Original Article

STUDY OF ACUTE BACTERIAL MENINGITIS IN CHILDREN BY GRAM STAIN, CULTURE AND ANTIGEN DETECTION

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ABSTRACT

Objective: Acute Bacterial Meningitis (ABM) is associated with a high mortality rate and morbidity in paediatric population despite recent advances in diagnostic methods, antimicrobial and supportive treatments and monitoring. This study is therefore undertaken to aid in rapid diagnosis of ABM by latex agglutination test (LAT) and to comparatively evaluate Gram stain, Culture and LAT in the diagnosis of ABM along with antibiotic susceptibility pattern of the isolates. We undertake this study in children with symptoms of meningitis to isolate and identify the pathogens in CSF and blood and to determine their antibiotic susceptibility pattern. We will compare and evaluate the three methods Gram stain, culture and Antigen detection.

Methods: A hospital-based prospective study conducted at Government General Hospital, Kakinada during December 2018–August 2020. A total of 50 clinically suspected cases of bacterial meningitis in the paediatric age group were taken. The CSF sample was collected and subjected to Gram stain, culture and antigen detection tests using PASTOREX ™MENINGITIS kit.

Results: Out of 50 samples collected, 15 cases were declared as laboratory confirmed cases as per WHO criteria. Gram stain was positive in 6 cases. CSF culture could identify 8 cases of ABM. CSF LAT was positive in 12 cases. *Group B Streptococcus* was the most common etiological agent in neonates, while *S. pneumoniae* in children. The isolates were 100% sensitive to cefoperazone, cefuroxime, cefepime and gentamicin.

Conclusion: Although Gram stain is simple and CSF culture is the gold standard, LAT has an advantage over Gram stain in terms of species identification. It was found to be more sensitive, rapid, easy to perform and could identify fastidious organisms like *S. pneumoniae, N. meningitidis and Group B Streptococcus.*

Keywords: Acute bacterial meningitis, Antigen detection, Culture, Gram stain

INTRODUCTION

Bacterial meningitis is a severe life-threatening infection of the meninges surrounding the brain and spinal cord. Acute Bacterial Meningitis (ABM) is seen more in children than adults. It is associated with a high mortality rate and morbidity in paediatric population despite recent advances in diagnostic methods, antimicrobial and supportive treatments, monitoring and prophylactic techniques [1].

The aetiology of bacterial meningitis varies by age group and region of the world, the reasons for this association remains incompletely understood. Increased availability, awareness and usage of vaccines may contribute to change in the epidemiological pattern of these pathogens [2].

Bacterial meningitis most commonly results from the haematogenous dissemination of microorganisms from the distant infection site, often from the respiratory tract, followed by infections of paranasal sinuses and otitis media. More than two-thirds of meningitis cases occur in the first two years of life due to high vascularity of the brain and decreased immunity [3].

The global burden of the disease was high in the pre-antibiotic era. Lethality was approximately 100% and the rare survivors developed serious neurological sequelae [4]. Community incidence of ABM in India varies from 0.5% to 2.6%8. The average incidence in India works out to be 1% of the total admissions to the paediatric ward and 0.8% of the total number of new paediatric cases seen in all outpatient departments [5]. The clinical signs and symptoms cannot always be relied upon due to the nonspecific nature of the clinical presentation, especially in children.

Although CSF culture is considered the definitive diagnostic test, is time-consuming (24-48 h) and can give false negative results if the specimen has been transported and stored under unsatisfactory conditions or if antibiotic therapy has been initiated before sample was taken [6].

Gram stain is the most rapid, least expensive and simplest method for the presumptive ABM diagnosis. Unfortunately presence of microbes in Gram stain depends on factors like volume of CSF, the number of organisms present, the technique of preparing slides, and prior use of antibiotics [6].

The detection of soluble bacterial antigens in CSF of patients with meningitis is an important diagnostic tool. Although it does not replace the conventional methods, it has distinct clinical advantages such as high sensitivity, specificity, simplicity in execution, rapidity and can be easily performed by laboratory technicians and no alteration of results by prior antibiotic therapy.

