Original Research Article

Acute Encephalitis Syndrome Due to Scrub Typhus: A Missed Diagnosis

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Abstract

Introduction- Clinically, a case of Acute Encephalitis Syndrome (AES) is defined as a person of any age, at any time of year with the Acute onset of fever and a change in mental status (including symptoms such as confusion, disorientation, come, or inability to talk) and/or new onset of seizures (excluding simple febrile seizures). Acute encephalitis syndrome (AES) is a major public health problem worldwide because of its high morbidity and mortality. Although incidence of AES varies according to different studies, it is generally between 3·5 and 7·4/1,00,000 patient-years, and is higher in children aged <1 year and adults aged > 65 years.(3). **Material and methods-** This study was Open Labeled, Prospective study and was performed over a period of 1 year and 6 months from January 2018 to June 2019 at Department of Microbiology, TNMC and BYL Nair Ch. Hospital, Mumbai and Molecular Diagnostic Reference Laboratory, Kasturba Hospital, Mumbai only after taking Approval from Institutional Ethics Committee and Informed consent of every patient enrolled in the study. **Results-** This study was done on adult population and the mean age was 42.5 years. The minimum age was 14 years and the maximum age was 78 years. Almost half of the patients were between 15 and 45 years of age, which suggest the disease has a higher incidence in the younger adults. A maximum number of patients came during the month of July -October, suggesting the seasonal occurrence of the disease. The most common clinical features of AES patients were fever and altered sensorium followed by behavioral changes, headache, and seizures. The most common finding of general examination of AES patients was pallor followed by tachycardia and tachypnea. Most of the patients had low GCS (<=7), hypotonia and abnormal movements during their CNS examination. In this study, 17(28.3%) the CSF R/M finding were suggestive of bacterial infection (white cell counts high with neutrophilic predominance, accompanied by elevated proteins and hypoglycorrachia) and in 5(8.4%) the CSF R/M findings were suggestive of viral infection (mild pleocytosis and lymphocytic predominance, slightly elevated protein, and normal glucose), however in 38(63.33%) patients CSF R/M findings were normal. **Conclusion-** To conclude, the etiology panorama of acute encephalitis syndrome including Scrub Typhus and viral agents (excluding JE) is not well documented especially in western India. The lack of published data from western India leads to lack of understanding of disease. Conventional methods like culture and routine CSF studies lead to identification of only few etiologic agents causing AES, Diagnosis of AES should be done by new molecular diagnostic technologies including PCR, nucleic acid sequence-based amplification, and branched-DNA assay. Multiplex PCR is a boon for CNS infections where "TIME IS PRECIOUS" and help in early diagnosis of infectious agents and thereby providing specific treatment. A surveillance system for undiagnosed infections should be developed which helps to monitor the trends of emerging infection and aid in their early diagnosis.

Keywords: Acute Encephalitis Syndrome**,** CNS infections, Multiplex PCR

Introduction

Clinically, a case of Acute Encephalitis Syndrome (AES) is defined as a person of any age, at any time of year with the Acute onset of fever and a change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk) and/or new onset of seizures (excluding simple febrile seizures).

(1) According to National Health Portal, India (March 2019), the most common organisms causing AES in India are Japanese encephalitis virus, Herpes simplex virus, Influenza A virus, West Nile virus. Though, viruses are the most common identified etiological cause, approximately in 70% of the cases the cause remains unidentified.

(2)The conventional methods for diagnosis of AES are time consuming and tedious. With the advent of multiplex PCR specific diagnosis is possible, thereby specific treatment such as acyclovir for HSV and VZV, cidofovir for adenovirus, valgancidovir for EBV, foscarnet for CMV, immunoglobulins for enterovirus, doxycycline for scrub typhus can be instituted to patients.

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Material and Methods

This study was Open Labeled, Prospective study and was performed over a period of 1 year and 6 months from January 2018 to June 2019 at Department of Microbiology, TNMC and BYL Nair Ch. Hospital, Mumbai and Molecular Diagnostic Reference Laboratory, Kasturba Hospital, Mumbai only after taking Approval from Institutional Ethics Committee and Informed consent of every patient enrolled in the study.

Material and methods

1) **Type of Study:** Cross sectional Study
2) **Sample size-**60
3) **Duration of study:** 18 Months
4) **Age group:** More than 12 Years

Inclusion Criteria

- Adult (Both males and female) patients admitted in wards and ICU in the Department of Medicine having Acute Encephalitis Syndrome as per case definition will be included.

