Aetiology and Laboratory Diagnosis of Acute Encephalitis Syndrome with Special Reference to India


Abstract

Acute encephalitis syndrome (AES) which encompasses infections of the central nervous system (CNS), such as encephalitis, meningitis, and meningoencephalitis, is quite commonly encountered in clinical practice and contributes to significant mortality and morbidity in the community. Several organisms including viruses, bacteria, fungi, and parasites are known to cause AES in humans. The cardinal clinical and laboratory findings are largely similar regardless of the causative agent and consist of fever, headache, and altered mental status, which are often accompanied by seizures and focal neurological abnormalities. Therefore, identification of the causative pathogen is paramount in AES, as rapid detection and confirmation of aetiological agent will have a tremendous impact on the management of outbreaks as well as patient's illness. This article reviews the aetiological and laboratory diagnostic aspects of AES with special reference to the Indian context.

Keywords: Acute encephalitis syndrome, viral encephalitis, aetiology, laboratory diagnosis.

Introduction

The World Health Organization (WHO) has defined Acute Encephalitis Syndrome (AES) as “Clinically any person of any age at any time of year presenting with acute onset of fever and a change in mental status (including signs and symptoms such as confusion, disorientation, coma, or inability to talk) and/or new onset of seizures (excluding simple febrile seizures).” 1 This broad definition of AES encompasses infectious as well as non-infectious aetiologies. It was introduced to facilitate surveillance for Japanese encephalitis (JE) as it enables the surveillance system to capture all the AES cases occurring in a community, thereby allowing for an enhanced sensitivity of case detection.

Aetiology of AES

Globally AES is known to occur in two forms, either as outbreaks of encephalitis or as sporadic encephalitis. The causative agents of outbreaks of encephalitis depend largely on the geographic distribution of the aetiological agent besides the seasonal, environmental, viral and host factors. For instance, St. Louis encephalitis, West-Nile virus, Western Equine Encephalitis, Eastern Equine Encephalitis and Venezuelan Equine Encephalitis viruses are common causes of outbreaks of encephalitis in the Americas, while Murray Valley Encephalitis virus is commonly encountered in Australia and Japanese encephalitis virus (JEV) is highly prevalent in South East Asian countries.2 In contrast, sporadic encephalitis is commonly caused by viruses belonging to the Herpesviridae family [Herpes Simplex Virus 1 and 2 (HSV), Varicella Zoster Virus (VZV), Human Herpes Virus-6 (HHV-6)] and Enteroviruses. In the immune-compromised individuals Mycobacterium tuberculosis, Toxoplasma gondii, Cryptococcus neoformans and JC virus are frequently encountered as aetiological agents of AES. A summary of the aetiological agents that are known to cause AES in India is presented in table 1.

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RNA VIRUSES | BACTERIA
---|---
Japanese Encephalitis (JE) | Streptococcus pneumoniae
West Nile virus (WNV) | Neisseria meningitidis
Dengue | Haemophilus influenzae
Chikungunya | Listeria monocytogenes
Enteroviruses | Group B Streptococci
Measles | Brucella species
Mumps | Salmonella species
Rubella | M.tuberculosis
Chandipura | Mycoplasma pneumoniae
Nipah | Treponema pallidum
Kyasanur forest disease (KFD) | Leptospira
Rabies | Orientia tsutsugamushi
Human Immunodeficiency Virus (HIV) | Rickettsia typhi
Lymphocytic choriomeningitis virus (LCMV) | FUNGI

DNA VIRUSES
---
Herpes simplex (HSV) | Cryptococcus neoformans
Varicella zoster (VZV) | Candida species

PARASITES
---
Human Herpes Virus-6 (HHV-6) | Plasmodium falciparum
Epstein Barr | Toxoplasma gondii
Adenovirus | Naegleria and Acanthamoeba
Paroviruses | Cysticercus cellulosae (Neurocysticercosis)
Cytomegalovirus (CMV) | 

| Table 1.Aetiological agents of AES identified in India |

**Aetiology of AES in India**

Published reports concerning AES in India are largely based on outbreak investigations. Most of these outbreaks predominantly affected children and were attributed to Japanese encephalitis virus (JEV) infection. In India, the history of AES has paralleled that of JE, which has been the most common virus responsible for causing acute encephalitis, especially in northern, north-eastern, and southern India. Approximately 7,500 cases of JE occur annually in India during epidemic years with a morbidity rate estimated at 0.3 to 1.5 in a population of 100,000.
For several decades, based on confirmed outbreak reports, JE has traditionally been considered as the only major cause of AES in India. However, several recent outbreak investigations and surveillance studies have increasingly reported non-JE aetiologies in AES, which may reflect a true changing epidemiological effect or the use of improved diagnostic tests. New viral agents such as Chandipura and Nipah virus, and other viruses such as HSV, VZV, Dengue, Enteroviruses, West Nile Virus, Chikungunya and Parvovirus B4 have been reported as causative agents of AES, largely in case-reports or case-series. Although AES occurs in non-outbreak settings and affects adults as well as children, there are very few published studies from India that describe the epidemiologic features of sporadic AES in adults in India.