MATERIALS AND METHODS

Study design: prospective study

Study group: This study was done in the Department of Microbiology, Government General Hospital, Kakinada, from December 2018–August 2020. A total of 50 clinically suspected cases of bacterial meningitis in the paediatric age group were taken.

Inclusion criteria

Children who were below 12 y of age having signs and symptoms (fever, vomiting, seizures, altered sensorium, refusal to feeds) of

meningitis. The samples were collected before administration of antibiotics whenever possible.

Exclusion criteria

Children developing meningitis following head trauma or neurological procedure were excluded.

WHO criteria for establishing a confirmed diagnosis included the following [7]

A case of clinically suspected meningitis was subjected to laboratory tests, including CSF and or blood culture. Positivity on culture for an organism known to cause meningitis, or the identification of the pathogen by Gram stain or by antigen detection methods, established the diagnosis of ABM.

Sample collection

1. CSF: After taking a detailed history and physical examination, lumbar puncture was done by a paediatrician. CSF sample (3 ml) was collected under all aseptic precautions.

The sample was transferred to three sterile containers of 1 ml quantity each, one given to the Pathology laboratory for cell count cell type, one to biochemistry laboratory for glucose and protein estimation and the remaining 1 ml was used for Gram stain, culture and LAT.

2. Blood: about 1-3 ml of blood was collected in BHI broth.

Laboratory examination

The CSF was inspected grossly for turbidity, colour and xanthochromia. The wet mount was done and observed for presence of bacteria, yeasts, RBCs, WBCs etc. After centrifugation, the supernatant was used for serological test (Latex Agglutination Test). The sediment was used for Gram stain and culture.

CSF–Gram stain: Smears were prepared by placing 1 or 2 drops of sediment of CSF on a slide, allowing the drop to form a large heap and air dried. Gram stain was done and observed for the presence of pus cells and organisms.

CSF Culture: The sediment was heavily inoculated into the following media:

1. Blood agar plate incubated at 37 °C in 5-10 % CO_2
2. Chocolate agar plate incubated at 37 °C in 5-10% CO_2
3. MacConkey agar incubated aerobically at 37 °C.
4. BHI broth incubated aerobically at 37 °C

The inoculated primary plates were incubated for 48-72 h. The plates were examined daily for 3 d before discarding them as negative. BHI broth was incubated for seven days and examined daily for presence of turbidity. The tubes showing turbidity were sub cultured on the Chocolate agar plate and MacConkey agar plate. Tubes that remained non-turbid were subcultured on 7th d before discarding.

Blood culture

Blood collected in BHI broth and incubated at 37 °C. if any turbidity noted it was subcultured onto Blood agar and Chocolate agar and incubated in CO_2 atmosphere for upto 48–72 h and onto MacConkey agar which is incubated aerobically overnight.

According to standard techniques, any growth on the above-mentioned media was identified based on their colony morphology, cultural characteristics and biochemical reactions.

Antibiogram

The antibiotic sensitivity test was done as per CLSI guidelines using Kirby Bauer method depending upon organism's presumptive identification.

eg.: *H. influenzae*–Chocolate agar *Pneumococci*–chocolate agar/blood agar Gram negative bacilli–Mueller Hinton agar

The choice of antibiotic discs based on the Gram reaction of the isolated pathogen. The diameter of zone of inhibition was measured and recorded and reported as sensitive or resistant depending upon zone size.

Latex agglutination test

The CSF samples were tested for bacterial antigen using PASTOREX MENINGITIS, a bacterial antigen kit to detect the antigen of 5 organisms.

Streptococcus pneumoniae

Group B Streptococcus

H. influenzae type b

N. meningitidis A, C, Y, W 135

N. meningitidis B/E. coli K_1

Meningococcus Group B polysaccharide antigen being structurally and immunologically related to *E. coli K_1*polysaccharide antigen, It is provided as a single test latex reagent. Depending upon the child's age, the positive reaction in neonatal specimen would suggest *E. coli K_1*infection, and in older children, *Meningococcus Group B* and also by correlating with direct smear examination of CSF.