Exclusion Criteria

- Adult patients having meningitis.
- Patients below 12 years of age.

Sample Processing

CSF routine microscopy, gram stain, culture and identification of organism was done using standard procedures and detection of Scrub Typhus by IgM enzyme linked immunosorbent assay (ELISA) was done as per kit insert at Department of Microbiology, TNMC and BYL Nair Ch. Hospital, Mumbai.

Multiplex PCR for 9 viruses which included Herpes simplex virus (HSV1,2), Enterovirus, Varicella- Zoster virus, Cytomegalovirus(CMV), Epstein -Barr virus(EBV), Adenovirus, Parechovirus, Human Herpes virus (HHV6,7), Parvovirus B19 and Real time PCR for detection of dengue virus were carried out using standard procedure as per instruction manual provided with the respective kits at Molecular Diagnostic Reference Laboratory, Kasturba Hospital, Mumbai.

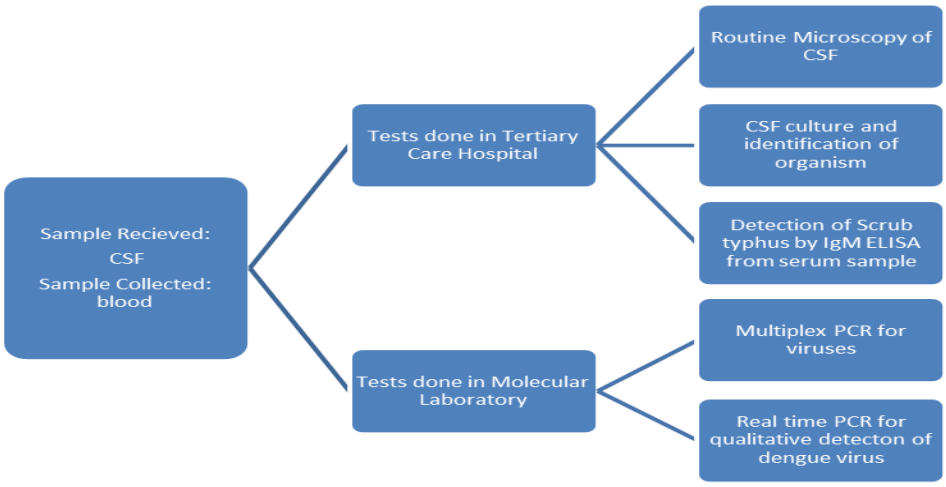

Fig 1:Conduction of process

Results

This study was done on adult population and the mean age was 42.5 years. The minimum age was 14 years and the maximum age was 78 years. Almost half of the patients were between 15 and 45 years of age, which suggest the disease has a higher incidence in the younger adults. A maximum number of patients came during the month of July -October, suggesting the seasonal occurrence of the disease. The most common clinical features of AES patients were fever and altered sensorium followed by behavioral changes, headache, and seizures.

The most common finding of general examination of AES patients was pallor followed by tachycardia and tachypnea. Most of the patients had low GCS (<=7), hypotonia and abnormal movements during their CNS examination. In this study, 17(28.3%) patient's CSF R/M finding were suggestive of bacterial infection (white cell counts high with neutrophilic predominance, accompanied by elevated proteins and hypoglycorrachia) and in 5(8.4%) patient's CSF R/M findings were suggestive of viral infection (mild pleocytosis and lymphocytic predominance, slightly elevated protein, and normal glucose), however in 38 (63.33%) patient's CSF R/M findings were normal.

Table 1: CSF R/M Examination Findings Suggestive of Infection (N=60)

CSF R/M Examination Suggestive of Infection	Number of Patients	Percentage
S/O Bacterial Infection	17	28.3%
S/O Viral Infection	5	8.4%
Normal	38	63.33%

In this study,8 (13.3.%) were positive for scrub typhus IgM ELISA while 52 (86.7%) were negative for the same. This study reveals that 37.5% of patients with scrub typhus encephalitis were farmers and 62.5% of patients with scrub typhus encephalitis were non-farmers, but the difference was not statistically significant.

