Original Research Paper

Volume : 5 | Issue : 8 | August 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

Comparison of Culture and Antigen Detection Method from Cerebrospinal Fluid for Diagnosis of Acute Pyogenic Meningitis Due to *Streptococcus Pneumoniae, Neisseria Meningitidis and Haemophilus Influenzae* Type B in a Tertiary Care Hospital

Medical Science

KEYWORDS : antigen detection, CSF, culture, pyogenic meningitis

Bhakare Prashant — Ex resident, Department of Microbiology, L.T.M. Medical College, Mumbai

*** Shinde Alka N** — Assistant Professor, Department of Microbiology, L.T.M. Medical College, Mumbai *Corresponding author

De Anuradha S — Professor, Department of Microbiology, T.N. Medical College, Mumbai

Baveja Sujata M — Professor and Head, Department of Microbiology, L.T.M. Medical College,Mumbai

ABSTRACT

Objectives: *This study was intended to isolate and identify and do antigen detection test for Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae type b from CSF samples in acute pyogenic meningitis.***Material & Methods:** *Fifty CSF samples from patients with acute pyogenic meningitis were cultured and identified as per standard techniques. Antigen detection was done by using PASTOREX MENINGITIS kit (Bio-Rad France).***Results:** *Majority were adults (64%), maximum were in the age group of 16-40 years (38 %). Only three Streptococcus pneumoniae grew in CSF samples but Hemophilus influenzae and Neisseria meningitidis did not grow. Antigen detection test positivity was 30%, maximum being Streptococcus pneumoniae (16%), followed by Neisseria meningitidis (10%) and Hemophilus influenzae (4%). It was significant than culture (p=0.002).***Conclusion:** *Antigen detection test of CSF sample is superior to culture in diagnosis of acute pyogenic meningitis, is rapid, easy to perform with not much expertise.*

INTRODUCTION

Bacterial meningitis (BM) is most common infection of central nervous system, which can progress rapidly and result in death or permanent debilitation. It is much more common in developing countries than developed countries [1, 2] The widespread use of antibacterial agents in the treatment of meningitis have drastically reduced the mortality caused by this disease. However, both the morbidity and the mortality of untreated and inappropriately treated bacterial meningitis patients remain high.[3, 4] In developed countries, the mortality from BM is < 10% but it may be ≥ 30% in developing countries.[5] Majority of patients with bacterial meningitis survive, but neurological sequelae occur in 10-35 % of all survivors (especially newborns and children).[6] The common bacteria recovered from acute pyogenic meningitis in children and young adults are *Streptococcus pneumoniae, Neisseria meningitidis* and *Hemophilus influenzae* type b.[7] Though culture methods are available for all the three bacteria but they take 24-48 hours to grow and further identification and antimicrobial susceptibility take another 48 hours.[8] Therefore rapid detection of these bacteria will help in early diagnosis and prompt patient management, resulting in reduction of morbidity and mortality of these patients. Bacterial antigen detection methods are simple, rapid and sensitive, as well as specific. As high concentrations of antigens are generally found in cerebrospinal fluid (CSF), these tests are particularly applicable to CSF samples.[9-11]. In view of the above, this study was undertaken to isolate and identify *Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae type b* from cerebrospinal fluid (CSF) samples by culture and antigen detection methods in cases of acute pyogenic meningitis. And also to compare the culture method with antigen detection method for the diagnosis of the same.

MATERIALS AND METHODS

A prospective study of cases of acute pyogenic meningitis was undertaken to isolate and identify *Streptococcus pneumoniae, Neisseria meningitidis* and *Haemophilus influenzae type b* from 50 cerebrospinal fluid (CSF) samples by culture method and antigen detection method, in the Department of Microbiology over one year (June 2012 to May 2013). Inclusion criteria were patients admitted in this hospital with signs and symptoms of acute pyogenic meningitis and patients of any age and sex. Exclusion criteria were HIV seropositive patients and patients not willing to participate in the study.

