ORIGINAL ARTICLE

Detection of meningococcal meningitis in cerebrospinal fluid of patients with neurological disorders in government hospitals of Karachi

Aneela Taj, Nusrat Jamil

Abstract

Objective: To investigate the microbiological yield from the apparently transparent cerebrospinal fluid samples of in-patients with suspected neurological disorders.

Methods: Samples of CSF were collected from Neurology and Neurosurgery Wards of the Jinnah Postgraduate Medical Centre and the Civil Hospital, Karachi, from December 2007 to March 2012, and comprised cerebrospinal fluid samples collected from neurologically compromised patients through lumbar puncture. The processing of the samples was done at the Department of Microbiology, University of Karachi. Moreover, 10ml of each sample was streaked separately on different culture media, i.e. Nutrient Agar, Blood Agar and Chocolate Agar, for the isolation of both aerobic and anaerobic bacteria.

Results: Of the 92 samples, bacterial meningitis was found in 21(22.8%), whereas 71(77.1%) samples did not yield any bacterial aetiology. Besides, 1(4.7%) sample revealed Gram-positive rods of *L. monocytogenes* while Gram-negative kidney-shaped *N. meningitidis* was found in 20(95.24%) samples.

Conclusion: Present study highlighted that apparently clear CSF samples predominantly revealed meningococcal meningitis. It is important to note that extracellular bacterial growth in CSF is not always the primary goal of pathogenesis, therefore establishing a fact that turbidity of CSF is not the cardinal symptom for the diagnosis of bacterial meningitis.

Keywords: Cerebrospinal fluid, Bacterial meningitis, *N. meningitides*, Culture, Central nervous system, Blood brain barrier. (JPMA 66: 1418; 2016)

Introduction

Bacterial meningitis (BM) is an acute infection in which the meninges, the subarachnoid space, and the brain parenchyma are all frequently involved in the inflammatory reaction. This disease is characterised by severe headache, fever, intolerance to light and sound, and rigidity of muscles, especially those of the neck. The central nervous system's (CNS) inflammatory reaction from bacterial meningitis may result in decreased consciousness, seizures, raised intracranial pressure and stroke.[1,2] *Neisseria meningitides* and *Listeria monocytogenes* are commonly responsible for this infection.[3,4]

Delays in diagnosis and treatment of meningococcal disease may contribute to its high morbidity and mortality.[4,5] Diagnosis of BM is normally done by physical and microscopic examination of the cerebrospinal fluid (CSF) in addition to clinical symptoms. However, direct microscopy of the CSF lacks sensitivity, hence may not promisingly contribute in the diagnosis. Therefore, once there is a suspicion of acute bacterial meningitis, CSF samples must be taken for culture in addition to routine examination to determine whether the CSF finding is consistent with the clinical diagnosis.[4]

Department of Microbiology, University of Karachi, Karachi, Pakistan.
Correspondence: Nusrat Jamil. Email: nusrat_91@yahoo.com

Investigations in the past have been limited to the concept that bacteria essentially cross the blood brain barrier to reach the meninges. Consequently, turbidity of the CSF may occur due to bacterial growth.[6] On the contrary, recent evidence suggests that bacteria may adopt certain other strategies to affect CNS.[7,8]

Moreover, administration of pre-admission antibiotics to patients with suspected invasive meningococcal disease has been supported by the clinicians.[9] Nevertheless, this may affect the physical appearance, i.e. transparency, and the efficacy of the microbiological examination of CSF. Consequently, suspected BM may be misdiagnosed.

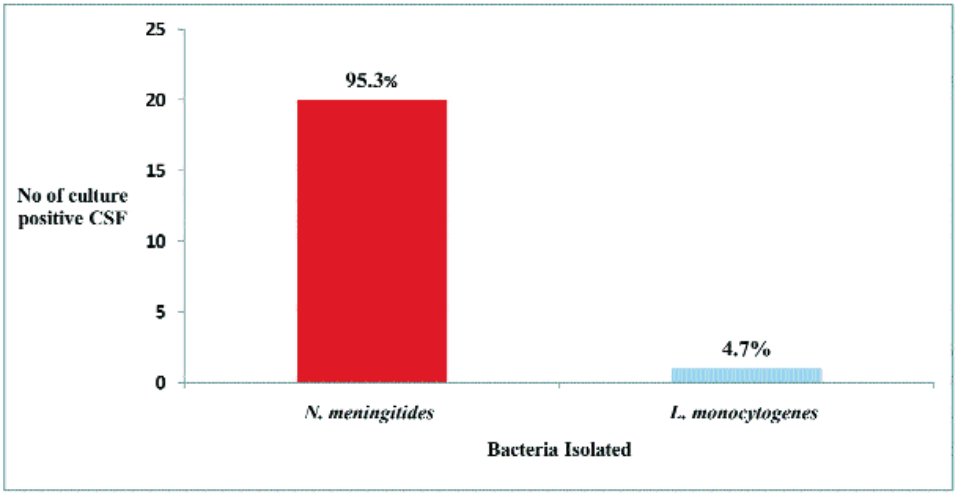
The present study was planned to investigate the microbiological yield from the apparently transparent CSF samples of the patients admitted with suspected neurological disorders. This study would establish a fact that turbidity of CSF is not the cardinal symptom for the diagnosis of bacterial meningitis.

