Evaluation of Usefulness of Latex Agglutination Test in Cerebrospinal Fluid for Diagnosis of Acute Bacterial Meningitis

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Abstract

Background: Early definite diagnosis and appropriate treatment is the cornerstone for reducing mortality and morbidity in Acute Bacterial Meningitis (ABM). In recent years, there has been considerable interest in rapid antigen detection tests that provide fast species identification. This study aimed to evaluate the usefulness of the Latex Agglutination Test (LAT) for diagnosing ABM.

Materials and methods: This cross-sectional study was carried out in the Department of Neurology, Medicine, and Pediatric of Chittagong Medical College Hospital. A total of 68 clinically suspected ABM cases of different ages were included in that study. Specimens of Cerebrospinal Fluid (CSF) were tested for routine CSF study including Gram stain with microscopy, LAT and culture. The entire group under the study got different antibiotics for a period of 24 to 72 hours before CSF sample was collected.

Results: Using LAT, an etiological diagnosis was achieved in 22 (32.4%) out of 68 cases of suspected ABM. In contrast, Gram stain and culture showed 23% and 7% positivity, respectively. *Streptococcus pneumoniae* was the most common causative pathogen as 17 of the cases were found to have this organism. This was followed by 2 cases of Haemophilus influenzae, 2 cases Neisseria meningitidis and 1 case of *Group b streptococcus.*

Conclusion: LAT seems to be a useful and efficient method, at least in parallel to CSF culture and Gram stain to detect the pathogens that are known to be the common cause of ABM. It can be added as an adjunct laboratory test for early diagnosis of ABM especially in pretreated cases for prompt initiation of proper antibiotics. Nevertheless, findings of the present study suggest further large-scale studies in order to confirm its usefulness.

Key words: Acute bacterial meningitis; Culture; Latex agglutination.

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Submitted on : 07.01.2023
Accepted on : 17.05.2023

Introduction

Meningitis can have infectious causes, such as bacteria, mycobacteria, viruses, fungi, or parasites, or be associated with autoimmunity, cancer, or reactions to medication.[1] Among these, ABM can rapidly become fatal and lead to severe disability in those who survive. The burden of ABM in terms of morbidity and mortality is high in South Asia, including Bangladesh.[2-4] Bacterial meningitis constitutes 25% of all meningitis cases, and the case fatality rate was 14% in Bangladesh.[5] Clinical diagnosis of meningitis is difficult as its early clinical manifestations are often not specific, especially in babies and small children. On the other hand, even if it is recognized early and prompt treatment is given, 8-15% of the patients die, typically within 24- 48 hours of symptoms onset. Furthermore, 10-20% of the survivors are prone to permanent neurological sequelae, including brain damage, hearing loss, and learning disabilities.[6,7]

The CSF examination is essential to establish the diagnosis and to identify the etiologic agent and its antibiotic sensitivity, is still considered the "gold standard".[8] However, the diminished sensitivity of the CSF culture in the patients who received antibiotics before the LP and the 72 hours test period hinder clinicians from reaching a prompt diagnosis and starting the treatment in the ideal period.[9,10] Other conventional methods for diagnosis of ABM is based on examination of CSF including physical, biochemical, cytological and Gram staining. Sensitivities of CSF Gram staining vary considerably for different microorganisms and Antibiotic therapy may cause alteration of CSF cytology, biochemical findings and also failure

of detection of causative organism, so the diagnosis may be delayed.[11] Moreover, in primary level hospital culture facility is not available in Bangladesh.

Due to low sensitivity of the above-mentioned conventional techniques some experts advocate the use of CSF bacterial antigen assay like Latex Agglutination Test (LAT). The LAT employ antigen-specific antibodies on latex particles to detect bacterial antigens. This method can detect antigens of five pathogens in CSF including *H. influenzae type B (Hib), S pneumoniae, N meningitidis, E coli K and S agalactiae (GBS).*[12] Its theoretical advantage is the detection of the bacterial antigens even after microbial killing, as is observed after antibacterial therapy.[13-15] LAT is rapid, reliable, less time consuming, easy to perform, sensitive and specific. However, a wide range of sensitivity and specificity has been seen in various studies.[15-19]

Contemplating this background, this study was conducted to evaluate the utility of LAT in CSF for rapid and definite diagnosis of ABM in a tertiary hospital in Bangladesh. If the study results demonstrated its usefulness, it would be expected to use LAT more confidently as an important supplement for managing patients with suspected ABM. In addition, the study would likely reveal the local pattern of organisms associated with ABM which would be imperative for the clinician to ensure empirical treatment.

Materials and methods

This was a descriptive cross-sectional study conducted in the Department of Neurology, of Medicine and Pediatric Medicine of Chittagong Medical College Hospital (CMCH) from July 2021 to June 2022. The study protocol was approved by the ethical and review committee of Chittagong Medical College (CMC/PG/2021/619, Dated:26/06/2021). Verbal and written informed consent was obtained from the caregivers of the patients.

