



Original Article

Etiological Agents of Acute Meningo-Encephalitis Syndrome: Study of 75 Cases in Rajshahi Medical College Hospital

N Begum¹, M A Salam², M I Rahman³, L Akter⁴,
S M Asafudullah⁵, I Mahmood⁶, I Ahmed⁷

Abstract

Acute Meningoencephalitis Syndrome (AMES) is a life threatening condition of all ages caused by different microbial agents. Etiological diagnosis is imperative for introduction of appropriate antimicrobial agents to treat the condition. This cross sectional prospective study included seventy five (75) clinically suspected patients of AMES of different age and sex groups, who were admitted at Rajshahi Medical College Hospital (RMCH) during August, 2005 to June, 2006. Cerebro spinal fluid (CSF) was studied by gram-stained smear examination, bacterial culture, Latex Agglutination Test (LAT), Cytological and Biochemical tests. The serum samples were also tested for qualitative C - reactive protein (CRP) by latex agglutination test and IgM antibody against Japanese Encephalitis (JE) virus by Enzyme-linked immunosorbent assay (ELISA). Direct microscopy on gram-stained smears of CSF was found positive for 10 (13.33%) cases while bacterial culture was positive in 17(22.66%) cases. Culture yielded *H. influenzae*, *S. pneumoniae* *N. meningitidis* and *Esch. coli* in 07 (41.17%), 05 (29.41%), 04 (23.53%) and 01(5.88%) cases respectively. Higher rate of isolation was noted among 0-5 years age group. Out of 75 CSF samples, LAT could be done for randomly selected 45 cases with positive results observed in 18 (40%) cases. Good correlation of increase total white cell count and protein level and decrease glucose level was observed among culture-positive cases in cytological and biochemical analysis of CSF. Serum CRP was found positive in 21 (28.00%) cases and it had also excellent (94.11%) correlation with culture-positive cases. Among 75 patients, whose CSF samples were found apparently clear on physical examination, 40 of them were tested for serum IgM antibodies against JE virus with 4 (10%) cases as JE-positive in ELISA. All bacterial isolates were 100% sensitive to Ceftriaxone and Ciprofloxacin except *S. pneumoniae* which showed 80% sensitivity to Ciprofloxacin in antimicrobial susceptibility testing. Variable sensitivity pattern was noted against Penicillin, Ampicillin, Cotrimoxazole, Gentamycin and Erythromycin. This limited study has revealed that clinically suspected cases of AMES can have varying etiology with JE virus is an important cause detected among patients admitted in RMCH and Ceftriaxone is the drug of choice for bacterial meningitis.

TAJ 2009; 22(2): 216-222

Introduction

Meningoencephalitis continues to be the life threatening infection in all ages throughout the

world. It is an important cause of death and disability especially in children in Asia¹⁻³. There are two predominant types of meningoencephalitis

¹ Clinical Pathologist, Department of Microbiology, Rajshahi Medical College, Rajshahi.

² Associate Professor, Department of Microbiology, Rajshahi Medical College, Rajshahi.

³ Lecturer, Department of Microbiology, Rajshahi Medical College, Rajshahi.

⁴ Assistant Professor, Department of Pathology, Islami Bank Medical College, Rajshahi.

⁵ Assistant Professor, Department of Microbiology, Rajshahi Medical College, Rajshahi.

⁶ Associate Professor, Department of Medicine, Rajshahi Medical College, Rajshahi.

⁷ Professor, Department of Microbiology, Enam Medical College, Dhaka.

based on causative agents such as bacterial and viral³. Nevertheless, meningitis can also be caused by protozoa and fungi⁴. Major vaccine-preventable etiologies of meningoencephalitis in Asia include Japanese encephalitis (JE) virus and bacteria such as *Haemophilus influenzae* type b (Hib), *Neisseria meningitidis* and *Streptococcus pneumoniae*. Public health initiatives to control these diseases are becoming more feasible with improved vaccine availability and affordability⁵. Recently a better terminology has emerged to cover both viral and bacterial etiologies of meningitis called Acute Meningoencephalitis Syndrome (AMES) and surveillance for AMES is underway in some of the Southeast Asian countries including Bangladesh where significant number of viral meningoencephalitis cases caused predominantly by Japanese Encephalitis (JE) virus has been reported³. The organisms usually enter the meninges by droplet infection or by direct invasion⁶. Although bacterial meningitis is a major cause of death and disability in the developing countries but viral meningitis is far more common in the developed countries including USA. More than one million people are infected with bacterial meningitis each year with around 200,000 deaths worldwide⁷.

