ORIGINAL ARTICLE

Study of the Role of Cerebrospinal Fluid C-reactive Protein and Adenosine Deaminase to differentiate Various Types of Meningitis

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Abstract

Objective: (1) To study cerebrospinal fluid (CSF) adenosine deaminase (ADA) and CSF C-reactive protein (CRP) levels in the differentiation of viral, pyogenic, and tuberculous meningitis (TBM). (2) To estimate the borderline levels of CRP in CSF in viral, pyogenic, and TBM.

Methods: A prospective and cross-sectional study was conducted at the Department of Medicine, SRN Hospital, Prayagraj, Uttar Pradesh, India, between August 2016 and September 2018. In this study, a total of 100 patients with meningitis were included applying specific inclusion and exclusion criteria after proper ethical approval.

Results: Out of 100 patients, 61 were TBM, 31 were pyogenic meningitis, and eight were viral meningitis (VM). CSF CRP level was significantly increased in pyogenic meningitis (1.05 ± 0.36 mg/dL) compared to nonpyogenic meningitis [TBM (0.42 ± 0.13 mg/dL) and VM (0.37 ± 0.09 mg/dL)]. At the cut-off level of CRP in CSF > 0.6 mg/dL, its diagnostic sensitivity in pyogenic meningitis was 93.55% and specificity 94.20%. While CSF ADA levels were higher in the TBM group (13.32 ± 3.21 U/L) compared to the other two groups [pyogenic meningitis (6.15 ± 1.27 U/L) and VM (4.86 ± 0.88 U/L)]. At a cut-off, CSF ADA level of >10 U/L, its diagnostic sensitivity for TBM was 91.67% and specificity 90%.

Conclusion: Cerebrospinal fluid (CSF) CRP levels were found to be raised in pyogenic meningitis, and CSF ADA was found to be elevated in TBM. While both ADA level and CRP level in CSF are found low in VM.

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Introduction

Infectious diseases are the leading cause of mortality and morbidity for millions of people worldwide, despite great progress in their prevention and treatment. Central nervous system (CNS) infection can lead to serious consequences and, in some cases, can lead to acute neurological conditions.[1] Delays in differentiating viral, bacterial, and TBM can lead to delays in treatment, leading to significant increases in morbidity and mortality. Most available tests for the early diagnosis of meningitis are not sensitive,[2] and other available useful tests may not be available for routine use.[3,4] It is, therefore, crucial to find a reliable, simple, and cheaper tool for rapid diagnosis and differentiation of different types of meningitis.

A CSF examination is important in the diagnosis and differentiation of meningitis. The estimation of CSF ADA is useful for making the diagnosis of TBM, while CSF CRP is helpful in diagnosing pyogenic meningitis. In the case of VM, both CSF CRP and CSF ADA were found to be low.

CRP is an acute phase reactant from the "pentraxins" group, discovered in 1930 by Tillet et al.[5] CRP is synthesized only in the liver,[6] secreted just in 6 hours after acute inflammation in the fluid or serum of affected tissues in a larger amount. The elevated level of CRP in patients with meningitis in the CSF is because of passive diffusion through the inflamed meninges. Thus, an increase in serum CRP indicates an acute phase response, and an elevated CSF CRP indicates meningeal involvement. Given this measurement, the CSF CRP level looks to be a good choice for diagnosing pyogenic meningitis.[6–11] Therefore, in this study, we estimated CSF CRP values to distinguish pyogenic meningitis from nonpyogenic meningitis and tried to develop a cut-off value of CSF CRP for the same.

Adenosine deaminase (ADA) is a polymorphic enzyme of the purine salvage pathway (Spencer et al.).[12] It has two isoforms of ADA—ADA1 and ADA2. Of these two, ADA1 is found mostly in body cells like lymphocytes and macrophages. It is mainly involved in the intracellular activity. While ADA2 is mainly found in human serum and plasma. ADA is the marker of T lymphocyte activation and is released during the cell-mediated immune response. The ADA2 isozyme constitutes the major fraction of increased ADA levels in CSF of TBM patients, suggesting the monocyto-macrophage origin of ADA. CSF ADA level in TBM patients may be increased due to a damaged blood-brain barrier and/or due to the proliferation of lymphocytic macrophages, indicating a local immune response.[13] Therefore, measuring ADA in CSF is a simple, fast, cheap, and specific test for establishing the diagnosis of TBM, especially when we need to distinguish tuberculous from nontuberculous etiology.[14]

Goals and Objectives

- To study CSF ADA and CSF CRP levels in the differentiation of viral, pyogenic, and TBM.
- To estimate the borderline levels of CRP in CSF in pyogenic, viral, and TBM.

Materials and Methods

A prospective and cross-sectional study was conducted at the Department of Medicine, Motilal Lal Nehru Medical College, and Swaroop Rani Nehru Affiliated Hospital, Prayagraj, Uttar Pradesh, India between August 2016 to September 2018. Applying the below-mentioned inclusion and exclusion criteria, 100 patients with meningitis were enrolled.

