Original Article

Evaluation of Latex Agglutination Test Efficacy in Diagnosis of Acute Pyogenic Meningitis

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ABSTRACT

Introduction: Acute pyogenic meningitis is one of the most common and devastating disease of Central Nervous System. Difficulty and delay in diagnosis adds to problem in early management of patients. Rapid tests need to be invented so as to curbing the acute pyogenic meningitis cases.

Aims: To identify the bacterial pathogens causing acute pyogenic meningitis and to evaluate the role of microscopy, Latex agglutination vis a vis culture in the diagnosis of pyogenic meningitis

Methods: Clinically diagnosed cases of Acute pyogenic meningitis were included in the study. Cerebrospinal fluid (CSF) was the specimen of choice. CSF was processed in the microbiology laboratory for bacteriological aerobic culture, microscopy and Latex Agglutionation test (LAT). All the results analyzed statistically.

Results: A total of 321 CSF specimens were screened. Among these 50 were from exclusive acute cases . On analysis gram stain positivity was 52%. Sensitivity of the gram staining was 64.29% and specificity was 52.78% while Culture positivity was 28%. Though LAT positivity (27.28%) was less as compared to microscopy and culture, LAT was found to be having higher sensitivity of 71.43% and specificity 80.55% .

Conclusion: LAT can be a valuable tool in early diagnosis of acute pyogenic meningitis cases even after administration of antibiotics. It can be used as an adjunctive to culture and microscopy. In country like India, use of LAT should be concentrated on highly suspicious cases to make it cost-effective.

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Introduction

Central nervous system (CNS) infections have been recognized as one of the most devastating of diseases. Among these CNS infections, bacterial meningitis found to be most common disease worldwide.[1] Approximately one million of pyogenic meningitis cases reported globally and around 2,00,000 of these die annually excluding epidemics. [2] In developed countries, mortality due to pyogenic meningitis is < 10% while in developing countries it may be 30% or more.[3]

Clinically there is always difficulty in diagnosis of bacterial meningitis, this may be due to frequent non-specific symptoms and signs. Clinical diagnosis is often difficult in case of young children as compared to older. As acute bacterial meningitis has very high morbidity and mortality, its early diagnosis and treatment is vital.[4] Unfortunately in developing countries like India, there is delay in specimen transport and in addition recommended conditions are often not maintained during storage or transport. Also administration of antibiotics before collection of sample can clear the organisms from CSF leading to low number of pathogens from CSF. So, culture can yield false negative results. On the other hand gram stain is skill based; results can vary according to observer. Latex agglutination test can yield rapid results and is not affected by antibiotics or delay in transport, also is not a skill based technique.[5] The present study was conducted to evaluate usefulness of rapid diagnostic tests in acute pyogenic meningitis.

Material and Methods

The current study was conducted in the Department of Microbiology, in a tertiary care hospital. An ethical committee approval was obtained prior to commencing study. A total of 321 cases of acute pyogenic meningitis admitted in pediatric and medicine wards were included in the above study.

Inclusion Criteria

1. The presence of clinical signs compatible with diagnosis of bacterial meningitis
2. CSF analysis with typical abnormalities suggestive of pyogenic meningitis
   - WBC counts >10 cells/mm3
   - Protein >40mg/dl and/or
   - Sugar <40mg/dl

Specimen were collected by clinician as per standard guidelines. Standard 3 tubes of CSF were collected for chemistry, microbiology, and cytology. If possible 3-4 ml (1 ml minimum) CSF were collected into sterile, screw-cap tubes. These all tubes were labeled and transported to laboratory as soon as possible. If there was inevitable delay, then samples were kept at room temperature.

CSF biochemical analysis was done by protein and sugar estimation. Simultaneously cytological examination was also performed and differential count estimated.

Microbiological Processing

On receipt of CSF into laboratory we looked for macroscopic examination. Specimen was preheated in boiling water bath for 3 min. After cooling, CSF was centrifuged for 5 min at 3000g. Supernatant separated and stored in a sterile tube. Sediment used for Gram staining and culture inoculation.

