Section: Microbiology

Original Research Article

IDENTIFICATION AND ANALYSIS OF BACTERIAL, VIRAL, AND FUNGAL INFECTIONS IN PATIENTS WITH ACUTE MENINGITIS AND MENINGOENCEPHALITIS IN A TERTIARY CARE HOSPITAL

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Abstract

Background: Acute meningitis or encephalitis (AME) is due to neurological infections with high fatality and severe conditions. The current study aims to assess AME's microbial aetiology in tertiary care hospitals. **Materials and Methods:** The cross-sectional study was conducted among 100 patients with AME for a one-year follow-up. The study was conducted in patients >18 years of age with AME. Cerebrospinal fluid and Blood(3-5ml) collected under aseptic conditions were used for microbial analysis. Direct Gram's stain, Bacterial culture for Aerobic bacteria, Antimicrobial susceptibility test, fungal culture, Enzyme-Linked Immunosorbent Assay(ELISA), and Latex agglutination tests were done. Positive and negative values were assessed after interpreting data from SPSS software. **Result:** From 100 study participants, 31 patients presented with clinically confirmed diagnosis of AME, majorly in the age group of 18-30 years with a male predominance. The study reported viral pathogens (74.1%) as the most common cause of AME among patients, followed by bacterial pathogens (19.3%). Patients presented with fever (100%), headache (84%), and altered sensorium (64%). Japanese encephalitis the most prevalent pathogen (43.47%), followed by Dengue virus (30.43%) and Herpes simplex virus (17.39%). Dual infections were reported in two patients. **Conclusion:** The study reports that most patients with AME are suspected to be with viral and bacterial pathogens. The study underscores that symptoms, signs, and routine laboratory tests have limited accuracy in predicting and diagnosing AME. Therefore, it is necessary to utilise specific tests that target specific viruses and assess the effectiveness of immunisation efforts.

INTRODUCTION

Acute meningitis or encephalitis results in substantial mortality and long-term neurological complications worldwide.[1-3] Several infectious agents, including bacteria, fungi, and viruses, are AME's causative agents.[1] The prevalence of infectious AME is estimated at 1.5-7 cases/ 100,000 inhabitants/year, with the highest prevalence among children.[2,4] The causative agents can spread to over one hundred viral and bacterial species, with viral pathogens as the most common causative agent.[5] The differentiation of AME is clinically difficult; hence the terms acute meningoencephalitis and meningitis are referred to as AME. Despite advanced diagnostic techniques and antimicrobial therapies, AME remains an emergent infectious disease with high mortality.[6,7]

The global burden of acute bacterial meningitis (ABM) has been estimated to range from 1 to 2 million cases annually.[8] However, the severity of this issue is particularly pronounced in low-resource countries in Latin America, sub-Saharan Africa, and South-East Asia.[9] *Neisseria meningitides, Streptococcus pneumoniae,* and *Haemophilus influenzae type b* are the predominant pathogens associated with bacterial meningitis on a global scale, contributing to nearly 90% of reported cases in children aged between 2 months and five years.[10] In developed countries, the overall incidence of ABM is approximately 2 to 3 cases per 100,000 population, with higher peaks observed in infants and adolescents.[11] Conversely, children residing in developing nations face a substantially greater incidence of bacterial meningitis, with rates ranging from 10 to 20 cases per 100,000, surpassing those observed in Western Europe and the United States by more than 10-fold.

