Cerebrospinal Fluid – A Clinicopathologic Analysis

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Abstract

Context and Objective: This study aims to emphasize the importance of an appropriate CSF examination in patients of suspected CNS disease and the necessity of correlating it with the clinico-radiologic findings which will help in early diagnosis of CNS diseases and guide the further management of the disease.

Design: In this 2 year study, 215 CSF samples from patients with clinically suspected diseases of the CNS were studied. The CSF samples were analyzed for gross examination, protein, sugar, adenosine deaminase (ADA) levels, microscopic examination and microbiologic examination. Culture was performed an all cases. A cytospin examination was done where malignancy (primary/metastasis) was suspected.

Results: Of the 215 samples analyzed, 97(45.1%) were found to have abnormal CSF findings. The maximum number of abnormal CSF samples were seen in the adolescents and adults (56.7%) age group. Very high protein levels were seen with bacterial, tuberculous and parasitic meningitis whereas fungal and viral meningitis showed moderate elevation of proteins. CSF ADA levels were raised in cases of tuberculous meningitis with mean value of 20.9 IU/L whereas all other types of meningitis showed normal level of ADA. A total of 3 cases of IVH and 2 cases of demyelinating disease were found in our study. Malignant cells were detected in two cases.

Conclusion: Thus, a study of CSF is vital as it provides an invaluable diagnostic window to the central nervous system atmosphere. A timely and appropriate analysis of CSF can help the clinician to direct the line of treatment and enhance patient care as well as reduce morbidity and mortality.

Introduction

Cerebrospinal fluid (CSF) is an ultrafiltrate of plasma secreted by the choroid plexus present in the ventricles of the brain. A study of CSF is vital as it provides an invaluable diagnostic window to the central nervous system (CNS) atmosphere. The most important indication for CSF analysis is when meningitis is suspected, detecting malignancies and a supportive investigation in demyelinating diseases and subarachnoid or intraventricular haemorrhage.¹ Even today, in spite of a wide range of investigations available to diagnose a variety of CNS diseases, CSF examination remains a rapid, sensitive, reliable and cost-effective test which provides valuable information and aids in timely diagnosis and therapy.

This study aims to emphasize the importance of an appropriate CSF examination in patients of suspected CNS disease and the necessity of correlating it with the clinico-radiologic findings which will help in early diagnosis of CNS diseases and guide the further management of the disease.

Materials and Methods

This prospective study was carried out over a period of 2 years in our hospital after institutional ethics committee approval. A total of 215 CSF samples from patients with clinically suspected diseases of the CNS were studied. Antemortem CSF samples received in the Pathology and Microbiology laboratories were included in the study and autopsy CSF samples were excluded from the study as they are often contaminated. All cases were analyzed with respect to the clinical presentation, clinical examination findings and

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radiologic features. The details of the cases were obtained from the clinical records of the patients.

The CSF samples were analyzed for the following parameters:

**Pathologic Examination**

Gross examination: Quantity, color, turbidity ±, coagulum ± were noted. The CSF was then centrifuged and the presence or absence of a sediment and the colour of the supernatant was recorded.

Protein: Protein estimation was performed by turbidimetry using 5% trichloroacetic acid to precipitate proteins and readings were taken using a semi automated analyser (ERBA Chem PRO) at a wavelength of 630nm.

Sugar: Enzymatic conversion of glucose to a coloured compound was done using glucose oxidase – peroxidase reagent, and readings were taken using a semiautomated analyser (ERBA Chem PRO) at a wavelength of 505 nm.

Adenosine deaminase (ADA) levels: CSF ADA levels were determined in majority of the cases. The CSF ADA level of > / = 10IU/L was considered as diagnostic of TBM.

Microscopic examination:

Total cell count was performed

**Table 1: Abnormal CSF findings in various diseases (n=97)**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Abnormal CSF findings (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningitis</td>
<td>90 (92.7)</td>
</tr>
<tr>
<td>IVH</td>
<td>3 (3.1)</td>
</tr>
<tr>
<td>Demyelinating disease</td>
<td>2 (2.1)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>2 (2.1)</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
</tr>
</tbody>
</table>