0.15 ml of deposit was inoculated on each of 5% sheep blood agar , chocolate agar and MacConkey Agar. 1 ml of deposit was deposited on brain heart infusion agar. [2,6] All plates were incubated at 37°C in 5-10% CO_2 for 48-72 hours while brain heart infusion agar were incubated for 7 days and examined for growth daily. Identification of different isolates were done by standard microbiological techniques. [2,6]

Latex agglutination test [2] was performed on CSF supernatant. CSF sample were tested for bacterial antigen detection using PASTOREX™ MENINGITIS (BIO-RAD), a latex antigen detection kit that included reagents: *H.influenzae b, S.pneumoniae, Gp B Streptococcus, N. meningitides A, C, Y/W 135, N. meningitides B/E.coli,* polyvalent negative Control and polyvalent positive control. A drop (40-50 µl) of the pre-treated sample supernatant placed in each of the agglutination card and one drop of latex regent delivered on the card following indicated distribution pattern. Sample and latex reagent mixed using a rod. The Card was rotated~120 rpm gently for 10 minutes and looked for agglutination visible to the naked eye within 10 minutes.

All the observations and results were analyzed further using In Silico statistical software. Chi-square and P-value was calculated by using this software for comparison of difference to know the statistical significance.

Results

CSF specimens were collected from 321 patients for analysis which were screened for presence of leucocytes. 50 of these screened specimens included in the study and were subjected to different laboratory tests. Maximum number of cases were in the age group 12-50(48%) years followed by 1-6(26%) years age group. Males(64%) were more commonly affected than females(36%).

Cytological analysis of CSF in patients of pyogenic meningitis was done . In 24 (48%) CSF samples (majority

of patient) cell count noticed were >1000 cells/cmm . In 14(28%) samples cell count were between 101-500 cells/cmm while Protein values were significantly higher (>200 mg/dL) in 23(46%) patients. In 25(50%) sugar concentration was between 11-30 mg/dL; in 17(34%) it was between 1-10 mg/dL (Table 1).

Out of 50 samples, total yield of gram stain positivity was 26(52%). Cocci (both gram positive and gram negative cocci) were common finding in microscopy. Sensitivity of the gram staining was 64.29% and specificity was 52.78% (Table 2). Out of 50 cases of acute pyogenic meningitis, Culture positivity was 28% in the present study. *S. pneumoniae* (10%) were the commonest isolates followed by *K. pneumoniae* (6%). (Table 3) . LAT was applicable to 43 samples. As LAT was not available for 7 (3 gram negative, 2 *Enterococci*, 1 *S. aureus* and 1 *Citrobacter spp*.) organisms isolated by culture, so those were excluded from analysis. One isolate was *Citrobacter spp*. which gave false positive reaction for *N.meningitidis* by latex agglutination. Among these 12 samples maximum identified organism was *S.pneumoniae* - 8(18.60%) followed by *N.meningitidis* – 2(4.65%) and *S.agalactiae*- 2(4.65%). In this test, no cases showed LAT positive for *H.influenzae* and *E.coli*. When we further analysed LAT was found to be having sensitivity 71.43% and specificity 80.55% while positive predictive value was 41.67%.(Table 5)

On further statistical analysis using In Silico statistical software, gram stain was found to be stastically insignificant while LAT was found statistically significant in rapid diagnosis of pyogenic meningitis. (Table 6)

Table 1: CSF Cytology 1nd Biochemical Parameters

Cytological parameters	Range	No. of cases (Percentage)
Cells/cmm	**<100**	6(12%)
	101-500	14(28%)
	501-1000	6(12%)
	>1000	24(48%)
Proteins (mg/dL)	**50-100**	15(30%)
	101-200	12(24%)
	>200	23(46%)
Sugar (mg/dL)	**1-10**	17(34%)
	11-30	25(50%)
	31-50	8(16%)
	Total	**50(%)**