Patients admitted in the respected department of CMCH during the study period with a diagnosis of suspected acute bacterial meningitis were subjected to detail history, physical examination and CSF analysis. Cases were finally included according to case definition and CSF criteria. We excluded the patients developing meningitis following head trauma ,neurosurgical procedure and patients or caregivers who refused participation.

The case definition of ABM included - acute onset of fever (Usually>38.5c rectal or >38c axillary) and one of the following signs: neck stiffness, altered level of consciousness, and other signs of meningeal irritation. CSF criteria included at least any of the followings- i) Turbid appearance ii) Leukocytosis (> 100 cells) iii) Leukocytosis (10-100) cells and Protein >100mg/dl or Glucose <40mg/dl.

CSF samples were tested for bacterial antigen detection using the bacterial antigen kit (BD Directigen Meningitis Combo Test). These kits provide a series of rapid latex tests for the qualitative detection of antigens of 5 organisms: *Escherichia coli* K1, *Neisseria meningitides* A, C, Y and W135, *Streptococcus pneumoniae , Group b streptococcus* and *Haemophilus influenza* type b respectively. Their reagents consist of polystyrene latex particles coated with antibodies to the bacterial antigen.

We analyzed the data with SPSS version 23.0 (IBM SPSS Inc., USA) and presented it as the frequency with percentage or as median with interquartile ranges. Patients were categorized as laboratory confirmed ABM and unconfirmed ABM cases based on the Gram stain, LAT and culture results. Finally, agreement between LAT, Gram stain and culture with confirmatory diagnosis were assessed by Kappa statistic. Between group differences were statistically assessed by Chi-square test. Results were considered statistically significant at the 95% confidence interval and $p < 0.05$.

Results

The study was conducted on 68 CSF samples obtained from patients with suspected ABM. The median age was 17 years and 63.2% were male. The entire group reported to received antibiotic before admission with a median duration of antibiotic exposure for 48 hours. The most frequent symptom was fever, followed by vomiting, headache, convulsion, altered consciousness and lethargy while, Photophobia and rash were reported very infrequently. Neck stiffness was found in 49 cases among the

patients. Duration of symptoms ranged from 1-5 days till admission. Leukocyte count > 100 was found in 80% cases. CSF protein >100mg was observed in about 65% cases while glucose is below 40 mg was found in only 40 % cases (Table I).

Table I Clinical and CSF characteristics of the patients with suspected ABM (n=68)

Attributes	Frequency (%)/Median (IQR)
Age, Years	17.0 (1.6-30.0)
Sex	
Male	43 (63.2)
Female	25 (36.8)
Received antibiotic before admission	68 (100.0)
Clinical manifestation	
Fever	68 (100.0)
Vomiting	45 (66.2)
Headache	38 (55.9)
Convulsion	35 (51.5)
Altered consciousness	31 (45.6)
Lethargy	28 (41.2)
Photophobia	16 (23.5)
Rashes	1 (1.5)
Neck stiffness	49(71)
CSF parameters	
Cell count, mm^3	
≤100	13 (19.5)
>100	55 (80.5)
Neutrophil (%)	70.0 (42.5-100.0)
Lymphocyte (%)	30.0 (0-60.0)
Protein, mg/dl	
<100	23 (33.8)
100-200	35 (51.5)
>200	10 (14.7)
Glucose, mg/dl	
<40	27 (39.7)
≥40	41 (60.3)

Table II shows that, none of the diagnostic tests were positive in more than three quarter (67.7%) of the patients. Rest of the 22 (32.4%) of the cases were confirmed case of ABM as per World Health Organization criteria. It was to be noted that, five cases had concordance of Gram stain, LAT and culture results.

Table II CSF Gram staining, LAT, and Culture results of the patients with suspected ABM (n=68)

Tests	Frequency (%)
Gram stain + LAT + culture positive	5 (7.4)
LAT + Gram stain	11 (16.2)
Only LAT positive	6 (8.8)
Negative in gram stain, LAT, & culture	46 (67.7)

Figure 1 shows, out of 68 suspected ABM cases LAT shows its superiority to diagnose pathogen than Gram Stain and Culture. Out of 68 cases LAT, Gram Stain and culture positivity were 32.4%, 23.5%, 7.4% respectively.

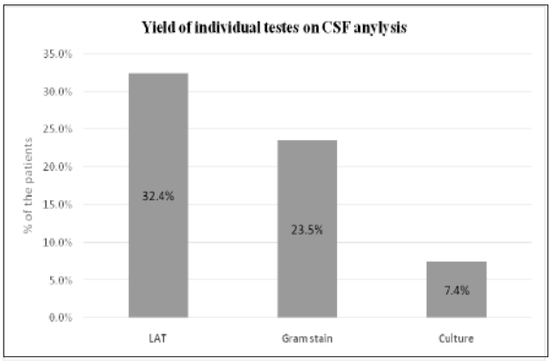

Figure 1 Overall findings (in percentage) of LAT, Gram stain and Culture in CSF to diagnose ABM

Table III shows, out of 68 samples LAT was positive in 22 samples and majority of the identified organism was *S. pneumoniae*-17 (77%) followed by *H. influenzae* and *N. meningitidis*-2 (9%) each and 1 (5%) Group b streptococcus. On the other hand, all of the identified organisms were *S. pneumonia* in CSF culture.