**Table 2: CSF findings in different types of meningitis**

<table>
<thead>
<tr>
<th>Type of meningitis</th>
<th>No. of samples</th>
<th>Gross appearance</th>
<th>Protein (mg/dL) (mean)</th>
<th>Sugar (mg/dL) (mean)</th>
<th>Total count (cells/μL) (mean)</th>
<th>N (%)</th>
<th>L (%)</th>
<th>M (%)</th>
<th>E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial meningitis</td>
<td>13</td>
<td>Slightly hazy to opaque white</td>
<td>134.2–854.2 (423.5)</td>
<td>5.12–42.2 (20.5)</td>
<td>94–3040 (559)</td>
<td>56–99 (86)</td>
<td>1–44 (14)</td>
<td>0</td>
<td>0–2</td>
</tr>
<tr>
<td>TB meningitis</td>
<td>38</td>
<td>Slightly hazy to opaque white</td>
<td>44.7–850 (185.4)</td>
<td>12.4–65 (34)</td>
<td>70–1050 (246)</td>
<td>0–48 (13)</td>
<td>50–100 (86)</td>
<td>0–6 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Fungal meningitis</td>
<td>11</td>
<td>Slightly hazy</td>
<td>53–174.1 (88.57)</td>
<td>12.6–62.4 (38.75)</td>
<td>8–120 (35)</td>
<td>0–20 (6)</td>
<td>80–100 (94)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Viral meningitis</td>
<td>9</td>
<td>Clear to slightly hazy</td>
<td>45–110 (77.35)</td>
<td>34.4–82.1 (55.45)</td>
<td>25–96 (63)</td>
<td>0–30 (13)</td>
<td>70–100 (85)</td>
<td>0–8 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Parasitic meningitis</td>
<td>1</td>
<td>Whitish hazy</td>
<td>646</td>
<td>28.2</td>
<td>56W</td>
<td>70</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Results**

Of the 215 samples analyzed, 97(45.1%) were found to have abnormal CSF findings (Table 1).

The maximum number of abnormal CSF samples were seen in the adolescents and adults (56.7%) age group. In neonates with CSF picture showing meningitis (9 cases); fever, lethargy and poor feeding had been the presenting complaints while the rest showed a variety of clinical features ranging from fever, headache, neck rigidity, vomiting, seizures, altered sensorium, neurological deficits and papilloedema.

The CSF findings in different types of meningitis are as depicted in Table 2.

The gross appearance of CSF varied from clear to slightly hazy to opaque whitish. Very high protein levels were seen with bacterial, tuberculous and parasitic meningitis whereas fungal and viral meningitis showed moderate elevation of proteins. Low sugar levels varied with the different types of meningitis.

CSF ADA levels were determined in 69 cases. The values were raised in cases of tuberculous meningitis with mean value of 20.9 IU/L whereas all other types of meningitis showed normal level of ADA.

Cell counts were markedly raised in bacterial and tuberculous meningitis. Neutrophils were predominantly seen in bacterial and parasitic meningitis, while lymphocytes were common in tuberculous, fungal and viral meningitis.

In majority of the cases of meningitis, no organism was
isolated (58.8%). Cryptococcus was isolated in 12.2% cases in adolescents and adults.

Of the total 90 cases of meningitis, 26 patients were found to be HIV positive (15 cases - TBM and 11 cases - Cryptococcal meningitis).

A total of 3 cases of IVH and 2 cases of Demyelinating disease were found in our study.

13 samples were received for suspected cases of malignancy where cytospin examination was done. Malignant cells were detected in two of the cases wherein cytospin examination showed field full of leukaeic blast cells with high nuclear cytoplasmic ratio and prominent nucleoli. The CSF was reported as positive for lymphoblasts, indicating metastasis of Acute lymphoblastic lymphoma (ALL) to the CNS.

59 cases (60.8%) showing abnormal CSF findings were discharged without any complications. Neurodeficits were seen in 7 cases (7.21) whereas 31 cases (31.95%) died during the hospital stay which was most commonly seen with pyogenic meningitis.

Discussion

Cerebrospinal fluid (CSF) analysis is a key tool in the diagnosis of a variety of CNS diseases. Proper evaluation of CSF depends on knowing which tests to order, normal ranges for the patient’s age, and the test’s limitations.

In our study, neonates and elderly presented with relatively nonspecific symptoms while older children and adults presented with more specific symptoms and signs of meningeal irritation. This explains the higher number of normal CSF samples in neonates (72.5%) and in elderly patients (83.3%) than in the other age groups. In children a large number of CSF samples were tapped to rule out meningitis in suspected cases of febrile or hypocalcaemic seizures even though signs of meningeal irritation are absent in them which explains the slightly higher number of normal CSF samples in them.