Table 2: Gram Stain Observations

Morphology	Number
Gram positive diplococci	7(14%)
Gram negative diplococci	2(4%)
Gram positive cocci in short chains	4(8%)
Gram positive cocci in cluster	4(8%)
Gram negative bacilli	7(14%)
Budding yeast cells	2(4%)
Total	**26(52%)**

Table 3: Pathogens Isolated from CSFSamples By Culture

Organisms	No of isolates	Percentage
Streptococcus pneumoniae	5	10%
Klebsiella pneumoniae	3	6%
E.coli	2	4%
Enterococci	2	4%
S.aureus	1	2%
Citrobacter spp.	1	2%
Total	**14**	**28%**

Table 4: Observations by Latex Agglutination Test

Organism	Number of LAT positive	Culture positive	Percentage of LAT positive
S.pneumoniae	8	5	18.60%
N.meningitidis	2	0	4.65%
H.influenzae	0	0	0
S.agalctiae	2	0	4.65%
E.coli	0	2	0
Total	**12**	**7**	**27.28%**

Table 5: Gram Stain Versus Lat

	Sensitivity	Specificity	PPV	NPV
Gram stain	**64.29%**	**52.78%**	**42.86%**	**82.76%**
LAT	**71.43%**	**80.55%**	**41.67%**	**93.54%**

Table 6: Stastical Analysis

		Culture		Total	Chi-square value	P value
		Growth	No growth			
Gram stain	**Positive**	9(18%)	17(34%)	**26(52%)**	**1.1758**	**0.2782**
	Negative	5(10%)	19(38%)	**24(48%)**		
	Total	**14(28%)**	**36(72%)**	**50(100%)**		
LAT	**Positive**	5	7(%)	**12(%)**	**7.8717**	**0.005**
	Negative	2(	29(%)	**32(%)**		
	Total	**7(%)**	**37(%)**	**43(100%)**		

