymphocytes, Proteins > 40mg/dl, Sugar < 40mg/dl. ZN, culture or manotux positive. Neuroimaging showing evidence of Meningeal enhancement, basal exudates and/or tuberculoma.

### Group III-Viral meningitis:

This group included 34 Patients with clinical and CSF laboratory findings consistent with VM. Clinical features being the Usually acute in onset with signs of meningeal irritation. CSF analysis showing Pleocytosis of > 25 cells/mm<sup>3</sup> predominantly lymphocytes, Proteins > 45mg/dl, Sugar normal.

While patients of Febrile seizures, patients with Non infectious conditions of CNS such as epilepsy, drug or vaccine induced, patients with acute infections at sites other than the central nervous system, those in whom lumbar puncture was contraindicated, and those with severe hepatic dysfunction were excluded from the study. Similarly Those cases after examination & investigation were not diagnosed as above three meningitis or diagnosed other than meningitis like febrile convulsion, cerebral malaria, subarachnoid hemorrhage were withdrawn from the study.

CSF CRP was measured by using CRP turbilatex kit

of agapee diagnostics in accustar semi-autoanalyzer based on agglutination of the latex particles coated with anti-human CRP.

CSF ADA was estimated by using ADA-MTB kit of Microxpress- A Division of Tulip Diagnostics [P] Ltd. Here ADA hydrolyzes adenosine to ammonia and inosine. The ammonia formed further reacts with phenol and hypochlorite in an alkaline medium to form blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue is proportional to the activity of ADA.

### Statistical Methods:

Descriptive statistical analysis has been carried out in the present study. Significance is assessed at 5 % level of significance. ANOVA test has been used to find significance of association of CRP & ADA with type of meningitis. Sensitivity, Specificity and Accuracy were calculated to know the diagnostic performance of CRP and ADA levels in relation to type of meningitis.

## RESULTS:

	TBM	BM	VM	ANOVA p value
CRP	4.85 ± 3.53	25.22 ± 10.38*	2.24 ± 1.63	< 0.001
ADA	22.50 ± 11.43*	4.55 ± 3.21	2.37 ± 1.41	< 0.001

Table 1: Mean Levels of CRP & ADA in different types of meningitis.

The comparison of CSF CRP & ADA in different types of meningitis are presented in Table 1. In TBM group mean CRP level was 4.85 ± 3.53 and mean ADA level was 22.50 ± 11.43. In BM group mean CRP level was 25.22 ± 10.38 and mean ADA level was 4.55 ± 3.21. While in VM group mean CRP level was 2.24 ± 1.63 and mean ADA level was 2.37 ± 1.41, in which mean level of CRP was significantly increased in BM group as compared TBM and VM, while mean level of ADA was significantly increased in TBM group as compared BM and VM.

At cut of level 15 mg/L, the sensitivity and specificity of the CRP for BM among these 100 patients was 86.11% and 98.43% respectively with an accuracy of 94%. While at cut of level 10 IU/L, the sensitivity and specificity of ADA for TBM among these 100 patients was 90% and 97.14% respectively with an accuracy of 95%.

## DISCUSSION

### CRP and meningitis.

The results of the present study show that CRP level was significantly increased in bacterial meningitis as

compared to tubercular & viral meningitis.

This increase in CRP level might be due to entry of CRP into CSF by passive diffusion across the highly inflamed meninges or *de-novo* synthesis in central nervous system.<sup>7</sup> Present study was consistent with the findings of various studies.

In a study conducted by Vaishnavi C *et al*, CRP in CSF was significantly higher in patients with pyogenic meningitis compared to tubercular meningitis. Authors concluded that the estimation of CRP in the differential diagnosis of meningitis might be made to give a preliminary diagnosis of meningitis.<sup>8</sup>

Riberio MH *et al* estimated the levels of CRP in CSF from 33 patients with bacterial meningitis, 21 patients with lymphocytic meningitis and 54 controls. 100% of these patients with bacterial meningitis were correctly classified on the basis of measurement of CRP levels in CSF. In conclusion authors recommend the estimation of CRP in CSF in the differentiation of bacterial from non-bacterial meningitis.<sup>9</sup>

A meta-analysis by Gerdes LU *et al* suggested that a negative CRP test in either CSF or serum can be used with a very high probability to rule out bacterial meningitis.<sup>10,11</sup>

#### **ADA and meningitis.**

Present study found that ADA level was significantly increased in tubercular meningitis as compared to bacterial & viral meningitis.

ADA is released by T cells during cell mediated immune response (CMI) to the tubercle bacilli. ADA is now being recognized as a marker of cell mediated immunity particularly as a marker of T lymphocyte activation. Adenosine deaminase levels (ADA) have also been considered by several researchers to differentiate tubercular disease from non-tubercular.<sup>12-15</sup>

The ADA2 isoenzyme is the major contributor to increased ADA activity in the CSF of patients with tuberculous meningitis, probably reflecting the monocyte-macrophage origin of the ADA.

