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Latex Agglutination Test for Early Detection of Causative Organism in Acute Bacterial Meningitis

Mishra B[1], Mahaseth C[2], Rayamajhi A[3]

[1]Dr. Baliram Mishra, MBBS, DCH, MD. MPH (Paediatrics), Head of department of Paediatrics, Janakpur Zonal Hospital, Janakpur, [2]Dr. Chandeshwar Mahaseth, Professor and Chief Consultant, Kanti Children Hospital, National Academy of Medical Sciences (NAMS), Kathmandu, [3]Dr. Ajit Rayamajhi, Associate Professor, Kanti Children Hospital, National Academy of Medical Sciences (NAMS), Kathmandu, Nepal.

Abstract

Introduction: Acute bacterial meningitis is one of the leading causes of mortality and morbidity in children. Identification of the causative organism is crucial to its management and outcome. The objective of this study was to see the usefulness of latex agglutination test in the early diagnosis of acute bacterial meningitis. **Materials and Methods:** A hospital based prospective cross-sectional study was conducted at Kanti Children's Hospital during December 2004 to August 2005. Cerebrospinal fluid from 150 consecutive clinically suspected cases of acute bacterial meningitis between the age group of 2 months to 14 years were analyzed. Bacterial culture and latex agglutination test was done on cerebrospinal fluid obtained from all 150 suspected cases of acute bacterial meningitis. Latex agglutination test was done using the BD Directigen™ Meningitis Combo test kit (Becton, Dickinson and company, USA) for *Streptococcus pneumoniae,* group B *Streptococcus, Escherichia coli, Neisseria meningitidis group A,C and Y/ W135,* and *Hemophilus influenzae type b.* Data was analysed by using *SPSS Version 11.5.* **Results:** Of the 150 Cerebrospinal fluid samples analysed bacterial culture identified only 4 meningitis cases giving an isolation rate of 1.3% whereas latex agglutination test identified 29 cases giving an isolation rate of 19.3% from 150 samples. *Streptococcus pneumoniae, Hemophilus influenzae type b* and *Group B Streptococcus* were the most common causative organism. **Conclusion:** Latex agglutination test has a better yield, higher sensitivity, provides microbiological diagnosis earlier than the traditional cerebrospinal fluid culture and is easy to perform.

Key words: Acute bacterial meningitis, Cerebrospinal fluid, Latex agglutination test.

Introduction

Acute bacterial meningitis (ABM) is a major public health problem. It is one of the most important causes of mortality and morbidity in children. ABM accounts for an estimated annual 5, 00,000 cases worldwide with at least 50,000 deaths and an equal number of neurological disabilities[1]. Even with best of care and antibiotic therapy, case fatality rates remain at 5% to 10% in industrialized countries and can reach up to 20% in developing countries. Between 10% and 30% of survivors develop permanent neurological sequelae such as paralysis, epilepsy, cognitive deficit or sensorineural deafness[2].

Identification of the causative organism in ABM is crucial to its management as it influences the choice of antibiotics, duration of therapy as well as the disease outcome. Three organisms namely *Neisseria meningitidis, Streptococcus pneumoniae and Hemophilus influenza* account for more than 80% of cases of ABM[3]. The diagnosis of meningitis in a suspected case is based on the analysis of cerebrospinal fluid (CSF) obtained by performing a lumbar puncture[4]. The CSF is examined for turbidity, white blood cell (WBC) count, Gram stain and the amount of protein and sugar. Bacterial culture of the CSF has long been the gold standard for the diagnosis of ABM, but due to its poor yield, longer time and unavailability in a resource limited country like ours we decided to conduct this study to see the usefulness of latex agglutination test (LAT) in the early diagnosis of ABM.

Address for correspondence
Dr. Baliram Mishra
Head of Department of Paediatrics
Janakpur Zonal Hospital
Janakpur, Nepal
E-mail: balirammishra@yahoo.com

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LAT relies on the detection of soluble antigen in the CSF. Pre-treated latex beads agglutinate in the presence of the bacterial antigen, allowing rapid visualization of positive agglutination to the naked eye. In contrast to the hours spent on bacterial culture LAT can be completed in approximately 20 min (including the time taken to heat and centrifuge the CSF sample). The LAT can differentiate between *N. meningitidis* serogroups A, C and Y/W135, *N. meningitidis* serogroups B/*Escherichia coli*, *Hemophilus influenzae*, *Streptococcus pneumoniae* and Group B *Streptococcus* hence allowing prompt institution of directed therapy that is specific for the bacteria detected.

Materials and Methods

Study site and study population. A hospital based prospective cross-sectional study was conducted at Kanti Children's Hospital during December 2004 to August 2005. This government hospital is situated in Kathmandu and is the largest children's hospital in the country providing multispecialty affordable care to children from all over the country. All children between 2 months to 14 years of age who were a suspected case of ABM were enrolled into the study. The study was approved by the Institutional Research Board of National Academy of Medical Sciences and ethical clearance granted.

