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LATEX PARTICLE AGGLUTINATION TEST AS AN ADJUNCT TO THE DIAGNOSIS OF BACTERIAL MENINGITIS

K Surinder, *K Bineeta, M Megha

Abstract

The present study aimed to review the results of microscopic examination, routine culture and antigen detection by latex particle agglutination test (LPAT), in order to evaluate the diagnostic value of the LPAT in establishing the aetiological diagnosis of bacterial meningitis. LPAT was done in 65 clinically suspected meningitis cases ranging from 5 days to 60 years of age and was compared with culture and Gram stain. Using LPAT, an aetiological diagnosis could be done in 10 out of 65 (15.4%) cases of bacterial meningitis. In contrast, Gram stain and culture showed 16.9 and 23.1% positivity, respectively. LPAT correlated well with Gram stain and culture and can be recommended as an adjunct laboratory test for rapid aetiological diagnosis of bacterial meningitis for prompt institution of proper antibiotics.

Key words: Culture, Gram stain, latex agglutination, meningitis

Bacterial meningitis is a serious disease and is potentially associated with a high rate of acute complications, risk of chronicity and death.1 Early implementation of appropriate antimicrobial therapy requires prompt identification of the infecting pathogen. The non-specific nature of clinical presentation, especially in children, and lack of laboratory facilities can delay or obscure diagnosis.2 Although culture is considered to be the definitive diagnostic test, microscopic examination of Gram-stained specimen of cerebrospinal fluid (CSF) may provide immediate information about the causative microorganism.3 Unfortunately, the yield of microorganisms on Gram stain depends on factors like the number of organisms present, prior use of antibiotics, technique of preparing slide and observer’s skill. Previous studies have suggested that the sensitivity of this technique ranges from 60% to 90% and the specificity approaches 100%.4 Culture is time consuming and can give false-negative results if the specimen has been transported and stored under unsatisfactory conditions or if an antibiotic therapy has been initiated before the specimen was taken. Several studies report poor CSF culture positivity under Indian conditions.5 The detection of soluble bacterial antigens in CSF of patients with meningitis could be an important diagnostic tool as it has distinct clinical advantages such as reported high sensitivity and specificity, simplicity in execution and interpretation, rapidity in being performed and reported by non-specialized laboratory technicians and finally no alteration of results by prior antibiotic therapy. However, some authors6 are of the opinion that CSF bacterial antigen detection did not enhance the diagnostic accuracy as there could be false-positive reactions and negative test never rules out bacterial meningitis.

In the present study we reviewed the results of microscopic examination, routine culture and antigen detection by latex particle agglutination test (LPAT) of 65 patients, in order to establish the diagnostic value of the LPAT for the aetiological diagnosis of bacterial meningitis.

Materials and Methods

The study included 65 cases of bacterial meningitis patients based on clinical suspicion. Demographic details, a detailed history including prior antibiotic intake and clinical examination, were recorded in a standard proforma. CSF procedures performed included total and differential white blood cells (WBC) count, protein and glucose concentration, Gram stain, culture and antibiotic sensitivity tests, and LPAT. Cerebrospinal fluid specimens were prepared for microscopic examination by centrifugation at 1500 rpm for 15 minutes at room temperature. After conventional Gram staining, slides were examined by light microscopy at ×100 magnification to allow quantitation of WBC and then at ×1000 magnification under oil immersion. The presence of morphology of organisms were noted. For culture, approximately 0.15 mL of the deposit of the centrifuged CSF specimen was inoculated onto each of one 5% sheep blood agar and one chocolate agar and 1.0 mL was inoculated into 3-4 mL of brain heart infusion broth. The blood agar plates were streaked with Staphylococcus aureus (Plazens) strain for demonstration of satellitism of Haemophilus influenzae. Agar plates were incubated at 37 °C in 5% CO₂ and examined daily for 3 days. Broth cultures were incubated at 37 °C and examined daily for 7 days. Any growth present on the plates was identified appropriately according to the standard recommendations.7 If any turbidity was seen in the
broth culture, a repeat smear was made for Gram staining and subcultures were done.

Cerebrospinal fluid was tested for bacterial antigen by LPAT using the BIO-RAD PASTOREX® MENINGITIS Kit. The kit was provided with sera to detect antigens of common aetiological agents of bacterial meningitis, namely *Neisseria meningitidis* A/B/C/Y/W135, *Escherichia coli* K1, *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, *Streptococcus* Group B. The test was carried out with the supernatant according to the manufacturer’s instructions.