Categorization of AMES is conventionally based on clinical findings, microscopical examination, bacterial culture, biochemical and cytological analysis of CSF. Bacterial culture and antimicrobial susceptibility testing (AST) of isolates is the gold standard for laboratory diagnosis and therapy of pyogenic meningitis but facility to perform culture and poor yielding rate are limiting factors. The detection of soluble bacterial antigens in CSF could be an important diagnostic tool for early empirical antibiotic therapy in bacterial meningitis⁸. Tunkel and Scheld (2000)⁹ published that soluble antigens of *H. influenzae* type b (Hib), *S. pneumoniae*, *N. meningitidis*, *S. agalactiae* and *Esch. coli* in CSF can be detected by Latex Agglutination test (LAT) with sensitivity and specificity ranging from 95 to 100%. The test is simple to perform and laboratory technicians can also interpret the results of the

tests. It requires minimum time and the use of antibiotics generally do not alter the results when used for short interval¹⁰. Although the laboratory test for C-reactive protein (CRP) is not usually considered as routine test but several investigators have found CRP in CSF and serum to be a reliable early diagnostic marker for the suspected bacterial meningitis cases with its diagnostic sensitivity and specificity as 100% and 94% respectively¹¹. CRP is an acute phase serum protein and a good indicator of local inflammatory activity and tissue damage which does not alter even after introduction of antibiotic therapy¹². Further, increase in white cell count and protein level and decrease level of glucose in CSF generally are associated with bacterial or pyogenic meningitis¹³.

Viral etiologies for AMES are gaining importance in the recent years with Japanese Encephalitis (JE) virus being the predominant agents reported from many countries of Southeast Asia¹⁴. JE virus appears to be a new emerging virus for meningoencephalitis reported from different parts of Bangladesh. In a prospective hospital based surveillance carried out at Dhaka, Maymensingh and Rajshahi Medical College Hospitals to find out the etiology of AMES, investigators reported 6% as JE encephalitis among all suspected cases³. After primary infection with JE virus, a rapid and potent monotypic IgM response occurs in serum and CSF usually within seven days. For this reason, detection of IgM antibody to JE virus in serum has gained diagnostic importance¹⁵.

The present study was designed to investigate cases of AMES by different laboratory methods with a special reference to JE viral case detection in RMCH.

Material and Methods

Selection of Patients: A total of 75 clinically suspected cases of acute meningoencephalitis of different age and sex who were admitted in the medicine and pediatric wards of Rajshahi Medical College Hospital (RMCH) during August, 2005 to June, 2006 were included in this study. Case definition of AMES as furnished below was followed for random selection of cases.

A person of any age at any time of year with

(i) Acute onset of fever

With one or more of the following

(ii) Change in mental status (i.e. Coma, lethargy, confusion, agitation, irritability, inability to talk)

(iii) New onset of seizures (excluding simple febrile seizures)

(iv) Meningeal signs, neck stiffness

Collection and utilization of Samples: After taking written informed consent from the patients or from their guardian, Cerebrospinal fluid (CSF) from 75 clinically suspected cases of AMES were collected by lumbar puncture (LP) done by the Assistant Registrar of the concern ward. With all aseptic measures, about 3 ml of CSF were collected and two aliquots were made. Test tube labeled as No. I contained about 1.0 ml of CSF utilized for culture, Gram-staining and LAT. Test tube labeled as No. II contained about 2.0 ml of CSF was utilized for cytological and biochemical tests. 2-3 ml of blood was collected with all aseptic measures and using disposable syringe from each case for separation of serum. All serum samples were utilized for qualitative CRP test and 40 samples collected from patients having apparently clear CSF on physical examination were used for IgM-ELISA for JE virus. Samples were collected before introduction of antibiotic or within 24 hours of antibiotic therapy. All the particulars were recorded systematically in a predesigned data sheet.