Processing of Cerebrospinal fluid (CSF)

CSF was collected by lumbar puncture with all aseptic precautions. It was collected in two sterile test tubes and was transported to the Microbiology Laboratory within two hours of collection. In the laboratory, the CSF from both test tubes was centrifuged at 1500 rpm for 15 minutes. From first tube, supernatant was transferred in another sterile tube for antigen detection test and deposit was utilized for gram staining. The deposit from second tube was used for subculture on Blood agar (BA), Chocolate agar (CA) and MacConkey agar (MA). Blood agar and Chocolate agar were incubated at 37º in a candle jar for 24 hrs and MA was incubated at 37º for 24 hrs. Antigen detection was done by latex agglutination test by using PASTOREX™ MENINGITIS kit (Bio-Rad France), as per manufacturer's instructions. The other part was plated on modified chocolate agar (MCA) incorporated with isovitalex & vitamin K2 and incubated at 37^{0}C with 5% CO_2 atmosphere for 48 hours. The remaining CSF was kept in the incubator at 37^{0}C as a backup for potential reculture.[12] The organisms isolated were identified by standard microbiological techniques.[8]

Ethical clearance

Clearance for the study was obained from the Institutional Review Board.

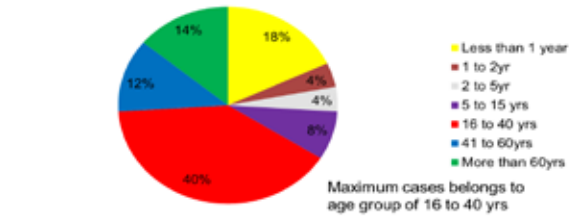
Statistical analysis find out

Data entry and analysis were done using statistical software SPSS for Windows Version 16.0 (SPSS Inc., Chicago, IL, USA)" The Chi−square test was used.

RESULTS

During a period of one year, 50 CSF samples from clinically suspected cases of meningitis were tested for culture as well as antigen detection test. Maximum cases in this study were males (69.37%), with male to female ratio of 2.26:1 (Fig. 1). Figures 2 & 3 show the ICU/Ward distribution and symptoms in 50 cases. Fever was most common symptom.

Figure 1 Age wise distribution of cases (%)



Total samples = 50

Figure 2 ICU and ward distribution (%)

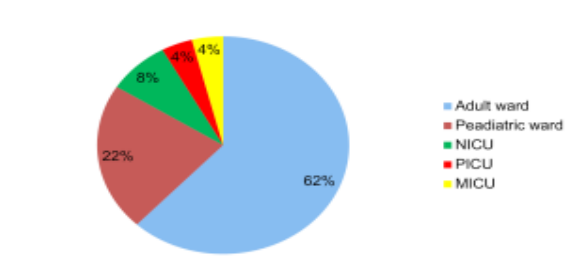
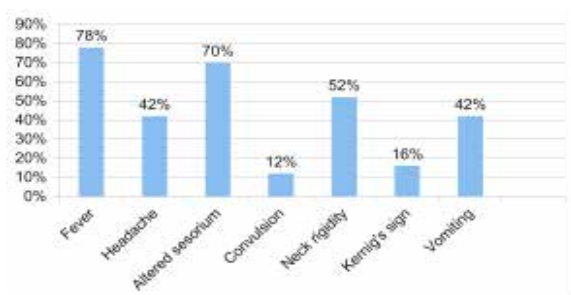


Figure 3 Symptoms in 50 cases



On Gram staining, 6% samples showed pus cells; 6% showed Gram positive cocci in pairs and 4% showed Gram negative cocci in pairs. Figure 4 shows colonies of *Streptococcus pneumoniae* on chocolate agar.