**P value < 0.05 , taken as statistically highly significant*

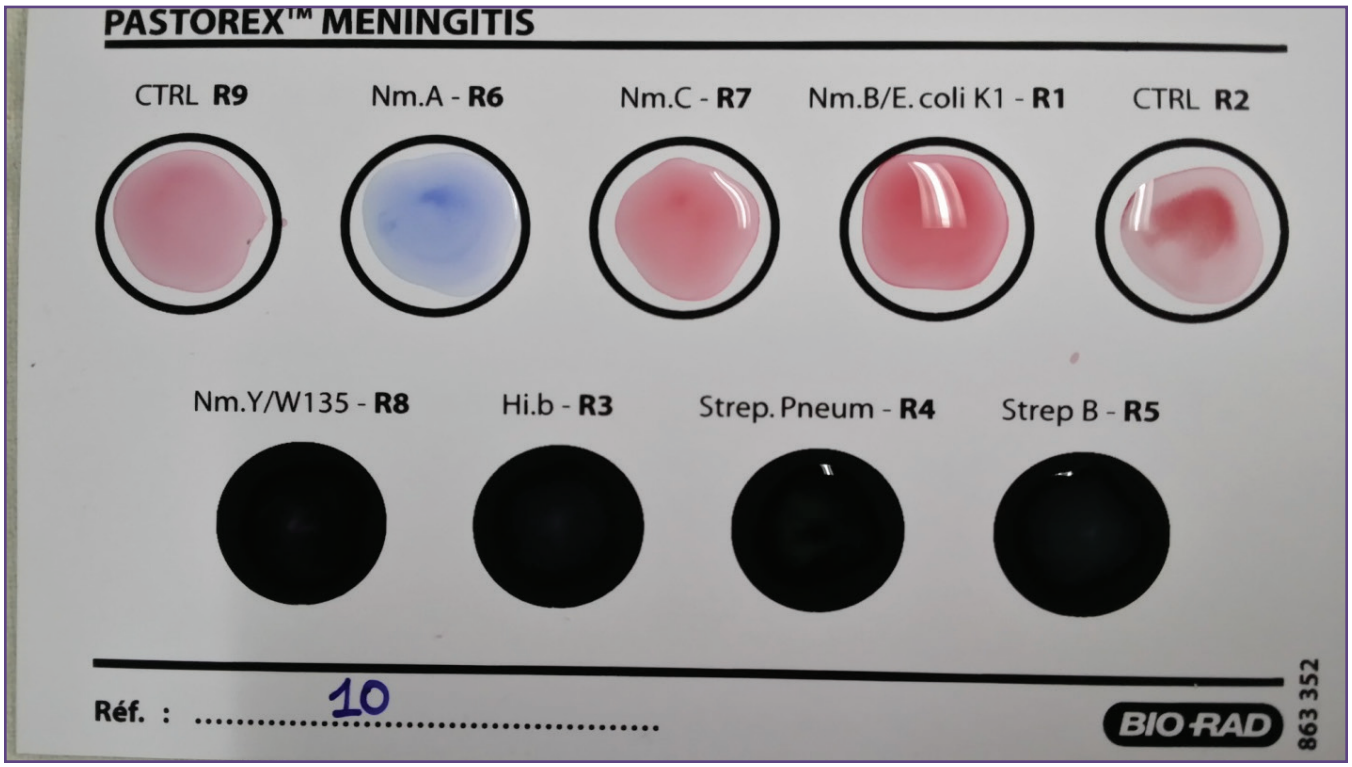

Fig. 1: Negative Latex agglutination test with positive and negative controls

Fig. 2: LAT showing Positive agglutination with N.menigitidis A (Left) and Group B Streptococci (Right) antigen

Discussion

An accurate laboratory confirmation is essential in proper management of case of acute bacterial meningitis. Nowadays there has been increase interest in early and rapid diagnosis of etiological agent of acute pyogenic meningitis. There has been much techniques evolved to fulfill above needs; one of the commonly used technique is latex agglutination test.[2] The present study was undertaken to study the bacteriological profile of meningitis and also to do a comparative evaluation of Gram stain, culture and LAT, in clinically suspected cases of acute bacterial meningitis.

Maximum samples in the present study, (table 1) showed cell count > 1000 cells/cmm which was 48%, followed by in 28% between 101-500 cells/cmm. Total 40% were showed CSF cytology between 101-1000 cells/cmm. In all the cases majority of cells were neutrophils. These findings were comparable to study conducted by Adhikary et al(7) in which maximum cell count was about, was >1000 cells/cmm in 60.53% .

Total 46% of cases showed protein concentration in the cerebrospinal fluid >200 mg/dL while in 30% cases, it was between 50-100 mg/dL while in remaining 24% of cases it was between 101-200 mg/dL. So majority of cases were above 50 mg/dL of protein concentration.It was seen that there was associated decrease in sugar levels in cerebrospinal fluids of patients. This typical picture of high protein and low sugar levels were found in all the cases. Typical high protein count and low sugar levels similar to the present study was also found in a study done by Adhikary et al[7], Gurley et al[8], Domingo et al[9] and Al-Marzoqi et al. [10]

In our study when the sensitivity of gram stain (table 2,5,6) was evaluated, it was 64.29% and specificity was 52.78%, positive predictive value 42.86% and negative productive value 82.76%. In the present study, gram stain found to be statistically non significant by applying chi-square test as P value was more than 0.05.

Mohammadi et al[11], had reported gram stain sensitivity 53.33%, specificity 83.52%,positive predictive value 36.36% and negative predictive value of 91.02%. in another study by Awari et al[12], had reported sensitivity 97.22%, but much lesser specificity of 14.28%, positive predictive value 74.47% and negative predictive value of 66.67%. While Vishwanath et al[13] in their study found gram sensitivity of 90%.

