Original Research Article

Utility of Cerebrospinal Fluid Adenosine Deaminase and C-Reactive Protein in patients with Meningitis

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Abstract

Introduction: We aimed to assess the utility of cerebrospinal fluid (CSF) Adenosine deaminase (ADA) and C-reactive protein (CRP) in differentiating various types of meningitis in adult population.
Materials and methods: The present observational study, conducted in the Department of Medicine, Lokmanya Tilak Municipal Medical College and Hospital from February 2016 till August 2017, included all meningitis and meningococcemia cases diagnosed according to the clinical and/or laboratory criteria during the study period were included in the study. Comparisons were made with respect to various biochemical investigations between patient groups diagnosed with various types of meningitis.
Results: 38% were diagnosed as bacterial meningitis, 54% as tubercular meningitis and rest as viral meningitis. CSF ADA was significantly higher in tubercular meningitis as compared to bacterial or viral meningitis. Similarly, CSF CRP was found to be significantly higher among patients with bacterial meningitis. In tubercular meningitis, CSF ADA and CRP were not found to be significantly associated with CSF cell count, CSF protein or ratio of CSF/Blood glucose. In bacterial meningitis, CSF CRP was found to be significantly higher among patients with CSF protein between 101 to 200 mg/dl. Using a cut off value of 5 IU/L, CSF ADA was found to have a sensitivity of 100% and specificity of 91.3% in diagnosing tubercular meningitis and with cut off value of 9 mg/L, CSF CRP had a sensitivity of 97% and specificity of 100% in diagnosing bacterial meningitis.
Conclusion: CSF ADA and CRP should be included in the workup of meningitis patients.

Key words

Bacterial meningitis, Cerebrospinal fluid, Tuberculous meningitis.

Introduction

Meningitis is an acute inflammation of the protective membranes covering the brain and spinal cord, collectively known as the meninges. Approximately 1.2 million cases of bacterial meningitis occur annually worldwide [1]. Meningitis is among the ten most common infectious causes of death and is responsible for approximately 135,000 deaths throughout the world each year. Because the management of tubercular meningitis is different from that of bacterial meningitis, early identification is essential [2]. Microbiological investigations are time consuming and lead to delay in accurate diagnosis. Adenosine deaminase (ADA) is found in most body cells, particularly lymphocytes and macrophages. Therefore ADA has been used in the workup of lymphocytic pleural effusions, peritoneal ascites and cerebrospinal fluid (CSF) in which high ADA levels points towards tuberculosis consideration. However, there is no consensus regarding its usefulness and sensitivity for tubercular meningitis diagnosis. C-reactive protein (CRP) is an acute phase reactant and inflammation in the body causes CRP to be detected in serum or other body fluids [3]. Furthermore, serum CRP values have been used to differentiate bacterial and viral infections [4]. Very few studies have investigated the role of CSF CRP in differentiating bacterial meningitis from non-bacterial types meningitis. In this study we aimed to assess the utility of CSF ADA and CRP in differentiating various types of meningitis in adult population.

Materials and methods

Study Design and Sampling

The present observational study was conducted in the Department of Medicine, Lokmanya Tilak Municipal Medical College and Hospital from February 2016 till August 2017. Our hospital is a tertiary care teaching hospital in Mumbai which caters to patients from Mumbai and adjoining districts and villages. All meningitis and meningococcemia cases diagnosed according to the clinical and/or laboratory criteria during the study period were included in the study. Patients with carcinomatous meningitis and degenerative neurological disorders were excluded from the study. Clinical criteria for diagnosis of meningitis included fever, headache, vomit, neck stiffness, signs of meningeal irritation, seizures, and/or rash. Laboratory confirmation included biochemical and microbiological examination of the cerebrospinal fluid (CSF), including culture and or antigen detection. Plain and contrast-enhanced computed tomography (CT) scan brain was done in all patients. Institutional Ethics Committee approval was obtained before starting the study. Written consent was obtained from the patients or their attendants before recruitment into the study. Refusal to consent for the study did not affect the management of the patients in any manner.

Maria's criteria [5] were used to diagnose tubercular meningitis, supported by findings of mycobacterial culture, CSF cytology and staining, polymerase chain reaction (PCR), and line probe assay (LPA). Bacterial meningitis was diagnosed based on Gram's staining and culture. A case of viral meningitis was defined on the basis of following criteria: fever, signs and symptoms of brain inflammation, CSF white blood cell count C10/mm^3 and routine CSF culture negative for common bacteria.

Data Collection and Data Analysis

Demographic data of the patients were obtained from the medical records. Clinical history of the patient, findings of the clinical examination and findings of the laboratory investigations were noted in a pre-designed semi-structured questionnaire. Data were summarized by descriptive statistics i.e., mean and standard deviation for numerical variables and frequency and percentages for categorical variables. Normality of the data was checked using the

Kolmogorov–Smirnov test. Differences in the frequency distribution or means of quantitative variables were analyzed among patients diagnosed with bacterial, tubercular and viral meningitis. Comparison of frequency distribution was done using chi squared or Fisher's exact test. Means and standard were compared using Kruskal Wallis or one way analysis of variance. Analysis was performed in SPSS version 21.0 (IBM Corp, New York) and all the results were considered to be significant at the 5% critical level.