Inclusion Criteria

- Meningitis patients of ≥18 years of age, both men and women.
- With clinical symptoms reminiscent of meningitis:
- Fever, headache, vomiting, and altered sensory perception.
- With meningeal irritation signs (nuchal stiffness and Kernig's sign)

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Exclusion Criteria

- Acute/chronic infections in non-CNS sites.
- Patients with hepatic encephalopathy.
- Patients already on immunosuppressive or steroid treatment.
- Patients with proven immunological disorders, e.g., systemic lupus erythematosus, rheumatoid arthritis.

Based on clinical symptoms and CSF reports, all selected patients were categorized into the following three groups.

Tuberculous meningitis (TBM)—included cases of TBM:

Clinical signs—duration of fever for <1 week, tuberculosis in any other organ, and signs of meningeal irritation.

Cerebrospinal fluid (CSF) analysis—appearance—clear/turbid, glucose—either <40% of plasma glucose or 20–40 mg/dL, protein >45 mg/dL (1–5 g/L), lymphocytopleocytosis (10–500 cells/µL), Ziehl Neilson staining if positive for acid-fast bacill or cartridge-based nucleic acid amplification test for mycobacterium tuberculosis.

Pyogenic meningitis—included cases of pyogenic meningitis:

Clinical signs—duration of fever <1 week and signs of meningeal irritation.

Cerebrospinal fluid (CSF) analysis—appearance—clear/turbid, glucose—either <40% of plasma glucose or <40 mg/dL, protein > 45 mg/dL, polymorphonuclear (PMN) leukocytosis (>100 cells/uL), positive gram stain and culture.

Viral meningitis (VM)—included cases of VM:

Clinical features—shorter duration of fever along with the presence of signs of meningeal irritation.

Cerebrospinal fluid (CSF) analysis—appearance—clear, glucose—normal, that is, > 60% of serum glucose, protein—normal or slightly elevated (20–80 mg/dL), pleocytosis 25–500 cells/µL/(predominantly lymphocytes, but PMN soon).

Adenosine deaminase (ADA) activity in CSF was measured in all patients by the colorimetric method, according to Galanti and Giusti. A threshold level >10 U/l was considered a symptom of TBM.[15]

Cerebrospinal fluid (CSF) CRP—A latex agglutination quantitative test was used to detect CSF CRP levels. CRP leads to the agglutination of latex particles which are coated with anti-human CRP. This agglutination of latex particles is proportionate to the concentration of CRP and measured turbidimetrically.[10,16] Any value >0.6 mg/dL was considered suggestive of pyogenic meningitis.

Statistical Analysis

Statistical Package for the Social Sciences, Excel 2013 was used for the analysis of data. All statistical analyses were done by applying the student's *t*-test. For comparing the two groups, pyogenic meningitis vs TBM or pyogenic meningitis vs VM or TBM vs VM, an unpaired *t*-test was used. While the analysis of variance test was used to compare all three groups. A *p*-value <0.05 was considered significant.

Result

Out of 100 patients, 61 patients had TBM, 31 patients had pyogenic meningitis, and eight patients had VM. At a CSF CRP cut-off of 0.6 mg/dL, 29 patients with pyogenic meningitis and 4 patients with nonpyogenic meningitis (TBM and viral) had CSF CRP levels of >0.6 mg/dL, whereas two patients with pyogenic meningitis and 65 patients without pyogenic meningitis had CRP levels in CSF of <0.6 mg/dL (Table 1).

The mean CSF CRP value was 1.05 ± 0.36 mg/dL in pyogenic meningitis cases, 0.42 ± 0.13 mg/dL in TBM, while 0.37 ± 0.09 mg/dL in VM patients. This difference was statistically significant ($p < 0.0001$) in pyogenic meningitis compared to TBM and VM.

Table 2 depicts the CSF CRP levels in meningitis cases.

When applying a CSF, ADA cut-off level of 10 U/L, 55 patients with TBM and 4 patients without TBM had a CSF ADA > 10 U/L, while six patients of TBM and 35 patients without TBM had CSF ADA of <10 U/L (Table 3).

The mean CSF ADA in TBM, pyogenic meningitis, and VM was 13.62 ± 2.88 U/L, 5.88 ± 0.94 U/L, and 4.86 ± 0.88 U/L respectively, which is statistically significant ($p < 0.001$). (Table 4)

The sensitivity of CSF CRP for making the diagnosis of pyogenic meningitis is 93.55%, while specificity is 94.20%, positive predictive value (PPV) is 87.88%, whereas negative predictive value (NPV) is 97.01%. The sensitivity of CSF ADA for diagnosing TBM is 91.67%, while the specificity is 90%. The PPV of CSF ADA is 93.22%, while NPV was 87.81%.