Procedure

CSF is preheated at 100 °C in a water bath for 3 min, cooled to room temperature, and centrifuged for 5 min at 3000g to remove proteinaceous material that would cross react with the antigen. The supernatant is then used for LAT. Disposable cards containing seven separate circles are provided with the kit. One drop of CSF is placed on the particular circle of the reaction card, and one drop of six different test latex reagents are added to separate circles, mixed thoroughly, and rotated at 120 RPM for 10 min, and observed for agglutination. Positive and negative controls were put up simultaneously.

Statistical analysis

Results were analysed and sensitivity, specificity, PPV, NPV, were calculated as per standard statistical methods.

RESULTS

Fifty cases of paediatric age group (<12 y), who presented with signs and symptoms of meningitis were subjected to laboratory confirmation of ABM.

Table 1: Age and sex distribution of cases (n=50)

Age	No. of cases	Male	Female
0-1 m	13	09	04
1-3 m	08	05	04
3 m–1 y	16	09	07
1-5 y	07	04	03
5-10 y	04	03	01
10-12 y	02	01	01
Total	50	31	19

As per table 1, most cases i.e. 16 (32%) cases were in the age group of 3m-1 y. The least number of cases i.e. 2(4%), were in the age group of 10-12 y. out of 50 cases, 37 (66.66%) cases were under one year of age. Males (31) were affected more than females (19) with a male to female ratio of 1.6:1.

Table 2: Laboratory-confirmed cases of ABM as per WHO criteria

Test	No. of cases
Culture, Gram stain and LAT	03
Culture and Gram stain	01
Culture and LAT	02
LAT and Gram stain	02
Culture only	02
LAT only	05
Gram stain only	00
Total	15

As per table 2, two cases that were identified by culture only were isolated from both blood and CSF culture. As per WHO criteria 15/50 (30%) cases were laboratory confirmed cases of ABM in the present study.

Table 3: Etiologic agents identified by CSF by culture, LAT, Gram stain (n=15)

Etiology	Total	Culture	LAT	Gram stain
S. pneumoniae	06	02	06	02
S. agalactiae	03	02	03	02
E. coli	02	01	02	01
Acinetobacter	01	01	00	01
N. meningitidis	01	00	01	00
*P. aeruginosa**	01	01	00	00
*K. pneumoniae**	01	01	00	00
Total	15	08	12	06

*Organisms were also isolated in blood culture

Table 3, shows the comparative analysis of culture, Gram stain, and LAT. CSF LAT could identify the maximum number of ABM i.e. 12/15 (80%) cases followed by culture 8/15(53.3%) and Gram stain 6/15(40%) cases.

CSF culture was positive in 8/15(53.6%) cases. P. aeruginosa and K. pneumoniae that were identified in CSF culture were also isolated in blood culture.

CSF Gram stain was positive in 06/15 (39.6%) cases. Pus cells with Gram-positive cocci were observed in 04 cases (26.4%) and Gram negative bacilli with pus cells in 02(13.2%) cases.

S. pneumoniae was the commonest organism identified by culture, Gram stain, and LAT i.e., 6/15 (40%) cases followed by *S. agalactiae* in 3/15(19.8%) cases and 2/15(13.3%) cases of *E. coli*. One case of *N. meningitidis* was identified by LAT only. Overall fastidious bacteria like *N. meningitidis, S. pneumoniae,* and *S. agalactiae* were more often identified by LAT compared to culture. *P. aeruginosa, K. pneumoniae*, *Acinetobacter spp.* were identified by culture.

Antibiotic susceptibility pattern

The antimicrobial sensitivity patterns of the isolates showed that all Gram-positive and Gram-negative organisms were 100% sensitive to third and fourth-generation cephalosporins (cefoperazone, cefepime) and aminoglycosides (gentamicin). Cotrimoxazole and ampicillin were the least effective.