Inclusion and exclusion criteria. All children between the age of 2 months to 14 years who fulfilled the case definition of a suspected case of ABM and had no exclusion criteria were included in the study. Children in whom lumbar puncture was contraindicated or refused; those receiving immunosuppressant's; those with a history of having undergone a neurosurgical procedure and those who had received parenteral antibiotics for more than 72 hours were excluded from the study.

Case definitions

Suspected case. A child between 2 months to 14 years of age presenting with clinical features of meningitis namely fever and/or headache and/or neck stiffness and/or bulging fontanel and/or altered sensorium and/or convulsions.

Confirmed case. A suspected case whose CSF has at least one of the following findings – (1) elevated protein to more than 40 mg/dl, (2) decreased glucose to less than 40 mg/dl, (3) a CSF to serum glucose ratio of less than 0.6, (4) a total leukocyte count of more than 10 cells/mm^3 with neutrophillic predominance, (5) organism in Gram's stain or bacterial culture.

Laboratory methods and data analysis. After obtaining written consent lumbar puncture was done using sterile technique (thorough cleaning of local part using povidone iodine and then alcohol skin preparation; sterile disposable needle and collection tubes). Two millilitres of CSF was collected in three different clear sterile plastic collection tubes and immediately transported to the laboratory for analysis of cells, sugar, protein, Gram's stain, bacterial culture and LAT. For bacterial culture 20 µL of CSF was inoculated into plates of blood agar and chocolate agar and incubated. All plates negative for growth after 48 hours were considered culture negative and discarded. LAT was done using the BD Directigen™ Meningitis Combo test kit (Becton, Dickinson and company, USA) for *Streptococcus pneumoniae,* group B *Streptococcus, Escherichia coli, Neisseria meningitidis group A,C and Y/W135,* and *Hemophilus influenzae type b.* CSF of all suspected cases enrolled in the study underwent bacterial culture and LAT. Data was analysed by using *SPSS Version 11.5.* Sensitivity, Specificity, Positive and Negative predictive values were calculated. Fischer's exact test and chi square test with Yate's correction were performed to assess statistical significance of relationship between variables and meningitis. A *'p' value* of <0.05 was considered significant.

Results

CSF from 150 consecutive clinically suspected cases of ABM between the age group of 2 months to 14 years were analyzed. Bacterial culture and LAT was done on CSF obtained from all 150 suspected cases of ABM. 38 (25.3%) patients were *confirmed cases* of ABM as per the case definition. Of the 150 CSF samples analysed organisms could be identified in 29 cases as shown in Figure 1.

Table 1: Organisms identified by CSF culture and LAT

Organisms	LAT	CSF bacterial culture
Streptococcus pneumoniae	12	2
Hemophilus influenzae type b	12	0
Group B Streptococcus	5	2
Neisseria meningitidis	0	0
Escherichia coli	0	0
Total	**29**	**4**

As shown in table 1 bacterial culture identified only four meningitis cases giving an isolation rate of 1.3% from 150 samples. Of the four organism identified two were *Streptococcus pneumoniae* and two *Group B Streptococcus.* LAT identified 29 cases giving an isolation rate of 19.3% from 150 samples. Of the 29 organisms identified by LAT 12 (41.4%) were *Streptococcus pneumoniae,* 12 (41.4%) *Hemophilus influenzae type b* and 5 (17.2%) *Group B Streptococcus.* The sensitivity, specificity, positive and negative predictive values of bacterial culture and LAT has been shown in table 2,3,4.

Table 2: Performance of Latex agglutination test

Latex agglutination test	Suspected case of meningitis (150)		Total	'p' value
	Confirmed case (38)	No meningitis (112)		
Positive	23	6	29	0.0001
Negative	15	106	121	
Total	**38**	**112**	**150**	

Table 3: Performance of bacterial culture of cerebrospinal fluid.

CSF culture	Suspected case of meningitis (150)		Total	'p' value
	Confirmed case (38)	No meningitis (112)		
Positive	4	0	4	0.0001
Negative	34	112	146	
Total	**38**	**112**	**150**	

Table 4: Comparison of performance of CSF culture and Latex agglutination test.