Results

During the study period a total of 100 patients were enrolled in the study, 38% were diagnosed as bacterial meningitis, 54% as tubercular meningitis and rest as viral meningitis. Age of the patients ranged from 13 to 82 years. Most common age group of the patients was 21 to 40 years among patients with the diagnosis of tubercular, bacterial and viral meningitis, however the proportion of patients in different age groups was not significantly different (**Table - 1**).

Table – 1: Comparing clinical characteristics of patients included in the study.

	Tubercular meningitis (n=54)	**Bacterial meningitis (n=38)**	**Viral meningitis (n=08)**	**p value**
Age distribution (in years)*				
Less than 20	19	6	03	0.08
21 to 40	26	16	03	
41 to 60	09	12	02	
61 to 80	00	03	00	
More than 80	00	01	00	
During of illness				
Less than 5 days	08	28	04	<0.001
More than 5 days	46	10	04	
Erythrocyte sedimentation rate				
Raised	50	24	00	<0.001
Normal	04	14	08	
Anemia				
Yes	36	20	01	<0.05
No	18	18	07	

*Frequency distribution compared using chi squared or Fisher's exact test

Table – 2: Comparing CSF adenosine deaminase and C reactive protein among patients.

	Tubercular (TB) Meningitis	**Bacterial (Bact.) Meningitis**	**Viral Meningitis**	**TB vs Bact.***	**TB vs Viral***	**Bact. vs Viral***
	Mean (SD), Median			**p value**		
CSF adenosine deaminase	11.46 (5.83), 9.35	4.40 (3.72), 4.00	0.85 (0.52), 0.65	<0.001	<0.001	<0.001
CSF C-reactive protein	0.84 (0.96), 0.60	14.09 (4.56), 13.00	0.35 (0.23), 0.30	<0.001	0.06	<0.001

*Calculated using Mann-Whitney U test

Duration of illness was more than five days in significantly higher proportion of patients with tubercular meningitis (p value <0.001). Similarly, erythrocyte sedimentation rate was

found to be raised in significantly higher proportion of patients (p value <0.001). Furthermore, there were more anemics among patients diagnosed with tubercular meningitis (p <0.05). **Table - 2** shows that CSF ADA was significantly higher among patients with tubercular meningitis as compared to those with bacterial or viral meningitis. Similarly, CSF CRP was found to be significantly higher among patients with bacterial meningitis. In patients with tubercular meningitis, CSF ADA and CRP were not found to be significantly associated with CSF cell count, CSF protein or ratio of CSF glucose/Blood glucose (**Table - 3**). Similarly, in patients with bacterial meningitis, CSF ADA was not found to be associated with CSF cell count, CSF protein or ratio of CSF glucose/Blood glucose. CSF CRP was found to be significantly higher among patients with CSF protein between 101 to 200 mg/dl (26.0 ± 12.66 mg/L) as compared to those with CSF protein less than 100 mg/dl (9.00 ± 4.22 mg/L) or more than 200 mg/dl (11.0 ± 7.94 mg/L) (p value <0.001). We did not find any association between CSF CRP and CSF cell count and ratio of CSF glucose/Blood glucose. Using a cut off value of 5 IU/L, CSF ADA was found to have a sensitivity of 100% and specificity of 91.3% in diagnosing tubercular meningitis and with cut off value of 9 mg/L, CSF CRP had a sensitivity of 97% and specificity of 100% in diagnosing bacterial meningitis (**Table - 4**).

Table – 3: Association between CSF cell count, protein and CSF/blood sugar ration with CSF ADA and CRP in patients with tubercular (TB) and bacterial meningitis.

	CSF ADA (IU/L)		**CSF CRP (mg/L)**	
	Mean ± SD	**p value**	**Mean ± SD**	**p value**
Tubercular meningitis				
CSF cell count (cells/μL)				
Less than 300	12.30 ± 7.31	0.52	0.92 ± 1.17	0.78
301-600	10.56 ± 3.48		0.73 ± 0.74	
>600	10.53 ± 3.11		0.75 ± 0.53	
CSF protein (mg/dl)				
<100	11.53 ± 6.08	0.89	0.87 ± 1.05	0.83
101-200	10.57 ± 5.32		0.63 ± 0.28	
>200	12.25 ± 4.73		0.83 ± 0.79	
CSF/Blood glucose ratio				
>0.40	10.08 ± 4.58	0.65	0.70 ± 0.58	0.87
0.21 to 0.40	11.89 ± 6.37		0.88 ± 1.04	
<0.20	10.40 ± 2.57		0.75 ± 0.93	
Bacterial meningitis				
CSF cell count (cells/μL)				
Less than 300	3.01 ± 0.22	0.81	12.0 ± 1.28	0.53
301-600	3.33 ± 0.58		11.53 ± 2.34	
>600	4.45 ± 3.91		14.38 ± 4.71	
CSF protein (mg/dl)				
<100	4.00 ± 2.34	0.98	9.00 ± 4.22	<0.001
101-200	4.35 ± 4.48		26.0 ± 12.66	
>200	4.56 ± 0.95		11.0 ± 7.94	
CSF/Blood glucose ratio				
0.21 to 0.40	4.64 ± 4.73	0.63	13.25 ± 4.14	0.16
<0.20	4.04 ± 1.08		15.37 ± 5.01	

Compared using Kruskal Wallis or one way analysis of variance

Table – 4: Accuracy of CSF ADA in tubercular meningitis and CSF CRP in bacterial meningitis.