Japanese encephalitis

JEV activity in India was first established by a serological survey in 1952 and clinical recognition of this disease was first made in 1955 at Vellore in Tamil Nadu state. Between 1955 and 2007, there had been an increase in the spread of infection throughout the country, except in arid and high land regions. Outbreaks of JE occurred in West Bengal during 1973–1976 and in Andhra Pradesh, Bihar, Tamil Nadu, and Uttar Pradesh during 1977–1979. Subsequently, outbreaks were reported in Assam, Karnataka, Pondicherry, and Uttar Pradesh in the 1980s. In recent years, JEV has spread to new geographical locations within India—Goa, Haryana, and Kerala states in the western half of India. JE occurrences have been reported from 16 states and Union Territories and among them Uttar Pradesh and Assam are the worst affected ones. In 2005, Uttar Pradesh faced a devastating epidemic outbreak of JE, reporting 6,061 cases with 1,500 deaths. This was followed by another outbreak in 2006 with 2,320 cases and 528 deaths.

Chandipura virus

An outbreak of acute encephalitis of unknown origin was investigated by Rao et al., in Andhra Pradesh in 2003. This outbreak which had a very high fatality rate of 55.6% mostly in children, was attributed to Chandipura virus, a member of the family Rhabdoviridae and genus Vesiculovirus. First identified in 1965, from the blood of two adult patients with febrile illness from Chandipura (Nagpur) in Maharashtra state, the significance of Chandipura virus, as a human pathogen was resolved only after this outbreak was conclusively linked to this virus. Subsequently, Chandipura virus was also identified as the aetiological agent in outbreaks from Gujarat in 2004, Maharashtra in 2003, 2005 and 2007 and in a hospital based surveillance of acute encephalitis among children from North Telangana in Andhra Pradesh.

West Nile Virus (WNV)

West Nile virus (WNV) fever is an endemic disease in India usually causing a mild, non-fatal illness in humans, but occasionally reported to cause encephalitis. Febrile illness and overt cases of encephalitis were reported in Udaipur area of Rajasthan, and in 3 districts in Maharashtra. WNV neutralizing antibodies have been detected in human sera collected from Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Gujarat, Madhya Pradesh, Orissa, and Rajasthan. Serologically confirmed cases were also reported from Vellore and Kolar districts. Isolation of WNV from fatal cases of encephalitis was reported from Mysore and Kolar districts of Karnataka. Recently, WNV has been reported from regions of Assam which are endemic for JEV. IgM antibodies to WNV were detected in 12/103 (11.6%) serum samples of patients with AES. A recent outbreak of WNV was also reported from Kerala in 2011, where 208 AES cases with 4 deaths were reported.

Enteroviruses

Enteroviruses have long been recognized as causative agents of acute encephalitis in India. In recent years, enteroviral infection was detected by reverse transcription PCR in the CSF samples of 66 out of 306 (21.5%) cases of AES, representing all 8 districts of eastern Uttar Pradesh. Sequencing and phylogenetic analyses of PCR products from 59 of 66 (89.3%) specimens showed similarity with EV-89 and EV-76 sequences. EV-75 was identified as the aetiological agent in 5 of the 106 (4.7%) children with AES during 2005-2007 in Bellary, Karnataka. Another study reported EV-71 as the most common aetiological agent, accounting for 42.1% cases in children hospitalized with suspected viral encephalitis in western Uttar Pradesh. JE infection was not detected in any of the 87 patients included in the study. EV-71 infection caused significant mortality (50%) and morbidity in children. EV-71 is emerging as a significant cause of AES, though it is most often associated with sporadic disease in India.

Nipah Virus

An outbreak of encephalitis, which was retrospectively identified to be due to Nipah virus,
was reported in 2001 from Siliguri, West Bengal. Sixty-six cases of encephalitis were identified, and the case-fatality ratio was 74%. Subsequently, in April 2007, 30 cases of fever with acute respiratory distress and/or neurological symptoms, with 5 deaths reported from Nadia district of West Bengal were also attributed to Nipah virus infection.

**Dengue and Chikungunya virus**

Globally, dengue is emerging as an important cause of AES. In India several reports, especially from Uttar Pradesh, have identified Dengue virus as an aetiological agent in AES. Chikungunya virus which re-emerged in 2005 in India after a long hiatus, has been reported to cause neurological manifestations and has emerged as one of the newer causative agents of AES in India.