Table 4: Accuracy indices of CSF Gram stain against CSF culture as a gold standard (n=50)

Test	Culture positive	Culture negative	Total
Gram stain positive	04	02	6
Gram stain negative	04	40	44
Total	08	42	50

Table 4 shows comparison of CSF Gram stain and CSF culture. Gram stain showed a sensitivity of 50%, specificity of 95.24%, the positive predictive value of 66.67% and a negative predictive value of 90.91%.

Table 5: Accuracy indices of CSF LAT against CSF culture as a gold standard (n=50)

Test	Culture positive	Culture negative	Total
LAT positive	05	07	12
LAT negative	03	35	38
Total	08	42	50

Table 5 shows comparison of CSF LAT and CSF culture. LAT showed a sensitivity of 62.5%, specificity of 83.3%, the positive predictive value of 41.6% and a negative predictive value of 92.1%.

Table 6: Comparison of sensitivity, specificity, PPV, NPV of CSF gram stain, CSF LAT with culture as a gold standard

Test	Sensitivity	Specificity	PPV	NPV
Gram stain	50%	95.24%	66.67%	90.91%
LAT	62.5%	83%	41.6%	92.10%

As per table 6, LAT was found to have a higher sensitivity i.e., 62.5%, when compared to Gram stain 50%, however, the specificity of LAT (83%) was lower than that of Gram stain (95.24%). NPV of LAT was only marginally higher i.e. 92.10%; however, PPV of LAT was lower i. e 41.6% than that of Gram stain (66.67%).

CSF cytology and biochemical parameters

In the present study, CSF leucocytes 28/50(56%), CSF protein 24/50(48%), CSF glucose 20/50(40%) were suggestive of bacterial meningitis. In our study, all laboratory-confirmed cases (15) showed positive CSF findings.

Case fatality rate

In the present study, the case fatality rate was 1/15 (6.6%) which was observed in a case of *S. pneumoniae.*

DISCUSSION

Bacterial meningitis is one of the most common infectious disease emergencies involving the central nervous system. The rapid laboratory diagnosis of meningitis is of particular concern to the clinician and clinical microbiologist. Gram stain and culture remain the standard tests for the laboratory diagnosis of meningitis. However, they are often augmented with antigen detection (latex agglutination) tests. These commercially available tests can be of help in the rapid recognition of the more common organisms causing ABM [8]. Early etiological diagnosis and appropriate treatment results in a higher cure rate and lowers incidence of fatal complications.

In the present study, male to female ratio was 1.6:1 and correlated with studies of Modi Gaurav B *et al.* [9], Jagadevi *et al.* [10] and Sayedafasiha *et al.* [11]. This male preponderance may be due to the relative absence of gene locus for elaboration of immunoglobulins, which has been documented to be located on the X chromosome [12].

In the present study, 37/50 (74%) children were below one year of age, of which 13(26%) were neonates. Similar observations were noted by Chinchankar N *et al.* [13]. The majority of meningitis cases occur in the first year of life owing to decreased immunity and high vascularity of the brain [14].

S. pneumoniae was the most common etiological agent, followed by S. agalactiae accounting for 6/15(40%) and 3/15(20%) cases respectively. Similar results were observed by Mishra B *et al.* [15] and Jagadevi *et al.* [10].

Comparative studies of gram stain

In the present study the percentage positivity of Gram stain was 40%. Our study correlates with studies of the author, Shilpa Dayanand *et al.* [16] and Shiva Prakash *et al.* [17]. The probability of visualizing bacteria on Gram stain CSF smear is dependent on the number of organisms present, 25% are positive with<1000 colony forming units (CFU) per ml of CSF, 60% with 1000–10000 CFU/ml and 97% with>10000 CFU/ml [18].