Discussion

In this study, mean CSF CRP levels were 1.05 ± 0.36 mg/dL in pyogenic meningitis, 0.42 ± 0.13 mg/dL in TBM, and 0.37 ± 0.09 mg/dL in VM. This study shows that CRP level in CSF was significantly increased in patients of pyogenic (bacterial) meningitis as compared to nonpyogenic (viral and tuberculous) meningitis ($p < 0.0001$). However, the difference between mean CSF CRP levels in VMs and TBM was statistically insignificant (*p*-value 0.2932). The elevated level of CRP in patients with meningitis in the CSF might be because of passive diffusion through the inflamed meninges or by *de novo* synthesis in the CNS. At a CSF CRP cut-off level of >0.6 mg/dL, its diagnostic sensitivity for pyogenic meningitis was found to be 93.55%, specificity was 94.20%, PPV was 87.88%, and NPV was 97.01%. The results of our current study are similar to the findings of studies conducted in India by Hemavani et al.[17] and Vaishnavi et al.,[16] who observed that the CSF CRP level was higher in pyogenic meningitis patients as compared to TBM patients significantly. So the measure of CSF CRP could be used for the diagnosis of pyogenic meningitis. In one of the studies, Gerdes et al. concluded that negative CRP levels, either in serum or CSF, can be used to rule out bacterial meningitis strongly.[18,19] Belagavi et al.[20] observed that with CSF CRP levels greater than or equal to 0.6 mg/dL, the specificity & sensitivity of CRP was 100 and 83.3%, respectively, with an accuracy of 98% and NPV 97.8%, suggesting that bacterial meningitis can be ruled out at the CRP level of <0.6 mg/L.

The CSF ADA level is elevated in TBM and is used to differentiate TBM from bacterial and VM.[21,22] In this study, mean CSF ADA levels was 13.32 ± 3.21 U/L in TBM, 6.15 ± 1.27 U/L

Table 3: Cerobrospinal fluid (CSF) ADA positivity in the TBM group and non-TBM group

CSF ADA	*TBM (n = 61)*	*Non-TBM (n = 39)*
Positive	55	4
Negative	6	35

Table 1: Cerobrospinal fluid (CSF) CRP in pyogenic and non-pyogenic meningitis

CSF CRP	*Pyogenic meningitis (n = 31)*	*Nonpyogenic meningitis (n = 69)*
Positive	29	4
Negative	2	65

Table 2: Cerobrospinal fluid (CSF) CRP values of TBM, pyogenic, and VM

	TBM (n = 61)	*Pyogenic meningitis (n = 31)*	*VM (n = 8)*	*p-value*
CSF CRP (mg/dL)	0.42 ± 0.13	1.05 ± 0.36	0.37 ± 0.09	<0.0001

Table 4: Comparison of mean CSF ADA levels between TBM, PM, and VM

	TBM (n = 61)	*Pyogenic meningitis (n = 31)*	*VM (n = 8)*	*p-value*
CSF ADA (U/L)	13.62 ± 2.88	5.88 ± 0.94	4.86 ± 0.88	<0.001

and 4.86 ± 0.88 U/L in pyogenic meningitis and VM, respectively. On comparing the ADA levels in three groups, it was found that the CSF ADA levels were significantly higher in the TBM group as compared to the other two groups ($p < 0.0001$). Using a CSF ADA cut-off level >10 U/L, its diagnostic sensitivity for TBM was 91.67%, specificity 90%, NPV was 87.81%, and PPV 93.22%. These values are consistent with other studies conducted by Mishra et al.[23] to compare CSF CRP and CSF ADA levels in partially treated pyogenic meningitis and TBM in children. In their study, the sensitivity of ADA was 62.5%, and the specificity of ADA was 88.9%, while the sensitivity and specificity of CRP were 75 and 100%. In their study, Choi et al.[24] found that the mean CSF ADA in patients with TBM was 12.7±7.5 U/L, and when compared to CSF ADA in viral and pyogenic meningitis, it was significantly higher. Sensitivity was 83% and specificity 95% using a cut-off of 7 U/L for CSF ADA. Chotmongkol et al.[25] proposed a cut-off value of 15.5 U/L for CSF ADA to differentiate non-TBM from TBM, having a specificity of 93% and a sensitivity of 75%. Other studies report a lower efficiency and show an overlap between bacterial and TBM.[26] Mean CSF ADA value in TBM patients was 9.61 ± 4.10 U/l in the study by Gambhir et al.,[23] and it was significantly high as compared to VM. This difference was not statistically significant as compared to pyogenic meningitis.

An elevated CSF CRP level in meningitis is highly suggestive of pyogenic meningitis, whereas an elevated CSF ADA level is highly suggestive of TBM. But any of these tests performed separately would cause confusion in distinguishing the three meningitis, as certain studies show overlapping of ADA levels between bacterial and TBM, such as Malan et al.,[13] Gambhir et al.[23] Distinguishing pyogenic meningitis and TBM by ADA alone in CSF is difficult. So it may be suggested to perform CSF CRP and CSF ADA both at the same time, which can increase the specificity of the test.

Conclusion

The measure of CRP and ADA both in CSF may help in differentiating viral, bacterial, and TBM. While CSF CRP is raised in pyogenic meningitis, CSF ADA has been found to be elevated in TBM. Both CSF ADA and CSF CRP have been found to be lower in VM. Testing of both ADA and CRP in CSF is simple and rapid to perform, and it may help reduce the diagnostic dilemma and ensure the rapid etiological diagnosis of different forms of meningitis.

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