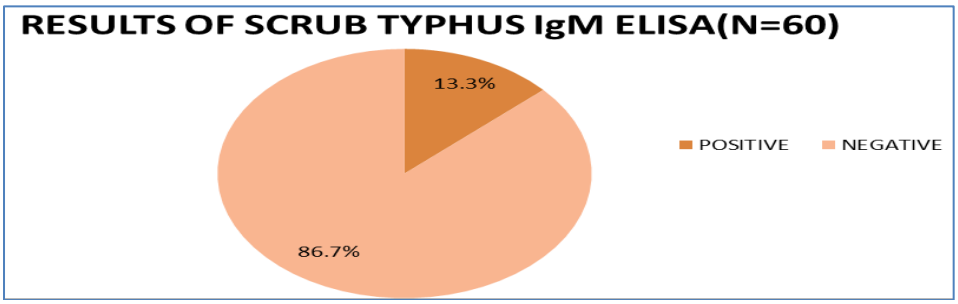

Fig 2:Results of scrub typhus IgM ELISA

Multiplex PCR for 9 viruses and Real time PCR for dengue virus was carried out. 2 out of 60 patients (3.4%) were positive for Epstein- Barr

virus and 1 out of 60 patient (1.6%) was positive for Parvovirus B19 and 1(1.6%) was positive for dengue virus. Rest all samples, 56 samples were CSF-PCR negative for the 10 viruses that were tested.

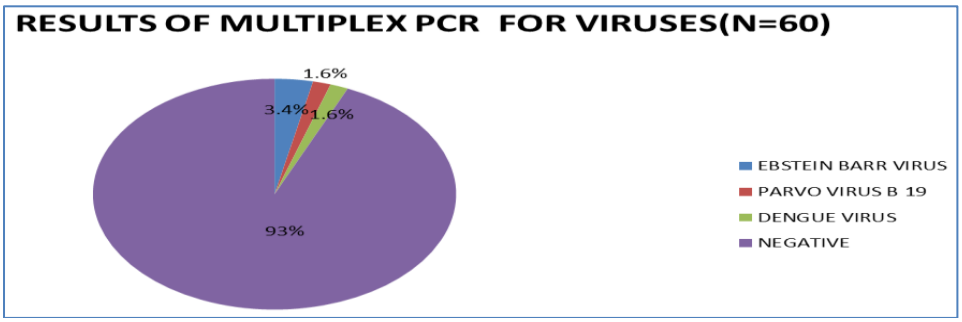

Fig 3:Results of multiplex PCR for viruses

Based on the above findings, the cases were labeled as follows:

- From all patients with AES, definitive diagnosis (using bacterial and fungal culture, scrub typhus ELISA and multiplex PCR for virus, real time PCR for dengue virus) could be made in 12 cases and these cases were called Diagnosed Infective Etiology cases.
- In 11 patients with CSF R/M suggestive of bacterial/viral infection, causative organism for AES was identified but it could not be identified in 11 patients with CSF R/M suggestive of infective etiology, and they were termed as Undiagnosed Infective Etiology cases.
- Rest 38 patients out of 60 had normal CSF R/M findings.
- One of the 38 cases with normal CSF R/M had diagnosed infective etiology ie dengue virus encephalitis.
- Presence of underlying diseases or systemic illness which may lead to encephalitis were observed in 38 patients out of 60 with normal CSF findings.
- In 14 patients out of 38 with normal CSF findings, underlying diseases and systemic illness including hepatic encephalitis, acute kidney injury, chronic renal failure, electrolyte imbalance, diabetic ketoacidosis, autoimmune diseases and others were found to cause AES, hence these were labelled as Non Infective Etiology cases.
- Also, in 23 patients out of 38 with normal CSF findings, there was no other systemic illness or underlying disease, all tests done in this study were negative hence, the etiology remained unknown and were named as Unknown Etiology cases.