Acute meningitis and meningoencephalitis (AME) can exhibit rapid progression, leading to long-lasting consequences within a relatively brief timeframe.[7] Timely initiation of treatment for meningitis patients relies heavily on the cerebrospinal fluid (CSF) examination results. The primary objective is to accurately diagnose bacterial meningitis while ensuring that viral meningitis is not mistakenly treated with inappropriate antibiotics or steroids.[12] It is imperative to conduct epidemiological and microbiological studies on AME to develop suitable clinical management strategies and implement effective preventive measures. There is a lack of data on aetiological agents for AME in India. Hence, the study aimed to identify and assess the aetiology of AME concerning bacterial, fungal, and viral infections in a tertiary care hospital.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology at the Madras Medical College, Rajiv Gandhi Government General Hospital, along with the Institute of Internal Medicine, Madras Medical College, Rajiv Gandhi Government General Hospital. This study was initiated after approval from the Ethics committee for one year (April 2016 to March 2017). Duly signed consent forms were obtained from all patients and every criterion was explained to the patient or their relatives

Inclusion criteria

Patients aged>18 years, patients with clinical signs and symptoms of AME admitted to the hospital, with clinical features such as fever, headache, vomiting, neck rigidity, altered sensorium, and seizures were included.

Exclusion Criteria

Patients <18 years of age, chronic meningitis patients, meningitis associated with neurosurgical procedures and a ventriculoperitoneal shunt, and meningitis secondary to carcinoma and auto-immune disorders like systemic lupus erythematosus and rheumatoid arthritis were excluded.

Materials

Patient blood and cerebrospinal fluid collected were subjected to various tests including Direct Gram's stain, bacterial culture for Aerobic bacteria, antimicrobial susceptibility test, fungal culture, Enzyme-Linked Immunosorbent Assay (ELISA) Latex agglutination tests Cryptococcal antigen detection test (CrAg) and serological tests (JEV capture IgM ELISA), Dengue tests, Herpes simplex virus test, varicella-zoster virus test, the procedure of each test can be found in supplementary data.

Interpretation of the results

Interpretation of the tests was done based on the standard guidelines (CLSI 2016) and as per the manufacturer's instructions given in the kit insert.

Interpretation of ELISA:

The absorbance mean of the two wells with the calibrator was computed and the cutoff values were calculated by multiplying the mean absorbance of the calibrator with a correction factor. The Quality control certificate was referred to for the determination of the correction factor value of the supplied lot of the kit. Samples with absorbance < 90% of the cut-off value were considered negative, and samples with absorbance >110% were considered positive. Samples with absorbance in the range of 0.90-1.10 of the cut-off value were considered as equivocal.

Statistical Analysis

The statistical analysis was conducted using the statistical package for social sciences software (SPSS). The proportional data of this study were analysed using Pearson's Chi Square Test.

RESULTS

Most individuals (51%) were between 18 and 30; the second largest age group was 31-40, comprising 19% of the total sample. The 41-50 age group accounted for 14% of the sample, followed by the 51-60 age group at 9%. The smallest age group was 61-70, representing 7% of the sample. When considering gender, 61% were males and 39% were females [Table 1].

Fever was the most prevalent clinical feature, reported in 100% of the cases. Headache was the second most commonly observed symptom in 84% of cases. Altered sensorium was reported in 64% of the cases, making it the third most prevalent clinical feature. Vomiting was observed in 45% of the cases, indicating a moderate prevalence. Neck rigidity was reported in 36% of the cases, while seizures were observed in 31%, making them the least prevalent clinical features in the sample population [Table 2].

The evaluation of cerebrospinal fluid reported a clear appearance in 94 patients, turbid/cloudy in 4 patients, and a blood-stained was reported in one patient.

Out of 100 individuals clinically suspected to have acute meningitis or meningoencephalitis, only 29 cases showed evidence of causative agents. In the study population, 31 central nervous system (CNS) pathogens were identified in these 29 patients. Interestingly, two patients were found to have simultaneous infections with two different viruses. One patient had dual infection with the Japanese encephalitis virus and Dengue virus, while another had dual infection with the Japanese encephalitis virus and Herpes simplex virus [Table 3].

In the age group of 18-30, there were 14 individuals (six males and eight females) and this age group represents 48.2% of the total.

Four males were included in 31-40 age group, and this accounts for 13.7% of the sample. Five males were included in 41-50 age group, and this accounts for 17.2% of the sample. The 51-60 age group has one male, with one individual accounting for 3.4% of the total, and no females were reported in 31-60 age group. In the 61-70 age group, there were four males and one female, making up five individuals. This age group represents 17.2% of the sample. Overall, in the entire sample population, there were 20 males (68.9%) and nine females (31.03%), totalling 29 individuals [Table 4].