Materials and Methods

Samples of CSF were collected from Neurology and Neurosurgery Wards of the Jinnah Postgraduate Medical Centre and the Civil Hospital, Karachi, from December 2007 to March 2012. The samples were collected aseptically from different mentally ill patients through lumbar puncture (LP) method and kept at -20°C till further use. The processing of the samples was done at the

Department of Microbiology, University of Karachi.

For the isolation of aetiological agents, 10µl of the CSF sample was streaked separately on different culture media i.e. Nutrient Agar, Blood Agar and Chocolate Agar (Merck). An experiment was run in duplicate for the isolation of both aerobic and anaerobic bacteria. One set of the media plates were incubated aerobically at 37°C. For the isolation of anaerobic aetiological agents, another set of above-mentioned media was incubated at 37°C in the presence of 5% carbon dioxide (CO_2). All plates were initially incubated for 24 hours; incubation was then continued up to 48 hours for late growers. Observations were made first at 24 hours and then after 48 hours. Bacteria were identified on the basis of colonial and cellular morphology and standard biochemical reactions.



CSF: Cerebrospinal fluid.
L. monocytogenes: Listeria monocytogenes.
N. meningitidis: Neisseria meningitides.

Figure-2: Aetiological Agents Isolated from apparently transparent negative CSF samples.

Results

Of the 92 CSF samples, 13(14.1%) were collected from Jinnah Postgraduate Medical Centre, 7(7.6%) from the diagnostic lab of Civil Hospital Karachi (CHK) and 72(78.26%) from the CHK branch of Dr. Essa's Laboratory and Diagnostic Centre. Moreover, 54(59%) samples related to male patients and 38(41%) to female patients.

Bacterial meningitis was found in 21(22.8%) CSF samples, whereas 71(77.1%) did not yield any bacterial aetiology (Figure-1). Of the bacterial culture samples, 1(4.7%) revealed Gram-positive rods of *L. monocytogenes* while Gram-negative kidney-shaped *N. meningitides* was found in 20(95.24%) samples (Figure-2).

Discussion

The present study highlighted the importance of applying culture-based methods in confirming the diagnosis of BM.

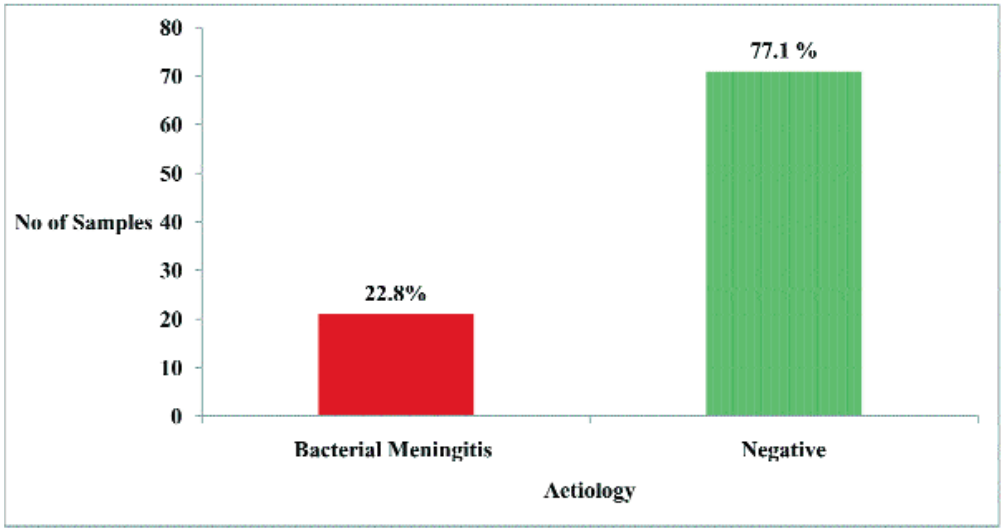


Figure-1: Graphical representation of the occurrence of meningococcal meningitis in neurological patients.