Table 2: Etiology Among The Study Population Suffering From AES (N=60)

Etiology	No. of Patients	Percentage
Diagnosed Infective Etiology	12	20%
Undiagnosed Infective Etiology	11	18.3%
Non Infective Etiology	14	23.3%
Unknown Cause	23	38.3%

Etiology among diagnosed infective cases is as follows:

Table 3: **Distribution of Study Population With Diagnosed Infective Etiology (N=11)**

Etiology Among Diagnosed Infective Cases	No. of Patients	Percentage
Scrub Typhus Encephalitis	8	66.66%
EBV Encephalitis	2	16.66%
Parvo Virus Encephalitis	1	8.33%
Dengue Virus Encephalitis	1	8.33%

Out of 22 cases s/o infective etiology, 17 CSF samples were suggestive of bacterial infection. Out of these 17 cases, 8(47.1%) were diagnosed as Scrub typhus encephalitis. None of the Scrub Typhus encephalitis patients had normal CSF. This difference was statistically significant (p value=0.001). Also, Out of 22 cases s/o infective etiology, 5 CSF samples were suggestive of viral infection. Out of these 5 cases, 2 cases were diagnosed with EBV Encephalitis and 1 case was diagnosed with Parvo Virus Encephalitis but difference was not statistically significant. Out of 38 patients with normal CSF R/M, 60.5% cases had unknown etiology, 36.8% had non infective etiology and 2.65% had dengue virus encephalitis.

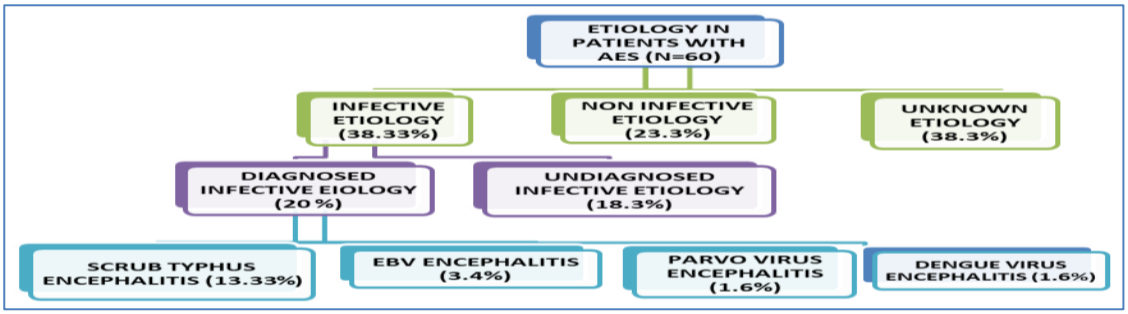

Fig 4:Graphical Representation of Diagnosis According to Etiology

The case fatality rate in patients with AES observed in this study was 41.7%. Poor outcome was more common in patients with low GCS. Maximum patients who died had unknown etiology. Also, 62.5% of Scrub typhus encephalitis cases died which was alarming as it is a treatable condition.

Discussion

Acute encephalitis syndrome (AES) is a major public health problem worldwide because of its high morbidity and mortality. Although incidence of AES varies according to different studies, it is generally between 3·5 and 7·4/1,00000 patient per years, and is higher in children aged <1 year and adults aged > 65 years.(3)

Etiology

In this study we found that, Scrub Typhus Encephalitis was seen in 13.3%, Epstein Barr virus Encephalitis in 3.4%, Parvo Virus Encephalitis and Dengue virus encephalitis in 1.6% cases. Hence, total diagnosed infective etiology cases were 20%. Also, patients with undiagnosed infective etiology were 18.3%, noninfective etiology were 23.3% and unknown etiology were 38.3%.

Gupta et al. from north India reported that in 59% of cases, etiology could not be known. Of the known etiologies of AES (41%), 27% were viral causes (JE, EV) and around 14% were bacterial causes.(4) Desai et al. reported that etiology could not be identified in 60.34% while identified in 39.66% which included JEV 21%, Scrub typhus IgM 26%, Dengue IgM 5%, HSV 0.62% and Enterovirus 0.16% amongst others.(5) Ravi et al. reported that etiology could be established in 49.2% cases which were JEV (16%), scrub typhus (16%) and DEN (5.2%) as the top three agents.(6)

In a study from California, USA by Glaser et al. only 16% had a confirmed etiological agent, 13% had a suspected agent and 8% had non-infectious cause identified, 66.66% had unknown etiology.(7)

Granerod et al. from England observed that in 37% cases cause was unknown, 21 % had non-infectious cause and 42% had infectious causes which included 24% viral cause (HSV, VZV) and 12.5% bacterial cause including Mycobacterium tuberculosis.(8)

Mailes et al. from France, did etiologic investigation which yielded an infectious cause of the encephalitis in 52% while 25% had suspected infectious cause and 23% had unknown cause. Among infectious cause 69% had viral encephalitis (HSV, VZV), 30% had bacterial encephalitis (MTB, Listeria monocytogenes) and 1 patient had fungal encephalitis.(9)