Among the 100 samples, 83 (83%) were acellular, meaning they had no detectable cells. There were 15 samples (15%) with a cell count ranging from 1 to 100 mm^3. Only one sample (1%) had a cell count between 100 and 500 mm^3. Similarly, one sample (1%) had a cell count greater than 500 mm^3. The table accounts for 100 samples, each falling into one of the mentioned categories [Table 5].

Among the identified bacterial organisms, *Streptococcus pneumoniae* was detected in 2 cases, representing 33.3%. *Escherichia coli* was found in 1 case, accounting for 16.6% of the total. Similarly, *Klebsiella pneumoniae* was detected in 2 cases, representing 33.3% of the total. *Pseudomonas aeruginosa* was identified in 1 case, accounting for 16.6% of the total. Overall, the table accounts for a total of 6 organisms, each falling into one of the specified categories [Figure 1].

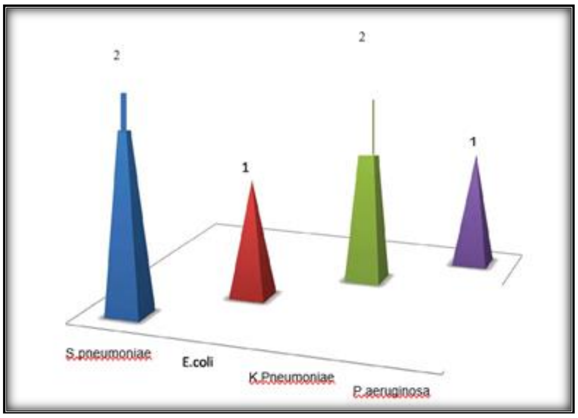

Figure 1. Profile of bacterial agents

The table summarises the susceptibility of different organisms to various antibiotics, including AK (Amikacin), GM (Gentamicin), CTX (Cefotaxime), CAZ (Ceftazidime), PT (Piperacillin-Tazobactam), and MERO (Meropenem). *Escherichia coli* (Organism 1), showed susceptibility (s) to all antibiotics listed. *Klebsiella pneumoniae* (Organism 2) was susceptible to AK, PT, and MRP and resistance (R) to GM, CTX, and CAZ in the first row. In the second row, *Klebsiella pneumoniae* (Organism 3) is susceptible to AK and CTX, resistant to GM, CAZ, and PT and susceptible to MRP. *Pseudomonas aeruginosa* (Organism 4) is susceptible to AK, GM, CAZ, PT, and MRP, was resistant to CTX [Table 6].

Japanese encephalitis virus was the most common of the identified pathogens, observed in 10 cases, making up 43.47% of the total. Dengue virus was detected in 7 cases, accounting for 30.43% of the total. Herpes simplex virus was found in 4 cases, making up 17.39% of the total. Varicella zoster virus was identified in 2 cases, representing 8.69% of the total. The table includes 23 pathogens, each falling into one of the specified categories [Figure 2].

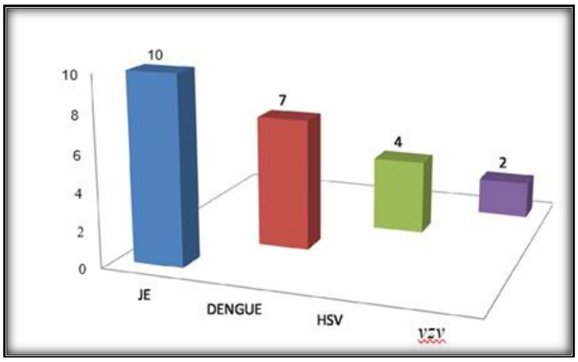

Figure 2: Viral aetiology among the study population

[Table 8] provides information on the viral etiology of cases and the results of serum IgM ELISA and CSF IgM ELISA tests. It also includes the total number of positive cases. Japanese encephalitis virus was identified in 10 cases, with positive results in serum IgM ELISA and CSF IgM ELISA tests. Dengue virus was identified in 7 cases, with positive results in serum IgM ELISA and CSF IgM ELISA tests.