The source of raised ADA in CSF of TBM patients may be the damaged blood brain barrier permitting ADA to enter into CSF from the blood or adjacent cerebral tissue and/or as a result of lymphocyte-macrophage proliferation indicating local immune response.<sup>12</sup>

Sang-Ho Choi *et al* studied ADA activity in CSF of 182 patients with meningitis. The mean ADA level in the tuberculous meningitis group was  $12.7 \pm 7.5$  U/L and it was significantly higher than the other groups ( $3.10 \pm 2.9$  U/L;  $p < 0.001$ ). The sensitivity and specificity was 0.83 and 0.95 respectively when a cut-off value of 7U/L was used.<sup>16</sup>

Pettersson *et al* reports sensitivity of 1.0 and specificity of 0.99 when a cut-off value of 20 U/L was used, but

in that study there were only 3 enrolled tuberculous meningitis patients.<sup>17</sup>

Chotmongkol V *et al* identified a CSF ADA level of 15.5 U/l as the best cut-off value to differentiate tuberculous meningitis and non-tuberculous meningitis, with a sensitivity of 75% and specificity of 93%. When tuberculous meningitis was compared with aseptic and carcinomatous meningitis, a CSF ADA level of 19.0 U/l was the best cut-off value for differentiation, with a sensitivity of 69% and a specificity of 94%.<sup>18</sup> Some studies have reported a lower efficacy of this test and show an overlap between tuberculous meningitis and bacterial meningitis.<sup>19</sup>

Malan C *et al* showed that in both bacterial and TBM groups, the mean ADA level in the CSF was significantly higher than in aseptic meningitis ( $p < 0.001$ ), but a significant difference was not shown between bacterial meningitis and TBM groups.<sup>12</sup>

Gambhir IS *et al* found that the mean CSF ADA levels in TBM patients was  $9.61 \pm 4.10$  U/l and was significantly elevated as compared to viral encephalitis and enteric encephalopathy cases; but the difference was insignificant in comparison to pyogenic meningitis and cerebral malaria.<sup>20</sup>

From above discussion, elevated CRP level in meningitis patient highly suggest Bacterial meningitis, while elevated ADA level in meningitis patient highly suggest Tubercular meningitis.

But either test done alone would still cause confusion in the probable diagnosis and differentiation of these three meningitis, since some studies shows overlap of ADA level between tuberculous meningitis and bacterial meningitis<sup>19</sup> like Malan C *et al*<sup>12</sup>, Gambhir IS *et al*<sup>20</sup>, in such situation Differentiation of BM from TBM by ADA alone is difficult. Also the cell type in tubercular meningitis initially can predominantly be neutrophilic leucocytosis, which favours diagnosis of Bacterial meningitis falsely, in which case the diagnosis of tubercular meningitis is never entertained until the patient shows no response to the antibiotics. Patients with partially treated meningitis can have lymphocytic predominance when tubercular meningitis is wrongly considered. To overcome this fallacy, it is essential to do CRP as well ADA simultaneously in order to increase the specificity of the test.

Even elevated ADA level in meningitis patient highly suggest Tubercular meningitis, it's low level in meningitis patient unable to differentiate BM from VM, as both cases shows low level of ADA which are not statistically different.

Similarly Even elevated CRP level in meningitis patient highly suggest Bacterial meningitis, it's low level in meningitis patient unable to differentiate TBM

from VM, as both cases shows low level of CRP which are not statistically different.

Thus, it is clearly evident that neither CSF ADA level nor CRP level alone could differentiate these three types of meningitis completely. But their simultaneous use along with other tests of meningitis may helpful in probable diagnosis and differentiation of these three meningitis. In which elevated CRP levels in meningitis are highly suggestive of Bacterial meningitis. High ADA and normal CRP suggest the diagnosis of TBM. On the other hand having both ADA and CRP negative can strengthen the diagnosis of viral meningitis. A high CRP in cases with high ADA would again favour the diagnosis of pyogenic meningitis thereby overcoming the false positive ADA.

### CONCLUSION:

From above discussion, study concludes that combine use of these two tests i.e. CSF CRP and ADA can be used for early differentiation of Bacterial, Tubercular, and Viral meningitis. This is necessary when gold standard test for meningitis like Smear and/or culture for AFB, smear and/or culture for bacteria, is not available or negative or time consuming. These tests for ADA and CRP in CSF are simple and can be carried out in a central laboratory with a rapid diagnosis, thus reducing unwarranted or harmful therapy for patients. Further studies may need in this regard considering appropriate sample size.

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