Gram-staining for direct microscopical examination: Two good quality smears were prepared from centrifuged deposits of sterile CSF immediately after collection and stained by Gram stain for detection of bacteria using oil-emersion objective of Olympus CH-20 light microscope.

Bacterial culture and sensitivity test: CSF was inoculated onto Blood agar, MacConkey's agar and Chocolate agar media and incubated aerobically at 37°C for maximum up to 48 hours. To ensure 5-10% CO₂, incubated Chocolate agar

plates were put under candle extinction jar. All the bacterial isolates were identified by their colony morphology, gram staining, motility testing by hanging drop preparation, pigment production and relevant biochemical tests¹⁶. All bacterial isolates were tested for their antimicrobial susceptibility by disc diffusion method against Penicillin (10µg), Ampicillin (30µg), Gentamycin (30µg), Cotrimoxazole (30µg), Erythromycin (10µg), Ciprofloxacin (5µg) and Ceftriaxone (10µg). The results of susceptibility were recorded as *Sensitive* or *Resistant*.

Latex Agglutination Test (LAT)

The kit reagents consist of latex particles sensitized with mouse monoclonal antibodies. In the presence of a sufficiently high concentration of soluble antigen present in CSF, the latex specific for the antigen present in the medium agglutinates on binding with the antigen and forms clumps, visible to naked eye. LAT was done as slide agglutination method for five bacteria viz. *N. meningitis*, *H. influenzae type b*, *S. pneumoniae*, *S. agalactiae* and *E. coli*. Results were interpreted as *Positive* or *Negative* on the basis of naked eye visible agglutination. Forty (40) randomly selected cases of CSF were tested by LAT due to resource constrain.

Reagents and accessories used were from the Slidex meningite kit-5 (France).

Detection of CRP in serum by latex agglutination test: Qualitative CRP detection by latex slide agglutination test was done for all serum samples using commercially prepared reagent kit as per manufacturer's instructions.

Detection of JE antibody by IgM antibody capture ELISA (MAC-ELISA)

The MAC-ELISA test for Japanese Encephalitis (JE) virus is an ELISA assay system for the detection of IgM antibodies in human serum to JE virus derived recombinant antigen (JERA). The procedure followed all standard steps of antibody capture ELISA. Results of ELISA were expressed as *Positive* or *Negative*.

Results

Results of different laboratory tests used for the detection of AMES cases are shown in Table-I. Out of 75 CSF samples examined, 10 (13.33%) bacteria were detected in gram-stained smears, culture yielded 17 (22.66%) bacterial isolates and CRP was positive in 21 (28%) cases. Latex agglutination test (LAT) was done for 40 cases and found positive in 40% cases.

Distribution of bacterial agents isolated from AMES cases are shown in Table-II. Out of 17 culture-positive cases, *H. influenzae*, *S. pneumoniae*, *N. meningitidis* and *Esch. coli* were noted in 07(41.17%), 05(29.41%), 04(23.53%) and 01(5.88%) cases respectively.

Figure-1 shows results of IgM-ELISA for JE virus carried out for randomly selected 40 cases having clear CSF on physical appearance. ELISA was found positive in 04 (10%) cases of 40 serum samples tested.

Cytological and biochemical test results of CSF are shown in Table-III. Culture-positive and negative samples had very good correlation with both cytological and biochemical findings. All culture-positive cases had cell count of 2000 ± 1175 SD, protein level of 300 ± 180 SD and glucose level 16 ± 12 SD which differed significantly from that of culture-negative CSF samples where cell count, protein and glucose levels of 1000 ± 975 , 180 ± 120 and 60 ± 26 respectively.

Antimicrobial susceptibility test (AST) results of the bacterial isolates from CSF are shown in table-IV. All bacteria except *S. pneumoniae* showed 100% sensitivity against both Ceftriaxone and Ciprofloxacin. Sensitivity of *S. pneumoniae* was 100% to Ceftriaxone but 80% to Ciprofloxacin. Bacteria showed variable levels of sensitivity against Penicillin, Ampicillin, Cotrimoxazole, Erythromycin and Gentamycin used in AST.