Similar findings were found by Laving et al,2 Garges et al3 and Brouwer et al.4 In the study by Laving et al,2 neonatal bacterial meningitis had a prevalence of 17.9% amongst cases of suspected sepsis. These findings reemphasize the need to perform lumbar puncture in neonates with symptoms and signs of sepsis. In elderly patients, neurological deficits are seen more commonly in addition to other symptoms.

Studies have found that neonates, infants and children with culture proven bacterial meningitis may have near normal cell counts, sugar and protein values, while adults usually have typical features of pyogenic meningitis.2,3 However in our study we have not found any case in any age group with positive CSF culture and normal CSF biochemical parameters and cell counts.

The yield of bacteria on Gram’s stain and culture depends on several factors like appropriate collection and transport of the specimen, the number of organisms present, prior use of antibiotics, technique used for smear preparation (centrifuged deposit, direct smear etc.), staining techniques and the observer’s skill and experience.5,6 The possible reason for isolating unusual bacteria like Staphylococcus aureus, Escherichia coli, Pseudomonas and Acinetobacter in a higher number of our cases could be due to the fact that 4 of the cases had a past history of hydrocephalus with a ventriculoperitoneal (VP) shunt in situ, and the meningitis was most likely a consequence of blockage and/or infection of the shunt.

In a study by Luby et al,7 twenty five cases of purulent meningitis after trauma or neurosurgery were reviewed and Staphylococcus aureus, Staphylococcus epidermidis, and Gram negative bacilli were found to be the most common pathogens. In our study, only 20 (64.5%) were culture positive while in 11 (35.48%) of the cases, no organism was isolated on Gram’s stain and culture. Other studies in India have also found similar low microbiologic yields.8-10 In a study conducted by Madhumita et al,5 in 120 adult patients with bacterial meningitis, bacterial pathogen could be demonstrated by the Gram stain in the CSF samples in only three (2.5%) patients, while 22 (18.3%) samples yielded bacterial growth on culture meningitis in this study.

In TB meningitis, though a predominance of lymphocytes was seen in most cases, a few of the cases a showed a considerable number of neutrophils (upto 48%). Other studies have also shown similar findings of variable cell differential counts ranging from a predominant lymphocytosis to a neutrophilic pleocytosis.11-13 This paradoxical response could be due to a hypersensitivity reaction related to the release of tubercular proteins during treatment.16

In our study, ZN stain for AFB was positive in only 2.85% of cases. Similar findings of low positivity of ZN smears and culture for identifying AFB has been reported in other studies. Besides low yield, another major drawback of culture is that it can take up to 8 weeks for the Mycobacterium colonies to grow.11,12 Hence newer modalities like CSF ADA are increasingly being used in the diagnosis of TB.17,18 The sensitivity and specificity of this test varies from 44 to 100% and 71 to 100% respectively. However standard cutoffs of ADA values in the diagnosis of TBM have not been established, and the values used in various studies range from 5 to 15 IU/L.16,17 In our study CSF ADA > /= 10 IU/L has been used as a cut off for diagnosing TBM. ADA may have both false positives as well as false negatives and should only be used as an ancillary investigation to diagnose TBM. Hence culture is still the gold standard.
standard for diagnosing TB and it also allows drug sensitivity testing of the mycobacteria. A review of literature shows that Cryptococcus neoformans is the commonest form of fungal meningitis and its incidence in HIV patients is variable.

In our study, all patients of viral meningitis were discharged without any neurodeficit. A review of literature also showed a similar low incidence of fatality and morbidity in cases of viral meningitis.

Subarachnoid (SAH) and intraventricular hemorrhage are diagnosed on CT scan or by xanthochromia of the cerebrospinal fluid (CSF). The use of CSF examination in the diagnosis of subarachnoid haemorrhage diminished considerably since the advent of advanced radioimaging modalities. However it is still of use when the imaging is normal in a suspected case of SAH.

The CSF findings in cases of demyelinating diseases in our study were of an albuminocytologic dissociation which concurs with the findings of other studies on inflammatory demyelinating neuropathies.

A review of literature showed that CNS relapse continues to afflict 3 to 5% of children with acute lymphoblastic leukemia (ALL). More than 60% of these occur in patients with no clinical or cytomorphic evidence of CNS involvement at initial diagnosis. These results stress the usefulness of spinal fluid cytology as a screening procedure in asymptomatic patients with leukemia and are of interest in regard to the pathophysiology of leukemia of the central nervous system.

Thus, a study of CSF is vital as it provides an invaluable diagnostic window to the central nervous system atmosphere. A timely and appropriate analysis of CSF can help the clinician to direct the line of treatment and enhance patient care as well as reduce morbidity and mortality.

References