Table III Etiological agents identified in CSF by LAT and culture methods (n=68)

Name of organisms	Total	Culture positive	LAT positive
S. pneumoniae	17 (25.0)	5 (7.4)	17 (25.0)
H. influenzae	2 (2.9)	0 (0)	2 (2.9)
N. meningitidis	2 (2.9)	0 (0)	2 (2.9)
Group B streptococcus	1 (1.5)	0 (0)	1 (1.5)
Total	22 (32.4)	5 (7.4)	22 (32.4)

Out of 22 laboratory confirmed cases only 5 were positive by culture and 17 of the confirmed cases were negative by CSF culture. There was fair agreement between culture test and confirmed diagnosis of suspected ABM. In CSF Gram Stain 16 of the 22 laboratory confirmed cases were positive indicating a good agreement between Gram stain and confirmed diagnosis of suspected ABM. All of the 22 confirmed cases were positive by LAT. There was perfect agreement between LAT test and confirmed diagnosis of suspected ABM.

Table IV Overall performance of CSF Gram stain, LAT and culture to differentiate confirmed ABM form the suspected ABM

Tests	ABM cases confirmed by any cases		Total (n=68)	Test statistics
	Yes (n=22)	No (n=46)		
CSF Gram stain				
Positive	16 (72.7)	0 (0)	16 (23.5)	<0.001
Negative	6 (27.3)	46 (100.0)	52 (76.5)	Kappa=0.821
CSF LAT				
Positive	22 (100.0)	0 (0)	22 (32.4)	<0.001
Negative	0 (0)	46 (100.0)	46 (67.6)	Kappa=1.0
CSF culture				
Positive	5 (22.7)	0 (0)	5 (7.4)	<0.001
Negative	17 (77.3)	46 (100.0)	62 (92.6)	Kappa=0.285

Data were expressed as frequency (Percentage)
p values were obtained from Chi-square test.

Discussion

The kit-based tests are easily available, easy to standardize, very simple to perform on a routine basis and provide very prompt results. Current study demonstrated a better sensitivity of LAT in comparison to gram staining and CSF culture with an overall of sensitivity of 32%, 23% and 7% respectively for LAT, Gram staining and Culture. Only 5 (7.4%) samples yield positive growth in CSF culture in the present study, which agreed to the previous studies.[4,20] This low yield in culture results could be attributed to the fact that antimicrobials are being dispensed without prescriptions in low and low to middle income countries.[21] In the present study the entire group received antibiotic for a minimum of 24 hours period before collection of CSF.

LAT was positive in 22 (32.4%) samples, and in other studies LAT positivity ranged between 19.3% to 40%.[13,17,19]Though the entire group of the present study had exposure to antibiotic prior LP, the LAT positivity rate was considerably higher. These results show that LAT is much more sensitive than the culture method in the patients who have received antibiotics before the collection of the CSF sample, and it correlated with the other studies.[22]

S. pneumoniae was the predominate organism identified by LAT and culture in the present study. Globally, the most prevalent bacterial pathogens isolated from acute meningitis cases are *S. pneumoniae* and *N. meningitides.*[23,24] In a large study from India, majority of the cases of bacterial meningitis were found to be due to S. pneumoniae followed by *H. influenzae* and *N. meningitidis.*[23] Decrease incidence of H. influenzae in recent studies especially in Bangladesh may be due to incorporation of Hib vaccine in EPI schedule from 2009.[2]

In the present study, according to WHO case definition of bacterial meningitis, 22 cases were confirmed. All these cases were positive by LAT, while Gram stain and culture were found to be positive in 16 and 5 cases respectively. LAT had a perfect agreement with confirmed ABM. There was no sample in which the CSF culture was positive, but LAT and Gram stain was negative. LAT was observed to be a more efficient method than the CSF culture and Gram stain in detecting the pathogens. This better detection rate of LAT in comparison to Gram stain and Culture might be due to all of the included cases were pretreated and LAT detects antigen which persist after lysis of bacteria.

Limitation

Small sample size with relatively fewer neonates and convenience sampling technique were some of the limited factor of this study. All cases received antibiotic before enrollment in the study.

Conclusion

Analyzing the findings, it can be concluded that LAT was observed to be a useful tool to diagnose ABM. It showed its superiority to detect pathogen than Gram stain and Culture especially in pretreated cases. Though it was found as a highly sensitive test, but negative test does not rule out other pathogens causing ABM.

Recommendations

Latex agglutination test can be added as an adjunct to culture and gram stain in the management of acute bacterial meningitis. It can be considered as a useful tool to guide the clinicians for administration appropriate antibiotics especially where resource facility is minimum. Expansion of current panel of LAT is required to detect more organisms causing acute bacterial meningitis. However, findings of the present study suggest further large scale studies in order to assess its usefulness and efficacy in the diagnosis of acute bacterial meningitis.

Acknowledgement
All the authors express their gratitude to the staffs of the Department of Neurology, Medicine and Pediatric, CMCH.

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Disclosure
All the authors declared no conflict of interest.

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