Figure 4 Colonies of *Streptococcus pneumoniae* on chocolate agar

Figure 5 Antigen detection positivity (30%)

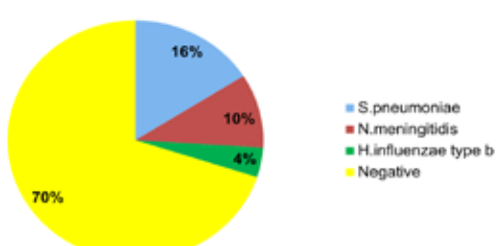
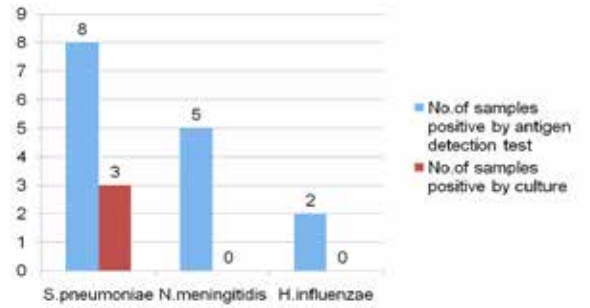


Figure 6 Detection of organism by antigen detection test and culture



By Chi square test along with Yate's correction (8.198), p value = 0.002. Therefore antigen detection test was highly significant than culture.

Table 1 Comparison of Antigen detection test and culture positivity

Total samples (50)	S. pneumoniae	N. meningitidis	H. influenzae	Total
Positive by both antigen and culture	3	0	0	3
Positive by antigen, negative by culture	5	5	2	12
Negative by antigen, positive by culture	0	0	0	0
Total	8	5	2	15

Antigen positivity was 30% (Fig. 5) but culture positivity was only 6% (3/50) (Table 1). By Chi square test along with Yate's correction (8.198), p value = 0.002. Therefore antigen detection test was highly significant than culture.

In 6% cases paralysis occurred as complication and in 4% cases muscular hypertonia was seen. Two patients who developed muscular hypertonia were diagnosed as *S. pneumoniae* by antigen detection test. A 65 years old female suffering from acute pyogenic meningitis developed paralysis as a complication and *N. meningitidis* type A was detected from CSF sample by LAT. Six percent patients expired.

DISCUSSION

As in the present study, almost all studies have shown male preponderance[2,13,14] A study by Singhal et al from Delhi have reported 71% male, which is almost similar to this study.[13] In this study, organisms were reported only in 10% cases on Gram stain. Mani et al[14] from Bangalore has reported Gram stain positivity in 65.7% CSF samples, which is very high as compared to this study. Less positivity in this study can be attributed to not using cytospin to concentrate the smear for Gram stain.

S. pneumoniae was isolated from 3 samples, but this study failed to recover *H. influenzae* and *N. meningitidis*, though selective media like Modified chocolate agar and Hemophilus test medium were used for these fastidious organisms. Overall culture positivity of *S. pneumoniae* in various studies varies from as low as 2.4% from Bangalore[15] to as high as 77% from Ghana[7]. Isolation of *N. meningitidis* in CSF is very low in India (1% from Bangalore) and varying from 1-25% in western countries.[7,16] However, a study from Niger had high isolation rate of *N. meningitidis* from CSF (63%).[2]

The *H. influenzae* type b (Hib) study working group has reported a high culture positivity of *H. influenzae* (34.62%).[17] In

Volume : 5 | Issue : 8 | August 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

all other studies,*H. influenzae* positivity rate varied from 0.9% to 12.6%.[2,14,16] The incidence of *H. influenzae* disease has remained low for the past several decades in India and vaccination of Hib, though not included in national immunization program, is recommended by Indian Academy of Pediatrics (IAP) and World Health Organization (WHO) for all children below 6 years of age with 3 doses at 6, 10 and 14 weeks.[18] Campagne et al have reported that 80% cases of bacterial meningitis were caused by three bacteria, i.e. *S. pneumoniae, N. meningitidis* and *H. influenzae*.[2]