Herpes simplex virus was detected in 4 cases, with positive results observed only in CSF IgM ELISA tests. Varicella zoster virus was identified in 2 cases, with positive results seen only in CSF IgM ELISA tests. There were 17 positive cases detected through serum IgM ELISA tests and eight positive cases detected through CSF IgM ELISA tests, resulting in 23 positive cases across all viral etiologies [Table 7].

[Table 8] represents information about two cases who were positive for Cryptococcal Antigen assay and HIV antibody.

First patient who was HIV positive was on ART and improved. Second patient who was HIV positive did not continue ART and succumbed to the disease.

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Table 1: Age distribution of patients

Age	Male	Female	Percentage
18-30	25	26	51 (51%)
31-40	14	5	19 (19%)
41-50	11	3	14 (14%)
51-60	7	2	9 (9%)
61-70	5	2	7 (7%)
Total	61 (61%)	39 (39%)	100 (100%)

Table 2: Clinical symptoms presented in patients with AME

Clinical features	Percentage
Fever	100%
Headache	84%
Vomiting	45%
Altered sensorium	64%
Neck rigidity	36%
Seizures	31%

Table 3: Distribution of aetiology among laboratory-proven cases (n = 31)

Microbiological agents	No. of positive cases	Percentage
Bacterial	6	19.3%
Viral	23	74.1%
Fungal	2	6.4%
Total	31	100%

Table 4: Age and gender distribution of laboratory-proven cases

Age	Male	Female	Total(%)
18-30	6	8	14(48.2%)
31-40	4	-	4(13.7%)
41-50	5	-	5(17.2%)
51-60	1	-	1(3.4%)
61-70	4	1	5(17.2%)
Total	20(68.9%)	9(31.03%)	29(100%)

Table 5: Cerebrospinal fluid among the study population

Cell count(mm3)	No of samples	Percentage
Acellular	83	83%
1-100mm3	15	15%
100-500 mm3	1	1%
>500mm3	1	1%
Total	100	100%

Table 6: Susceptibility profile of bacterial agents

Organisms	AK	GM	CTX	CAZ	PT	MERO
Escherichia coli	s	s	s	s	s	s
Klebsiella pneumoniae (l)	s	R	R	R	s	s
Klebsiella pneumoniae (2)	s	s	R	s	s	s
Pseudomonas aeruginosa	s	s	-	R	s	s

Table 7: Serology of viral agents

Viral etiology	Serum IgM ELISA	CSF IgM ELISA	Total no of positives
Japanese encephalitis virus	10	6	10
Dengue virus	7	5	7
Herpes simplex virus	-	4	4
Varicella zoster virus	-	2	2
Total	17	8	23

Table 8: Correlation of cryptococcal meningitis with HIV status

Cases	Age I sex	HIV status	Stage of HIV	CD4 count (cells/mm3)	Other opportunistic infections	ART& ATT	Amphotericin B therapy	Outcome
1	29/F	Positive	IV	21Ocells/mm3	nil	On ART	Started & completed	Improved
2	55/M	Positive	IV	32cells/mm3	Pulmonary tuberculosis	not started	Started & not completed	Expired

DISCUSSION

The present study aimed to investigate etiology and clinical presentation of acute meningitis and meningoencephalitis (AME) among clinically suspected cases. The data was collected from 100 individuals admitted to the medical wards of Rajiv Gandhi Government General Hospital. The study findings shed light on several important factors, including age distribution, gender distribution, clinical features, cerebrospinal fluid (CSF) evaluation, etiological agents, antibacterial

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susceptibility patterns, and the association with HIV status. In our study, regarding age distribution, most individuals (51%) fell within the 18-30 age range, indicating higher susceptibility to AME in this age group. The second largest age group was 31-40, accounting for 19% of the total sample. This information suggests that AME affects individuals primarily in their young adulthood. Additionally, the distribution of cases across different age groups highlights the importance of age-specific prevention strategies and targeted interventions. Regarding gender distribution, males constituted the majority (61%) of the total sample, whereas females accounted for 39%. This finding suggests a potential gender-related difference in susceptibility to AME. Further investigation is warranted to explore the underlying factors contributing to this observed gender disparity. The current study reports a male-to-female ratio of 1:6:1.