**Results**

The study population consisted of 65 patients clinically diagnosed with meningitis, 40 (61.5%) males and 25 (38.5%) females in the age group of 5 days to 60 years. The mean age was 9.88 years and the average length of hospital stay was 10.2 days. Twenty (30.8%) of them had history of antibiotic intake before presentation. The clinical presentation was frequently non-specific; the most common features were fever in 53 (81.5%), coma in 32 (49.2%) and convulsions in 24 (36.9%) patients.

Cerebrospinal fluid culture was positive only in 15 (23.1%) cases, *Neisseria meningitidis* being the most frequent organism encountered in four (26.7%) cases. One isolate of coagulase negative *Staphylococcus* (CoNS) was counted as ‘contaminant’, because the corresponding blood culture was not positive for CoNS and the patient neither was on any shunt nor was clinically septic. Of these 15 samples, the Gram stain of CSF showed positive results in 11 (73.3%) cases. In the remaining 50 samples, two (4%) were thought to yield a misleading false-positive result on Gram stain. The culture yielded no growth and aerobic spore bearers in the first and second samples, respectively and the direct examination was negative for pus cells in both. Of the remaining 48 samples that were negative for microscopic examination, there were five (10.4%) specimens in whom the culture yielded organisms that were judged to be contaminants (aerobic spore bearers).

Latex agglutination test detected bacterial antigens in 10 (15.4%) cases of bacterial meningitis. The common aetiological agents identified were *Neisseria meningitidis* A in 4 (40%), *Streptococcus pneumoniae* in 3 (30%), *Escherichia coli* in 2 (20%) and Group B *Streptococcus* in 1 (10%) cases. In 55 (84.6%) cases, no organisms could be detected. No CSF specimen was positive for *Neisseria meningitidis* B/C/Y/W35 and *Haemophilus influenzae* type b. Interestingly, two of 4 (50%) culture negative specimens were positive with latex agglutination for *Neisseria meningitidis* group A. Table shows the correlation of culture, latex agglutination and Gram stain findings for the CSF specimens obtained from 65 cases of bacterial meningitis.

All the patients diagnosed to have bacterial meningitis by culture had cell count >50 cells/mm³, glucose ≤45 mg/dL and proteins >100 g/L. One case that showed CoNS on culture, however, showed normal glucose and protein values and only a modest increase in cell count (40 cells/mm³).

**Discussion**

Bacterial meningitis plays a major role in morbidity and mortality, despite the availability of effective antibiotics. The diagnosis of bacterial meningitis with the identification of the causal agent plays a crucial role in preventing unnecessary associations or indiscriminate use of broad-spectrum antibiotics.

The existence of 76.92% of aetiological indetermination observed in 50 of 65 cases in the present study, much higher than previous reports,⁴ points out the need for the improvement of laboratory procedures, storage and transport conditions of CSF samples. The inability of establishing a definitive aetiological diagnosis may be due to several factors such as the inoculum size or the previous use of antimicrobial drugs.
The LPAT for bacterial antigen detection was positive in 10 (15.4%) cases. Our results demonstrate that latex agglutination positivity correlated well with the culture results. Only in two of 65 cases (3.1%) did LPAT show positive results in the absence of any growth on culture; and in both these cases, the patients gave history of antibiotic intake prior to collection of CSF samples. Previous studies have reported low sensitivity of culture when performed after the initiation of antibiotic treatment. Other workers have advocated the usefulness of bacterial antigen detection test for the diagnosis of bacterial meningitis, especially in situations where patient has received prior antibiotics. The results of the present study demonstrate the same.

The present study shows that the microscopic examination of Gram-stained CSF is likely to suggest the correct aetiologic agent in 11 of 15 (73.3%) cases of bacterial meningitis when compared to culture or LPAT. The presence and quantity of WBC on the CSF Gram stain is useful to identify specimens that deserve closer scrutiny in the laboratory as, except for one sample that showed no pus cells on Gram stain, all the samples that were positive on culture or by LPAT showed the presence of more than five to six pus cells/high power field on Gram stain.

The study concludes that along with greater ease of performance, LPAT for specific bacterial antigens compared favourably with the conventional tests and is rapid enough to guide the clinician for institution of proper antibiotics; hence, it can be included as an adjunct laboratory test for establishing the aetiological diagnosis of bacterial meningitis particularly in pre-treated cases.

References


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