Test characteristics	Latex agglutination test	CSF culture
Sensitivity	60.5%	10.5%
Specificity	94.6%	100%
Positive Predictive Value	79.3%	100%
Negative Predictive Value	87.6%	16.4%

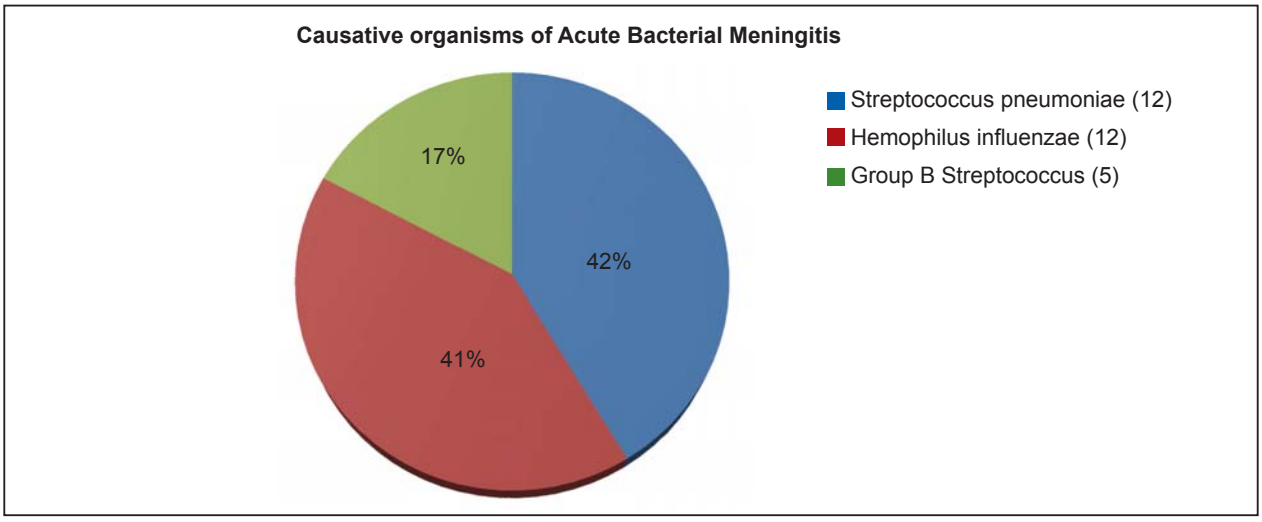

Fig 1: Showing the causative organisms of acute bacterial meningitis.

Discussion

ABM in children is a potentially life threatening condition requiring early diagnosis, prompt identification of the causative organism and institution of effective therapy[5]. The yield of bacterial culture in our country is low, hence there was need to evaluate a test that could perform better and could rapidly identify the causative organism of ABM. Ours is the first study to evaluate LAT against bacterial culture in Nepal.

Streptococcus pneumoniae, Hemophilus influenza and *Group B Streptococcus* were the commonest causative organisms isolated in our study. This is in accordance to the organisms isolated from CSF in other studies[6,7,8,9]. Seasonal variation in the incidence of meningococcal meningitis and vaccination against it might have accounted for the fact that *Neisseria meningitidis* was not isolated in any of the CSF samples in our study. Of the 150 CSF samples analysed culture could identify organism in only 4 and LAT in 29 giving an isolation rate of 1.3% and 19.3% respectively. The sensitivity of LAT (60.5%) was higher than that of CSF culture (10.5%). Similar results have been observed in other studies with sensitivity of LAT ranging from 60 to 95% and specificity from 70 to 98%[10,11,12,13,14]. However there have been conflicting results in the literature regarding the utility of LAT with studies suggesting that it is no better than using Gram's stain and CSF culture

together[15,16,17]. These studies are mostly from countries where the yield of CSF culture is high as compared to our country.

One of the drawbacks of our study is the fact that we did not include Gram's stain in our study protocol. Secondly, in the definition of confirmed case only 4 had positive culture and the rest were classified as confirmed on the basis of CSF criteria's which are not the gold standard for the diagnosis of ABM and this might have affected the sensitivity and specificity of LAT. Thirdly, we did not look into how early diagnosis using LAT affected disease outcome in terms of mortality, reduction in the duration of hospital stay, cost of therapy and unnecessary use of empirical antibiotic therapy instead of specific therapy based on the organism identified. However this study forms a foundation on the basis of which further studies to look into cost effectiveness of using LAT can be performed. Study to see whether early detection of organism and subsequent specific therapy brings about a decrease in mortality and morbidity, duration of hospital stay, antibiotic resistance needs to be done.

The strength of our study is that in a country where the yield of culture is low and where culture is not readily available simple and rapid test like the LAT can be used in the peripheral set up to promptly identify the organism and start specific therapy. This becomes especially more important as steroids have a role in reducing mortality and morbidity in ABM due to *Hemophilus influenza type b* if they are given for the first 2 days. Secondly, LAT can be extremely useful if there is an outbreak of ABM. It is yet to be studied whether the implementation of LAT in the peripheral hospitals will bring about an effect similar to that brought by the use of Rapid antigen capture test for *Plasmodium falciparum* malaria. Though the technology for detection of bacterial antigen in CSF by latex agglutination is still imperfect for widespread use, it still can have a major impact in a country like ours.

In conclusion as LAT has a better yield, provides microbiological diagnosis earlier than the traditional CSF culture and is easy to perform, it should be provided by the government as a part of national programme to the peripheral centres.

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Permission from IRB: Yes

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