Table-I : Results of different laboratory tests of 75 AMES cases

Tests	Number positive	Percentage
Gram stain	10	13.33
Culture	17	22.00
CRP	21	28.00
LAT	18	40.00

Table -II : Distribution of bacterial isolates among culture-positive cases (n=17)

Bacterial isolates	Number	Percentage
<i>H. influenzae</i>	07	41.17
<i>S. pneumoniae</i>	05	29.42
<i>N. meningitidis</i>	04	23.53
<i>Esch. coli</i>	01	05.88
Total	17	100.00

Table-III : Results of biochemical and cytological examination of CSF samples

Samples	Cytological	Biochemical	
	Cell count / cumm	Protein (mg/ 100ml)	Glucose (mg /100ml)
Culture positive (n=17)	2000 ± 1175 SD (825-3175)	300 ± 180 SD (120 - 480)	16 ± 12 SD (4 - 28)
Culture negative (n=58)	1000 ± 975 SD (25 - 1975)	180 ± 120 SD (60 - 300)	60 ± 26 SD (34- 86)

Figures indicate the mean value with standard deviation. Figures within the parenthesis indicate range.

Figure-1

Results of IgM- ELISA for JE Virus

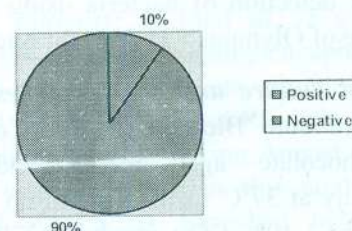


Table - IV : Antibiogram of bacterial isolates from CSF

Anti-microbial agents	Susceptibility pattern	Name of bacterial isolates			
		<i>H.influenzae</i> (n=7)	<i>S.pneumoniae</i> (n=5)	<i>N.meningitidis</i> (n=4)	<i>Esch.coli</i> (n=1)
Amp	S	5 (71.42)	4(80.00)	3(75.00)	0(0)
	R	2(28.58)	1(20.00)	1(25.00)	1(100)
Co	S	6(85.71)	4(80.00)	4(100)	0(0)
	R	1(14.29)	1(20.00)	0(0)	1(100)
G	S	5(71.42)	2(40.00)	3(75.00)	1(100)
	R	2(28.58)	2(40.00)	1(25.00)	0(0)
Cip	S	7(100)	4(80)	4(100)	1(100)
	R	0(0)	1(20)	0(0)	0(0)
Cef	S	7(100)	5(100)	4(100)	1(100)
	R	0(0)	0(0)	0(0)	0(0)
P	S	5 (71.42)	3(60.00)	3(75.00)	1(100)
	R	2(28.58)	2(40.00)	1(25.00)	0(0)
E	S	6(85.71)	4(80.00)	2(50.00)	1(100)
	R	1(14.29)	1(20.00)	2(50.00)	0(0)

Figures within the parenthesis indicate percentage

Amp = Ampicillin Co = Cotrimoxazole G = Gentamycin Cip = Ciprofloxacin Cef = Ceftriaxone P = Penicillin
E = Erythromycin S=Sensitive R=Resistant

Discussion

The epidemiology and public health burden of JE and bacterial meningitis are poorly understood in many Asian countries including Bangladesh. Rapid diagnosis of microbial infections especially for bacterial and viral causes in the central nervous system has become increasingly important. Prevention of spread of disease and differentiation from infections caused by agents sensitive to antibiotics may be the important consequences of a virus specific diagnosis gained early in the disease. Further early etiological diagnosis and appropriate treatment of meningitis results in higher survival rate as well as lower incidence of fatal complications.

In the present study, rate of detection of bacteria both in microscopy and culture is consistent with results of other investigators from both at home and abroad¹⁷⁻²⁰. Cerebro spinal fluid is considered to be a stat specimen, which requires immediate processing for laboratory demonstration of bacteria apart from strict aseptic collection and transportation. More over introduction of antibacterial drugs before CSF collection is

another important factor for poor detection rate. We appreciate that all these limiting factors contributed in our setting resulting in low isolation rate as usual.

As far as the pattern of bacterial isolates is concerned, our findings are in accordance with that of others²¹. *H. influenzae* was the leading bacterial isolates recovered from 0-5 years age group predominantly and it reflects the general facts of incidence and prevalence of *H. influenzae* meningitis in that age group. The comparatively high rate of detection of *H. influenzae* could also be due to the fact that our children are yet to be vaccinated with Hib.