The clinical features observed in the study population provided valuable insights into the characteristic manifestations of AME. Fever emerged as the most prevalent clinical feature, reported in 100% of the cases, followed by headache (84%) and altered sensorium (64%). Vomiting was observed in 45% of the cases, indicating a moderate prevalence. Neck rigidity and seizures were relatively less prevalent, with percentages of 36% and 31%, respectively. These findings align with the typical symptoms associated with AME, highlighting the importance of prompt recognition and early management of these infections. Adhikary et al,[13] reported a similar finding that altered sensorium was one of the most common symptoms among AME patients. In addition, the triad of symptoms, including fever, neck rigidity, and headache, was observed in 36% of our patients, similar to the findings of Merkelbach et al,[14] and Van de Beek D et al., respectively.[15] CSF evaluation revealed that most patients (94%) had a clear macroscopic appearance, indicating a lack of cellular abnormalities. However, a small proportion of patients presented with turbid/cloudy CSF (4%), and one had blood-stained CSF. These findings emphasise the significance of CSF analysis in diagnosing and managing AME, as it provides valuable information regarding the inflammatory and infectious processes occurring within the central nervous system.

Identifying etiological agents in AME cases is crucial for the appropriate management and targeted interventions. Among the 100 clinically suspected cases, evidence of causative agents was detected in 29 cases. A total of 31 CNS pathogens were identified, with Japanese encephalitis virus being the most prevalent (43.47%), followed by Dengue virus (30.43%), Herpes simplex virus (17.39%), and Varicella zoster virus (8.69%). Notably, dual infections with different viruses were observed in two patients, underscoring the complexity and potential for coinfections in AME cases. In addition, similar study findings were reported by Giri et al., who reported 38 patients with CNS pathogens, of which 33 patients had dual infections.[16] These findings highlight the importance of comprehensive laboratory testing and multiplex diagnostic approaches for accurately identifying and characterising etiological agents in AME.

In the present study, we observed viruses as the most common aetiological agents for causing AME comprising 21% of the study population, followed by bacteria (6%) and fungus(2%). Giri et al.[16] also reported a similar study finding, which reported viral pathology in 28% of patients, and bacterial pathogens in 14% of the population. Viral aetiology was also the commonest in a study conducted in Kazakhstan among 556 patients; 494 patients were diagnosed with viral pathology, 37 patients with bacterial, and 19 patients were of unknown etiology.[17]

The susceptibility patterns of identified organisms to various antibiotics provided insights into the potential treatment options for AME. The results revealed varying degrees of susceptibility and resistance among the identified organisms. *Escherichia coli* demonstrated susceptibility to all listed antibiotics, whereas *Klebsiella pneumoniae* showed resistance to certain antibiotics such as Gentamicin, Cefotaxime, and Ceftazidime. In our study, the four major bacterial pathogens diagnosed were *S.pneumoniae, E. coli, Klebsiella Pneumoniae,* and *Pseudomonas aeruginosa*. However, Bumburidi et al. reported *N. meningitidis, S.pneumoniae, and Hemophilus influenza* as the most common cause of AME.[17] These findings emphasise the importance of antimicrobial stewardship and the need for appropriate antibiotic selection based on susceptibility patterns and individual patient characteristics.

Lastly, the association between AME and HIV status was explored in this study. The information provided includes the stage of HIV infection, CD4 counts, presence of other opportunistic infections, ART, ATT, Amphotericin B therapy, and the outcomes of two cases. It is evident that individuals with HIV infection are susceptible to AME, and managing such cases requires a comprehensive approach addressing both the underlying HIV infection and the associated opportunistic infections.