Overall positivity of antigen detection test was 30% in this study. S. *pneumoniae* positivity was 16%, followed by *N. meningitidis* type A 10% and *H. influenzae* type b 4%. No antigen was detected in 70% cases. Mani et al have reported antigen detection positivity in 54.6% cases.[14] A study from Bangalore has reported 2.85% positivity of *S. pneumoniae* by latex agglutination test (LAT) in CSF in cases of invasive pneumococcal disease.[15] A study from Niger reported antigen detection positivity from CSF samples to be 77.9% for *N. meningitidis,* 11.9% for *S. pneumoniae* and 10.2% for *H. influenzae*.[2]

Shah et al from Bangalore in 2009 also showed better LAT positivity (7.7%), than culture positivity (2.9%) in cases of acute pyogenic meningitis due to *S. pneumoniae* in children <5 years of age.[15] A study by Minz et al in 2008, has shown superiority of LAT over culture positivity for cases of *H. influenzae* type b meningitis.[19] LAT may be most useful for the patient who has been pretreated with antimicrobial therapy and whose Gram stain and CSF culture results are negative.[20]

In 90% cases, no complications were seen in this study. The common complications seen in cases of acute pyogenic meningitis are cranial nerve palsy, hemiparesis/quadriparesis, stroke, cerebral/cerebellar herniation and thrombosis of dural venous sinuses.[21] Beek et al have reported cranial nerve palsy in 19% of their cases, paralysis in 5% and aphasia in 2%.[21] However, none of the patients developed cranial nerve palsy and aphasia in this study.

About 30% of survivors of *H. influenzae* type b meningitis suffer from major disabilities.[22] Out of two cases of *H. influenzae* type b meningitis diagnosed by LAT in this study, one patient expired but no complications were seen in both these patients. Mortality due to *S. pneumoniae* in

<5 years age group of children has been reported to be as high as 73% by CDC.[22] In a study from Pondicherry, mortality reported was 20% in meningitis due to *S. pneumoniae*,[23] and in this study, mortality was 25% in meningitis due to *S. pneumoniae* (2/8). Singhal et al from Delhi have reported 14% mortality in meningitis due to *N. meningitidis* serogroup A in 2007.[13] None of the cases of *N. meningitidis* meningitis in this study expired, though one developed paralysis.

CONCLUSION

Antigen detection test by latex agglutination from CSF sample is superior to culture and Gram stain in diagnosis of acute pyogenic meningitis. Though latex agglutination test is slightly expensive but it is rapid, easy to perform and does not require much expertise.