Higher sensitivity in other studies correlates with the concentration of bacteria in cerebrospinal fluid of the patients of pyogenic meningitis. The likelihood of increased positivity or sensitivity is also depends on specific pathogen leading to meningitis. Gram stain results were observed to be ~20% lower in patients of pyogenic meningitis who received prior antimicrobial therapy.[14]

Bacterial culture(table 3 and 4) detected 28% of etiological agents causing acute pyogenic meningitis. In the majority of patients, 16% isolates were showed gram positive organisms. Among gram positive bacteria, *S.pneumoniae* (10%) was the commonest organism isolated. Gram negative bacteria isolated in culture were 12%. Among gram negative bacteria, *K.pneumoniae* (6%) was the major cause of meningitis followed by *E.coli* (4%) and *Citrobacter spp*.(2%). These findings correlates with results reported by Adhikary et al[7], Mohammadi et al[11] and Bajaj et al[15].

Reasons for low culture yield may be:

- False negative results can be seen in case of if specimen been transported or stored under inappropriate conditions[16]
- If an antibiotic therapy started before collection of CSF specimen[16]
- Low bacterial load[11]

Detection of bacterial antigen in the present study was done in 43 patients by using latex agglutination and the test was positive in 12(27.90%) samples. Among these 12 latex positive samples 8(18.60%) were *S.pneumoniae* and 2(4.65%) were *N.meningitidis, S.agalctiae* each. Similar results were observed by Shivaprakash et al[17], Ceyhan et al[18], Mishra et al[19], which had 28.94%, 23% and 19.3% latex agglutination positivity respectively.

In the present study, no *N.meningitidis*, *H.influenzae b* and *E.coli* isolated. Detection of *N.meningitidis* especially group b antigen poses a problem by immunological techniques as it has been suggested that it had poor immunogenicity of this antigen. Also it is possible other organisms including *N.meningitidis* which are not detected in LAT or produced false negative results, reason for this might be that the antiserum included in latex agglutination kit does not detect all capsular serotypes prevalent in geographical area.[11] Also *Enterobacteriaceae* other than *E.coli*, that were culture isolated not included in the antigen kit.

In the present study (table 5 and 6) on culture, 7 were grown the organisms that are not included in latex agglutination ; so latex agglutination test was applicable to 43 cases. Also LAT was observed to be statistically significant, as its P value was < 0.05. LAT also found statistically significant in Bajaj et al[15] study in diagnosis of bacterial meningitis. Similar accuracy indices results correlating with our studies were reported by Mohammadi et al[11] study, in which sensitivity of latex agglutination was 66.66%, specificity 87.91%, positive predictive value 35.29% and negative predictive value was 96.38%.

Statistical analysis such as sensitivity, specificity, positive predictive value and negative predictive value considering CSF culture as gold standard has its own limitations which could affected sensitivity, specificity and positive predictive value negatively. In the present study, LAT had less sensitivity as it cannot detect all bacteria which are included in panel. And from results which showed low culture yield of fastidious organisms, it is clear that LAT is superior in detecting fastidious organisms such as *S.pneumoniae*, *H.influenzae* and *S.agalctiae.* Though it had low positive predictive value(41.67%) high negative predictive value(93.54%) rules out acute bacterial meningitis. [11]

Though the culture is considered as gold standard for diagnosis bacterial meningitis, it has a certain limitations in diagnosis. It is time consuming, give false negative results due to improper storage and transport, use of poor growth media or antibiotic therapy before specimen collection. So, a culture is less sensitive in diagnosis of bacterial menigitis.[16, 15]

Latex agglutination test also has certain limitations. Cross reactions are common with bacteria which share common antigen which can be nullified or minimized by heating the CSF before testing. It cannot detect other bacteria that are also important cause of acute pyogenic meningitis such as *K.pneumoniae, L.monocyotogenes* and other gram negative bacilli. [20]

Conclusion

In spite of drawbacks of LAT, When culture is compared with LAT in various previous studies it is observed that it is useful rapid diagnostic test for acute pyogenic meningitis. Also it had been an important diagnostic tool in bacterial meningitis cases where poor diagnostic facilities or resources available and also important in identifying pathogen in whom preadmission antibiotics administered. Thus, LAT provides rapid microbiological diagnosis of acute bacterial meningitis earlier than culture so as to guide the clinicians for administration of appropriate antibiotics. Considering its high cost we advice its use based on the individual case history appears to be rational.

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Competing Interests

None Declared

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