Bacterial meningitis cases usually have high cell count, increase protein and decrease glucose levels and culture-positive cases of the present study were in accordance with these facts like that of other studies¹⁷⁻¹⁸. Some of the culture-negative cases in this study also had slightly raised cell count and protein content; those cases could be of viral origin or treated convalescent meningitis cases where moderate increase of these parameters may occur.

Latex agglutination test for detection of soluble bacterial antigens in CSF was done for randomly selected 45 patients and higher rate of detection was noted in comparison to culture. This could be correlated with the fact that LAT remains positive even after introduction of antibiotics which many of our patients have had in their history. LAT results of this study are also equally comparable with other similar studies^{19,22}. There was very good concordance of CRP and culture-positive cases and our results coincide well with that of others²³. In clinical cases suspected for viral encephalitis or meningoencephalitis, the estimation of virus-specific serum antibodies by enzyme immunoassay has been found to be highly sensitive²⁴. We took an attempt to look for Japanese encephalitis viral cases among clinically suspected AMES patients in RMCH with randomly selected 40 cases on the basis of physical appearance of CSF. Serum IgM-ELISA for JE virus was found positive in 10% cases in our study and this is consistent with previous reports on JE case detection rate from RMCH³. Aseptic meningitis or encephalitis can be caused by a handful number of viral agents including Enterovirus, Measles virus, Mumps virus, varicella-Zoster virus, Herpes simplex-1, Echo virus, Cytomegalovirus etc. We had limitation with the present study that we could not include investigation for other viral agents so it is logically speculated that JE-negative cases were caused by other viruses of aseptic meningitis.

Antimicrobial susceptibility testing carried out in this study showed Ceftriaxone to be the drug of choice being 100% effective against all bacterial isolates followed by Ciprofloxacin. Regarding in-vitro susceptibility of bacteria causing meningitis, similar results were also found by other investigators²⁵.

In conclusion we would like to mention that the etiology of AMES varies significantly in different geographical areas and even among places within same area. For proper case management, clinical suspicion must be verified with appropriate laboratory tests especially to make distinction between septic and aseptic meningitis. In this context, rapid and sensitive diagnostic tools need

to be developed and evaluated through multi-centre study. We appreciate the AMES surveillance currently being held for 4 tertiary-care hospitals in Bangladesh and looking forward with optimism that with further strengthening of this programme in future will enable us to reveal not only the facts of AMES in our context but also to adopt appropriate measures for many of these vaccine-preventable diseases.

Acknowledgement

We would like to acknowledge with thanks the cooperation extended by doctors and staffs of all medical and pediatric units of RMCH for conduction of this study.

References

1. Tsai TF. New initiatives for the control of Japanese encephalitis by vaccination: minutes of a WHO/CVI meeting, Bangkok, Thailand, 13-15 October 1998. *Vaccine* 2000; 18: 1-25.
2. Chotpitayasunondh T. Bacterial meningitis in children: etiology and clinical features, an 11-year review of 618 cases. *Southeast Asian J Trop Med Public Health* 1994; 25: 107-115.
3. Hussain SM, Ekram ARMS, Hossain MJ, Gurley E, Labeys. Japanese Encephalitis: Bangladesh perspective: A Review. *Journal of Teachers Association (TAJ), Rajshahi Medical College* 2004; 17 (1): 57-70.
4. Jewetz F, Melnick JL, Adelberg EA. *Medical Microbiology*. 22nd ed. Mc Graw Hill Company. 2000: 192-213.
5. Beasley DW, Lewthwaite P, Solomon T. Current use and development of vaccines for Japanese encephalitis. *Expert Opin Biol Ther* 2008; 8: 95-106.
6. Crook DW, Phupradit P, Warrel DA. Bacterial Meningitis. In: Weatherall D, Ledingham JG, Warrell DA, editors. *Oxford Text book of Medicine*. 3rd ed. New York, Oxford University Press Inc. 1996: 4050-4064.
7. Hwang MY. Meningitis in Children. *JAMA and Archives Journals* 1999; 281:15-60.
8. Das BK, Gurubacharya RL, Mohapatra TM, and Mishra OP. Bacterial antigen detection test in meningitis. *Indian Journal of Pediatrics* 2003; 70(10): 799-801.
9. Camargos PAM, Almeida MS, Cardoso. Latex particle agglutination test in diagnosis of *Haemophilus influenzae* type b, *Streptococcus*