CONCLUSION

In conclusion, this study provides valuable insights into various aspects of AME, including age and gender distribution, clinical features, CSF evaluation, etiological agents, susceptibility patterns, and the association with HIV status. These findings contribute to our understanding of AME's epidemiology, clinical presentation, and management. Further research and collaborative efforts are warranted to improve AME prevention, diagnosis, and treatment, ultimately leading to better outcomes for affected individuals.

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REFERENCES

1. Chaudhuri A, Kennedy PGE. Diagnosis and treatment of viral encephalitis. Postgrad Med J. 2002;78:575–583.
2. Boucher A, Herrmann JL, Morand P, et al. Epidemiology of infectious encephalitis causes in 2016. Med Mal Infect. 2017;47:221–235.
3. Zunt JR, Kassebaum NJ, Blake N, Glennie L, Wright C, Nichols E, et al. Global, regional, and national burden of meningitis, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2018;17:1061-82.
4. Venkatesan A, Michael BD, Probasco JC, Geocadin RG, Solomon T. Acute encephalitis in immunocompetent adults. Lancet. 2019;393:702–716.
5. McGill F, Griffiths MJ, Solomon T. Viral meningitis: Current issues in diagnosis and treatment. Curr Opin Infect Dis. 2017;30:248–256.
6. Quagliarello VJ, Scheld WM. Treatment of bacterial meningitis. N Engl J Med. 2001;336:708–716.
7. Robbins JB, Schneerson R, Gotschlich EC. Surveillance for bacterial meningitis by means of polymerase chain reaction. Clin Inf Dis. 2005;40:26–27.
8. Bennet JV, Platonov AE, Slack MPE, Mala P, Burton H, Robertson SE. Haemophilus influenzae type b (Hib) meningitis in the pre-vaccine era: A global review of incidence, age distribution, and case fatality rate. Geneva (CH): World Health Organization; 2002.
9. Van de Beek D, De Gans J, Tunkel AR, Wijdicks EF. Community-acquired bacterial meningitis in adult. N Engl J Med. 2006;354:44–53.
10. Tzanakaki G, Mastrantonio P. Aetiology of bacterial meningitis and resistance to antibiotic of causative pathogens in Europe and in Mediterranean region. Int J Antimicrob Agents. 2007;29:621–629.
11. Ramakrishnan KA, Levin M, Faust SN. Bacterial meningitis and brain abscess. Medicine. 2009;37:567–573.
12. Chavanet P, Schaller C, Levy C, Flores-Cordero J, Arens M, Piroth L, et al. Performance of a predictive rule to distinguish bacterial and viral meningitis. J Infect 2007;54:328–36.
13. Adhikary M, Chatterjee R. Laboratory evaluation of cases of meningitis attending a tertiary care hospital in India: An observational study. Int J Nutr Pharmacol Neurol Dis 2013;3:282.
14. Merkelbach S, Sittinger H, Schweizer I, and Muller M. Cognitive outcome after bacterial meningitis. Acta Neurol Scand. 102:118-123.
15. Van de Beek D, Schmand B, De Gans J, Weisfelt M, Vaessen H, Dankert J, et al. Cognitive impairment in adults with good recovery after bacterial meningitis. J Infect Dis 2002;186:1047–52.
16. Giri A, Arjyal A, Koirala S, Karkey A, Dongol S, Thapa SD, et al. Aetiologies of central nervous system infections in adults in Kathmandu, Nepal: a prospective hospital-based study. Sci Rep 2013;3:2382.
17. Bumburidi Y, Utepbergenova G, Yerezhepov B, Berdiyarova N, Kulzhanova K, Head J, et al. Etiology of acute meningitis and encephalitis from hospital-based surveillance in South Kazakhstan oblast, February 2017-January 2018. PLoS One 2021;16:e0251494.