Comparative studies of CSF culture and blood culture

In the present study, CSF culture detected 8/15 confirmed cases of ABM, i.e. 53.3%. The culture was negative in 7/15 (46.7%). Our study correlates with studies of Jyothi R *et al.* [19], Sayedafasiha *et al.* [11] and Chinchankar *et al.* [13]. Reasons for low CSF culture yield may be, low bacterial load, use of antimicrobial agents prior to CSF collection, poor culture media, less quantity of CSF which is not sufficient for centrifugation, stored in unsatisfactory conditions, delayed processing of CSF specimens, autolytic enzymes.

Blood culture detected 2/15 (13%) cases of ABM which correlated with the study of Ashok Garg *et al.* [3].

Comparative studies of % positivity of LAT

In the present study, Bacterial antigen detection test could detect 12 lab-confirmed cases of ABM (80%) viz 6 S. pneumoniae, and 3 Group B Streptococci, 2 E. coli, 1meningitidis. 20% were LAT negative. No case of H. influenzae was detected in present study. Our study correlates with studies of Shiva Prakash *et al.* [17], Das K *et al.* [20] and Jyothi R *et al.* [19]. In developing countries like India, where majority of neonatal meningitis is caused by Enterobacteriaceae, culture is superior to LAT as the latter is not designed to detect Enterobacteriaceae other than E. coli.

Comparative studies of CSF gram stain and culture

The accuracy indices of Gram stain against culture showed a sensitivity of 50%, specificity of 95.2%, PPV value of 66.67%, and a

NPV of 90.91%. Our study correlates with Sayeda *et al.* [11] and Shilpa *et al.* [16]. The frequency of bacterial meningitis is high among patients with the positive Gram stain to warrant presumptive treatment, pending the results of CSF culture. On the other hand, PPV is low enough that unless there are other factors that increase the risk of bacterial meningitis.

Comparative studies of CSF LAT and culture

In the present study, accuracy indices of LAT against culture were showed a sensitivity of 62.5% and specificity of 83.3%, Positive Predictive value of 41.6% and negative predictive value of 92.1%. Our study correlates with Sayeda *et al.* [11] and Shilpa *et al.* [16]. Out of the 15 confirmed cases of ABM, 7 cases were culture-negative. All these 7 cases were LAT positive, 4 cases were S. pneumoniae, 1 case was Group B Streptococcus and 1 case was E. coli. The fastidious nature of these bacteria and the effect of prior antibiotic therapy in most of our cases probably explain the poor sensitivity of culture in these cases. Conversely, LAT was better at identifying these bacteria. LAT was positive in 5 of the 8 culture positive cases, 2 cases were S. pneumoniae, 2 cases were Group B Streptococcus and 1 case was E. coli. Among these remaining 3 culture-positive cases (K. pnemoniae, Acinetobacterspp and P. aeruginosa), the reagents were not included in the panel of the kit. Hence LAT failed to detect these organisms. Despite the good specificity of LAT 83.3% in the present study, the low positive predictive value of 41.6% is of concern, especially in view of the high cost of the test. Never the less, because of the high NPV of 92.1%, a negative LAT fairly rules out ABM in clinically suspected cases.

CONCLUSION

S. pneumoniae, S. agalactiae were the most common organisms isolated in CSF culture. The simple gram stain smear of CSF was the most useful single test for identifying bacterial meningitis. LAT has an advantage over Gram stain in terms of species identification. It was also more sensitive compared to Gram stain and culture in identifying fastidious organisms like Neisseria meningitidis, S. pneumoniae and S. agalactiae. CSF culture is the gold standard and no test can replace the utility of culture, especially in neonates as LAT does not identify Enterobacteriaceae other than E. coli. It was also found that CSF LAT is a valuable tool in the rapid etiological diagnosis of ABM. The cost of LAT is prohibitive factor in resource poor countries. No single test possesses the quality of an ideal diagnostic for the diagnosis of ABM. Thus the combination of tests based on individual's case histories will yield more productive results than any of the tests done alone.

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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