Operating characteristic	Percentage (95% confidence interval)
CSF ADA at cutoff value of 5 IU/L in tubercular meningitis	
Sensitivity	100 (93.36-100)
Specificity	91.30 (79.68-96.57)
Positive predictive value	93.10 (83.57-97.29)
Negative predictive value	100 (91.62-100)
Accuracy	96 (90.16-98.43)
CSF CRP at cutoff value of 9 mg/L in bacterial meningitis	
Sensitivity	97 (86.5-99.5)
Specificity	100 (94.17-100)
Positive predictive value	100 (90.54-100)
Negative predictive value	98 (91.54-99.72)
Accuracy	99 (94.55-99.82)

Discussion

Diagnosing tubercular meningitis is a clinical dilemma for majority of the physicians as these patients do not present with classical symptoms of meningitis and untreated cases are always fatal. High mortality associated with tubercular meningitis has been reported by numerous authors previously and has been found to be associated with multiple patient related factors [6]. Lack of classical symptoms of meningitis and atypical CSF findings are some of the prominent reasons which makes it difficult to diagnose the condition early in its clinical course [7].

In a multivariate analysis by Youssef et al, history of the disease more than 5 days, headache, clear CSF, cell count of CSF less than 1000/dL, percentage of lymphocyte more than 30%, and protein content more than 100 mg/dL were independently predictive of distinction between tubercular meningitis and bacterial meningitis [8]. Our study found disease history more than five days to be significantly higher among patients with tubercular meningitis as well. Furthermore, CSF ADA levels have been recommended to be useful for the detection of tubercular meningitis. A recently published meta-analysis provides robust evidence to support the use of CSF ADA in detecting tubercular meningitis [9]. The authors calculated a summary sensitivity and specificity were 0.89 and 0.91 respectively, indicating a sufficient level for overall diagnostic accuracy. However, they cautioned that a negative ADA could not be used alone to discontinue anti-tuberculosis treatment and microscopic examination should be factored in. Using CSF ADA cut-off value of 5 IU/L, we obtained a sensitivity of 100% (93.3 to 100%) and specificity of 91.3% (79.68 to 96.57%), with overall accuracy of 96%. Baheti R, et al. using a higher cut-off value (6.5 IU/L) demonstrated a sensitivity of 95.83% and specificity of 92.85%, positive predictive value 95.83%, negative predictive value 92.85% and overall accuracy of test was 94.73% [10]. Rana, et al. in their study took an even higher cut-off value (10 IU/L) and calculated a lower sensitivity (66.6%) and specificity (90%) [11].

Role of serum CRP levels in diagnosing bacterial meningitis has been studied in great detail in previous studies and how it can be used to differential it from viral meningitis. However, very few studies have looked at the utility of CSF CRP in differentiating meningitis types. We found CSF CRP levels to be significantly higher among patients with bacterial meningitis as compared to tubercular or viral meningitis. Similar results were reported by Tankhiwale, et al. [12] Furthermore, Gojan, et al. reported higher CSF CRP levels in patients with gram-negative pyogenic meningitis compared to gram-positive pyogenic meningitis [13]. Endotoxin lipoprotein-S present in gram-negative bacteria might be responsible for increasing the permeability of blood brain barrier to CRP. Moreover, lipopolysaccharide-S produced by the gram-negative bacteria is known to induce local production of CRP. Using CSF CRP cut-off value of 9 mg/L we calculated the sensitivity to be 97 % (86.5 to 99.5%) and specificity of 100 % (94.17 to 100%) with overall accuracy of 99% (94.55 to 99.82%). In pediatric population, Khanam et al used a CSF CRP cut-off of 6 mg/L

and reported low sensitivity of 35% with perfect specificity of 100% [14].

There are a few limitations of this study. Firstly, the sample size was small which led to wider confidence intervals. Secondly, CSF-CRP was measured using a semi-quantitative method and thus exact values could not be measured. Lastly, the clinical outcome of the patients could be assessed at the time of data collection.

Conclusion

In this study we found hospital stay more than five days, raised erythrocyte sedimentation rate and anemia to be significantly more common among patients with tubercular meningitis. CSF ADA levels were found to be significantly higher in tubercular meningitis as compared to bacterial or viral meningitis. CSF CRP levels were found to be significantly higher in bacterial meningitis. Both CSF ADA and CRP demonstrated high sensitivity and specificity in diagnosing tubercular and bacterial meningitis respectively. Further investigations studying the measurement of various biomarkers for differentiating meningitis types are suggested.

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