In order to screen the presence of neuropathogens, we analysed 92 apparently transparent and Gram-negative CSF samples. Aetiological agents were identified on the basis of physical, biochemical and microbiological examination of the collected samples. Of all, 21 samples revealed bacterial aetiology. *L. monocytogenes* was found in 4.7% of the CSF samples whereas 95.3% yielded *N. meningitides* (Figure-2). The results of this study

indicated that most of the hospitalised patients were suffering from mening-ococcal meningitis. These results matched with the findings of other authors[10,11] who performed surveillance study during the meningococcal sero group A epidemic which occurred in Karachi in 1988, in which out of 112 cases 20% had septicaemia and 80% had meningococcal meningitis. Our results can be further supported by the findings of other researchers[11-15] who suggested that over 30% of bacterial meningitis in adults and 20% in children was due to *N. meningitidis*.

Interestingly, all the CSF samples used in this study did not reveal any aetiology on Gram staining. Although Gram stain examination of CSF permits a rapid, inexpensive and accurate identification of the causative bacterium in 60-90% of the patients suspected with bacterial meningitis, the probability of having a positive Gram stain result mainly depends on the specific bacterial pathogen causing the disease.[4,16] Concentration of bacteria in CSF on the other hand promisingly contributes in the likelihood of visualising the bacteria. Our results extended these findings and suggested microbiological culture is the gold standard technique in isolation and identification of the causative agent of the meningococcal disease.

N. meningitides, being an extracellular pathogen, expresses capsule as a main virulence factor that prevents bacterial phagocytosis mediated by the host immune response.[17,18] *L. monocytogenes*, on the other hand, is a facultative intracellular bacterium which may gain the CNS in infected cells, such as circulating leucocytes, which are known to be able to cross themselves the blood-brain barrier.[19] Therefore once inside the CSF, bacterial multiplication is thought to be uncontrolled due to the local deficiency in complement and immunoglobulins and despite the influx of polymorphonuclear leukocytes induced by the local inflammatory response. Consequently, the clinical manifestation of the BM shows no presence of apparent turbidity that mainly occurs when bacteria grow extra cellularly in CSF. Furthermore, Gram staining may lead to negative results due to internalisation of the bacteria. In view of the fact that considering turbidity as an essential symptom of BM may lead to the misunderstanding of BM as neurological disorder, microbiological culture of the CSF is pivotal.

Findings of our study showed that 71 CSF samples (77.1%) did not yield any bacterial aetiology (Figure-1). These findings are in line with Zaidi et al.[20] who reported that 3.6% cases of meningitis were due to *N. meningitides* whereas no aetiology was found in 80% of the cases due to high rates of antibiotic usage prior to lumbar puncture. This can be considered significant bearing in mind that antibiotics given prior to CSF collection caused noteworthy reduction in the rate of isolation of bacteria which, on the other hand, considerably increased the number of negative cultures. Although prior antibiotic therapy effectively killed the bacteria, bacterial lysis yields certain bacterial components. Bacterial Lipopolysaccharide (LPS), peptidoglycan, deoxyribonucleic acid (DNA), capsular polysaccharide and exotoxins are the known biologically active components to date. Furthermore, potential of these components to exert their deleterious effects on CNS is now a well-established fact.[6,7] The existence of viral or aseptic meningitis could be another possible explanation for the negative results of bacterial culture.

Conclusion

Cerebrospinal fluid samples predominantly revealed meningococcal meningitis. Furthermore, CNS infections can be misdiagnosed and mistreated. The detection and diagnosis of neurological infections on the basis of clinical signs in addition to physical and microscopic examination, i.e. Gram stain, were unsatisfactory. Therefore, bacterial culture must be performed in conjunction with the routine CSF diagnostic tests as they serve as a pivotal diagnostic tool for the diagnosis of BM. On the contrary, negative results of bacterial culture would highlight the likelihood of viral meningitis. Therefore, bactriologically negative CSF samples must be checked for the presence of viral aetiology.

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Conflict of Interest: None.

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