Srey et al. reported that pathogens could not be identified in 36% cases.(10) Quist-Paulsen et al. reported unknown etiology in 57% cases but among pathogens identified more were viral than bacterial.(11)

Lohitharajah et al. from south Asian population reported Gram stain positivity 0% and Bacterial culture positivity also 0% in their study which was similar to our study where positivity was 0% in the gram stain and culture.(2)

Scrub Typhus Encephalitis

In our study, amongst 36.7% cases with CSF R/M s/o infective etiology, 28.33% were suspected to have bacterial infection and from these 13.3% were diagnosed as Scrub Typhus Encephalitis. In our study, most cases of Scrub Typhus Encephalitis were seen in monsoon and post monsoon.

Studies from north India have reported high rates of Scrub typhus encephalitis. Mittal et al. reported IgM scrub typhus positivity to be as high as 63% and Murhekar et al. as high as 62.7%.(12,13)

Incidence of Scrub typhus encephalitis was lower in the study by Thangaraj et al. who reported IgM scrub typhus positivity of 17.9% and Mane et al. 13.6%.(14,15) From south India, Desai et al. reported scrub typhus positivity to be 26% and Ravi et al to be 16% which was conforming with our study.(5,6) However, despite of through literature search, no study was found in western India.

In this study, Cut off value of optical density (OD) 0.8 was determined using geographically relevant serum samples taken from 15 normal human or human with unrelated infections. Similar cut off was taken in a study done in North India using 40 samples by Gupta et al. (16) Several studies from India have used a cutoff-OD from 0.5 to 1. (16) But, published ICMR guidelines in 2015 recommend a cutoff-OD value of 0.5(17) A nationalized cutoff OD cannot be determined because a wide geographical variation which has been noticed in different studies.

Season: As observed in our study, this increase in scrub typhus cases in monsoon and post monsoon seasons in India occurs due to increased exposure to trombiculid mites (chiggers) during harvesting season as well as the growth of new vegetation, which serves as habitat for this vector.(18) Also in these season, humidity is increased which is associated with increased number of cases of Scrub typhus. Similar observations were made in a study done from North India.(19)

Environment: In our study, urban population of Mumbai and suburbs were affected by scrub typhus. On the contrary population working in the rural area are said to be associated with scrub typhus in higher percentages, but now–a-days population working in areas like palm plantations, primary forest, beaches, gardens have also shown to high incidences of scrub typhus.(20) Exposure of the affected individuals to beaches and gardens could account for these scrub typhus cases. Urban population in our study could acquire the infection due to prior rural residence, visits to rural areas to help with farming, collecting bamboo shoots, hunting and fishing which is also stated in the study by Vallae et al.(20)

Individual behavior: Individual behavior may also affect the disease acquisition. Not changing underclothes and overclothes before sleeping, resting on mud or grass, and lack of toilets in the house, open defeacation, involvement in gardening activities may be associated with infection as seen by Goerge et al.(21)

Viral Encephalitis

For viral diagnosis multiplex PCR which included 9 viruses and Real Time PCR for dengue virus were used. From the 60 samples tested for Real time multiplex PCR, 3 cases were positive. Among these, 5% cases had definitive diagnosis which included 3.4 % cases of EBV Encephalitis and 1.6% cases of Parvo Virus Encephalitis. From the 60 samples tested for Real time PCR for dengue virus, 1(1.6%) case was positive for Dengue Virus Encephalitis.

Viral Etiology: Similar findings were noted by others. Joshi et al. from central India saw that 79% of cases had undiagnosed viral etiology.(22) Tiwari et al. from North West India documented viral confirmation in 22.7% cases with HSV group as most common.(23) Other Indian studies have reported viral positivity from 17.20% to 29.81%, at Odisha, Uttar Pradesh, West Bengal, but studies from Uttar Pradesh, Karnataka and New Delhi reported higher positivity from 50% to 71.92%.(23)

Lohitharajah et al. from south Asian population noted that despite a wide array of stringent laboratory testing, a viral etiology was identified in only 25% patients presenting with AES.(2) In a study from Finland, 34% of the cases from total AES cases remained undiagnosed, despite extensive use of PCR based diagnostic tests.(24)