REFERENCES

1. Brouwer MC, Tunkel AR, van de Beek D. Epidemiology, Diagnosis and Antimicrobial Treatment of Acute Bacterial Meningitis. Clin Microbiol Rev 2010; 23: 467–92.
2. Campagne G, Schuchat A, Djibo S, Ousseini A, Cisse L, Chippaux JP. Epidemiology of bacterial meningitis in Niamey, Niger, 1981-96. Bull WHO 1999; 77: 499-508.
3. Hambleton G, Davies PA. Bacterial meningitis: Some aspects of diagnosis and treatment. Arch Dis Childhood 1975; 50: 674-84.
4. Tacon CL, Flower O. Diagnosis and
5. Management of Bacterial Meningitis in the Paediatric Population: A Review. Emergency Med International, Vol 2012, Article ID 320309, 8 pages, 2012. doi:10.1155/2012/320309.
6. Cartwright KAV. Bacterial meningitis. In: Topley & Wilson's Microbiology & Microbial Infections. Bacteriology Vol 1, 10th Ed. Eds. Borriello SP, Murray PR, Funke G. Hodder Arnold ASM press, London, 2005; pp 555-80.
7. Chavez-Bueno S, McCracken GH.
8. Bacterial Meningitis in Children. Pediatr Clin North Amer 2005; 52: 795– 810.
9. Owusu M, Nguah SB, Boaitey YA, Badu Boateng E, Abubakr AR, Lartey RA, et al. Etiological agents of cerebrospinal meningitis: A retrospective study from a teaching hospital in Ghana. Ann Clin Microbiol Antimicrob 2012; 11: 28-35.
10. Koneman EW, Allen SD, Janda WM,
11. Schreckenberger PC, Winn WC. Koneman's Color Atlas and Textbook of Diagnostic Microbiology.6thEd. Lippincott, Philadelphia 2006; pp 92-5, 431-52, 591-603, 643, 683, 712, 1443-7, 1453-8, 1462-3, 1469-70 and 1474.
12. Sippel JE, Prato CM, Girgis NI, Edwards
13. EA. Detection of Neisseria meningitides
14. group A, Haemophilus influenzae type b and Streptococcus pneumoniae antigens in cerebrospinal fluid specimens by antigen capture enzyme linked immunosorbent assays. J Clin Microbiol 1984; 20: 259-65.
15. Ingram DL, Pearson AW, Occhiuti AR. Detection of Bacterial Antigens in Body Fluids with the Wellcogen Haemophilus influenzae b, Streptococcus pneumoniae and Neisseria meningitidis (ACYW135) Latex Agglutination Tests. J Clin Microbiol 1983; 18:1119-21.
16. Ajello GW, Bolan GA, Hayes PS, Lehmann D, Montgomery J, Feeley JC, et al. Commercial Latex Agglutination Tests for Detection of Haemophilus influenzae Type b and Streptococcus pneumoniae Antigens in Patients with Bacteremic Pneumonia. J Clin Microbiol 1987; 25:1388-91.
17. Gray LD, Fedorko DP. Laboratory Diagnosis of Bacterial Meningitis. Clin Microbiol Rev 1992; 5: 130-45.
18. Singhal S, Purnapatre KP, Kalia V, Dube S, Nair D, Deb M, et al. Ciprofloxacin Resistant Neisseria meningitidis, Delhi, India. Emerging Infect Dis 2007; 13: 1614-6.
19. Mani R, Pradhan S, Nagarathna S, Wasiulla R, Chandramuki A. Bacteriological profile of community acquired acute bacterial meningitis: A ten-year retrospective study in a tertiary neurocare centre in South India. Indian J Med Microbiol 2007; 25: 108-14.
20. Shah AS, Nisarga R, Ravikumar KL, Hubler R, Herrera G, Kilgore P. Establishment of population based surveillance for invasive pneumococcal disease in Bangalore, India. Indian J Med Sci 2009; 63: 498-507.
21. Schuchat A, Robinson K, Wenger JD, Harrison LH, Farley M, Reingold AL, et al. Bacterial meningitis in the United States in 1995. New England J Med 1997; 337: 970-6.
22. Gupta M, Kumar R, Deb AK, Bhattacharya SK, Bose A, John J, et al. Multicenter surveillance for pneumonia & meningitis among children (< 2 yr) for Hib vaccine probe trial preparation in India. Indian J Med Res 2010; 131: 649-58.
23. Principles of epidemiology and epidemiologic methods. In: Park's Textbook of Preventive and Social Medicine. 21st Ed. Banarsidas Bhanot Publishers, Jabalpur, 2011; pp 113-4.
24. Minz S, Balraj V, Lalitha MK, Murali N, Cherian T, Manoharan G, et al. Incidence of Haemophilus influenzae type b meningitis in India. Indian J Med Res 2008; 128: 57-64.
25. Tunkel AR, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, Scheld WM, et al. Practice Guidelines for the Management of Bacterial Meningitis. Clin Infect Dis 2004; 39: 1267–84.
26. Van de Beek D, de Gans J, Spanjaard L, Weisfelt M, Reitsma JB, Vermeulen M. Clinical Features and Prognostic Factors in Adults with Bacterial Meningitis. N Engl J Med 2004; 351: 1849-59.
27. Vashishtha VM. Introduction of Hib Containing Pentavalent Vaccine in National Immunization Program of India: The Concerns and the Reality. Indian Pediatr 2009; 46: 781-2.
28. 23. Kanungo R, D'lima D, Rajalakshmi B, Kumar A, Badrinath S. Emerging antibiotic resistant pneumococci in invasive infections in South India: Need for monitoring. Indian J Pharmac 2002; 34: 38-43.