- pneumoniae and *Neisseria meningitidis* A and C meningitis in infants and children. *J Clin Epidemiol* 1995; 48: 1245-1250.
10. Naik DG, Seyoum M. *Haemophilus influenzae* type b meningitis in children in Eritrea. *Emerg Infect* 2004; 10 (1): 30-32.
 11. Macfarlane DE, Narla VR. Cerebrospinal fluid C-reactive protein in the laboratory diagnosis of bacterial meningitis in children. *Acta Paediatr Scand* 1985; 74: 560-563.
 12. John M, Raj IS, Macadens R, Raghuvor TS, Yeswanth M and Meund DM: Cerebrospinal fluid C-reactive protein measurement a bed side test in the rapid diagnosis of bacterial meningitis. *J Trop Paediatr* 1990; 36: 213-217.
 13. Harish LD, Fedorku DP. Laboratory diagnosis of Bacterial Meningitis. *Clinical Microbiology Reviews* 1992; 5: 130-195.
 14. Kari K, Liuw, Gautama K, Mammen MP, Nisalak A, Subrata K. A hospital-based surveillance for Japanese encephalitis virus in Bali, Indonesia. *BMC Med* 2006; 4: 8.
 15. Grandie M and Olding-Stenkvis E. Rapid diagnosis of viral infections in the central nervous system. *Scand J Infec Dis* 1984; 16(1): 1-8.
 16. NCCLS (National Committee for Clinical Laboratory Standards). Performance standards for antimicrobial disc susceptibility tests. Tentative standard. 4th ed. Villanova. PA. USA. 1988.
 17. Chowdhury MZU, Rahman KM, Miah RA, Sattar H and Hussain T. Bacterial meningitis in children. *Bangladesh Medical Journal* 1992; 21: 3-7.
 18. Mazed MA. Detection of bacterial antigen by LPAAT & estimation of cytokines (Tumor necrosis factor- α Interleukin-1B) in CSF for the diagnosis of bacterial meningitis in Paediatric patients, Dept of Microbiology, BSMMU, Dhaka (M.Phil Thesis), 2002.
 19. Deivanayagan N, Ashok TP, Neduchelian K, Ahmed SS and Mala N. Bacterial Meningitis. Diagnosis by latex agglutination test and clinical features. *Indian Paediatr* 1993; 30: 495-500.
 20. Shaha SK, Baqui AH, Miah MRA, Hanif M, Arifen EL, Oishi K, Santos ML, Nagatake T, Black RE. Invasive Hib disease in Bangladesh. *Journal of Paediatrics* 2005; 146: 227-233.
 21. Shaha SK, Rkitomi N, Miah MRA, Watanaba K, Ahmed K, Biswas D, Hanif M, Khan WA, Islam M, Matsumoto K, Nagatake T. The increasing burden of disease in Banladeshi children due to *Haemophilus influenzae* type b meningitis. *Ann Trop Ped* 1997; 17: 5-8.
 22. Venkatesh VC, Steinhoff MC, Moses P, Zadhav M and pereira SM. Latex-agglutination an appropriate technology for the diagnosis of bacterial meningitis in developing Countries. *Ann Trop Ped* 1985; 5: 33-36.
 23. Derrel Clarke and Karen Cost. Use of serum C reactive protein in differentiating septic from aseptic meningitis in children. *The Journal of Pediatrics* 1983; 12(5): 718-720.
 24. Dennin RH and Herb E. Immunological diagnosis in viral infections of the central nervous system: course of antibody titres against homo- and heterologous viruses. *Med Microbiol Immunol* 1989; 178(5): 255-268.
 25. M Rabiul Alam, Samir Kumar Saha, Tania Nasreen, Farzana Latif, Sabita Rezwana Rahman, Donald James Gomes. Detection, Antimicrobial Susceptibility and Serotyping of *Streptococcus pneumoniae* from Cerebrospinal Fluid Specimens from Suspected Meningitis Patients. *Bangladesh J Microbiol* 2007; 24(1): 24-29.

All correspondence to:

M A Salam

Associate Professor

Department of Microbiology

Rajshahi Medical College, Rajshahi