EBV Encephalitis: Herpes group remains the most common causative agent in acute sporadic encephalitis cases in the developed world and in India too but in our study we found cases of EBV encephalitis instead of HSV. In the study by Tiwari et al., EBV was positive in 5.6% patients and by Lohitharajah et al. it was 3% similar to the present study.(2,23)

Parvo Virus Encephalitis: In India parvovirus encephalitis is rare. Arankalle et al. detected parvovirus 4 in 19.5% cases by Real-time PCR in serum sample,(25) Benjamin et al. detected Parvovirus 4 in 16.6% patients with AES.(70) 2 cases of Parvovirus 4 were reported by Prakash et al. from UP from a cases of AES.(71) Several case reports all round the world have detected Parvovirus B19 in AES patients with immune-compromised and immune-competent conditions by Druschky et al., Barah et al., Erik et al. and Filipoo et al.(26-29)

Dengue Virus Encephalitis: Desai et al. from southern India reported Dengue Encephalitis in 5% of cases. (5) Ravi et al. reported

that etiology could be established in 49.2% cases among which Dengue encephalitis was diagnosed in 5.2% in the study done at Uttar Pradesh, West Bengal, and Assam. (6) Koshy et al. observed 2.6 % cases of dengue encephalitis, similarly Varatharaj et al. and Verma et al. observed CSN manifestations of dengue in their study.(30-32). These findings were similar to our study. Various case reports of dengue encephalitis were reported from India by Borawake et al., Madi et al, Rao et al. etc.(33-35).

Dengue encephalitis is observed in most countries in the world. Solomon et at. from southern Vietnam reported dengue encephalitis in about 4.2% case and Aruujo et al. reported around 15% of cases with dengue encephalitis amongst many others. (36, 37)

In this study, for diagnosis of viral encephalitis, Multiplex PCR for 9 viruses and Real time PCR for dengue virus was done which excluded various other viral etiological agents causing encephalitis. As JEV is not endemic in this area, testing for the same was not done. This may have lead to under diagnosis of sporadic cases of Japanese encephalitis in this study.

In this study, as the case definition of AES was adopted from the national vector-borne disease control programme (NVBDCP), this included all patients with fever and mental abnormality but could miss some cases of AES that present only with seizures, paresis or headache. On the other hand, this case definition can include some cases of meningitis that can present with ill-defined altered sensorium without showing frank signs of meningitis. This would lead to more number of suspected case of AES without actual diagnosis.

Timing of sample acquisition during CNS infections affects test positivity and this factor might have contributed to failure to identify organisms. As the patients first visit the nearby hospital and then get referred to a tertiary care hospital, there is delay in sampling of the patients from actual onset of disease which leads to low positivity.

Even if the sample reaches the laboratory on time, the positivity varies from place-to-place, this depends on the type of samples included in the study(blood/CSF), storage conditions, test used for detection of organisms, detection techniques, number of viruses included in the PCR detection panel, etc. In this study, real time Multiplex PCR was used for simultaneous detection of 9 viruses. Real time multiplex PCR requires processing a minimum of 20-25 samples in one batch for optimal usage of consumables provided in the kit. This would lead to delay in processing of samples thereby affecting immediate management. This can be obviated by using film-array technology where continuous loading of samples and processing is done and reports are available in 1-2 hours. This would be beneficial for providing appropriate therapy.

In our study the number of cases with unknown diagnosis is more than the ones with specific identified cause, which may be due to the failure to identify non-encephalitic syndromic mimics, inadequate case investigation by clinicians, and the presence of novel infectious agent causing encephalitis. The poor outcome in etiological diagnosis of AES emphasises the importance of using improved, highly sensitive tests for identification of infective pathogen.

Conclusion

To conclude, the etiology panorama of acute encephalitis syndrome including Scrub Typhus and viral agents (excluding JE) is not well documented especially in western India. The lack of published data from western India leads to lack of understanding of disease.

Conventional methods like culture and routine CSF studies lead to identification of only few etiologic agents causing AES, Diagnosis of AES should be done by new molecular diagnostic technologies including PCR, nucleic acid sequence-based amplification, and branched-DNA assay. Multiplex PCR is a boon for CNS infections where “Time is Precious” and help in early diagnosis of infectious agents and thereby providing specific treatment. A surveillance system for undiagnosed infections should be developed which helps to monitor the trends of emerging infection and aid in their early diagnosis.

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