**Other viruses causing AES**

Several other viruses including HSV, VZV, CMV, HHV-6, Epstein Barr, JC, Measles, Mumps, Rubella, and Rabies have been reported to cause AES in India, most often as sporadic cases (table-1).

**Non-viral aetiological agents of AES in India**

Several non-viral pathogens can lead to a clinical presentation compatible with AES. They include Plasmodium falciparum (cerebral malaria), leptospira, bacteria causing meningitis like Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae, and Mycobacterium tuberculosis (table-1).

Scrub typhus is an acute febrile illness reported from several parts of India, caused by rickettsial pathogen Orientia tsutsugamushi, and is characterized by an eschar, lymphadenopathy, multisystem involvement and a rapid response to doxycycline. CNS involvement in Scrub typhus in the form of meningitis/meningoencephalitis has been recently reported from India and it has emerged as a new aetiological agent causing AES.

**Non-infectious aetiology of AES**

In addition, a number of non-infectious conditions may account for encephalopathic syndromes that mimic CNS infections. These include neoplastic diseases, intracranial tumours and cysts, medications, acute disseminated encephalomyelitis, cortical venous thrombosis, neuroleptic malignant syndrome, collagen vascular disorders and other systemic metabolic illnesses.

The cause for a seasonal AES outbreak that occurs in Muzaffarpur, Bihar for several years has remained undetermined; hence it continues to be called a mystery disease. It occurs as annual seasonal outbreaks during the months of April–July, affecting hundreds of children with 40–60% mortality, according to local physicians. JE as the aetiological agent, as earlier attributed, has been ruled out in recent studies. The outbreaks have been associated with the Lychee harvesting season. Recently a hypothesis of toxic origin related to consumption of Lychee fruits by undernourished children has been proposed.

**Laboratory Diagnosis of AES**

**The Need for Laboratory Confirmation**

Clinical management of AES cases is mostly symptomatic; however laboratory confirmation of the aetiological pathogen helps in prompt initiation of therapy especially in potentially fatal cases of bacterial meningitis. In addition, identification of the aetiological agent is also necessary to understand the epidemiology, identify targets for immunization, chart preventive strategies, implement appropriate control measures especially in outbreak situations, and to help formulate rational empirical treatment especially for non-viral causes of AES.

**Challenges in Laboratory Diagnosis of AES**

There is a paucity of data about the regional epidemiology and aetiology of AES in India. Though several pathogens can cause AES, the clinical presentation in many of these infections can overlap. Indeed, aetiological confirmation of the agent causing AES is one of the major diagnostic challenges in clinical medicine. Several factors contribute to the difficulty in the rapid identification of the aetiological agent. Firstly, the number of infectious pathogens known to cause AES is sizeable (table-1); they include several viruses, bacteria, fungi, and protozoa. Secondly, several conventional methods available for testing are time-consuming, expensive, lack adequate sensitivity, and/or specificity and may not be easily accessible. Thirdly, the small volume of cerebrospinal fluid (CSF) available from patients is quite often insufficient for laboratory testing for all the pathogens. Lastly, at
present, there is no single method available for simultaneous detection of all pathogens causing AES.

**Laboratory Investigations**

**Basic investigations**

Basic blood investigations should be carried out in all patients with AES, which will help in prompt clinical management. They include a complete blood count including platelet count, blood glucose, serum electrolytes, liver and kidney function tests, blood culture, arterial blood gas, and lactate (if available). A chest X-ray, peripheral smear for malarial parasite and rapid diagnostic test for malaria should also be done.  

**Lumbar Puncture and CSF Analysis**

Since CSF analysis offers vital clues to diagnosis and management, a lumbar puncture should be performed on patients who are haemodynamically stable, and do not demonstrate features of raised intracranial pressure. When lumbar puncture is contraindicated, neuroimaging should be performed; however empirical treatment should be initiated pending results of neuroimaging/CSF analysis.  

CSF analysis may provide invaluable information about the nature of the infectious process, and cytological changes of the CSF generally indicate the nature of the infecting agent. CSF cell pleocytosis is observed in encephalitis or meningitis; polymorphonuclear leucocytes increase in bacterial meningitis while lymphocytosis is seen in viral encephalitis. While cell counts in bacterial infections are very high ranging in thousands, in viral encephalitis the numbers range from above normal to hundreds. CSF lymphocytosis is also seen in tuberculous meningitis or fungal meningitis, as well as in cases of partially treated pyogenic meningitis. In rare instances like enteroviral meningitis, West-Nile virus meningoencephalitis, cytomegalovirus radiculomyelitis and arboviral infections, there may be a predominance of polymorphonuclear leucocytes, early in the disease process.

The CSF glucose is usually normal during viral infections, and reduction of CSF glucose levels relative to blood glucose is characteristic of meningitis due to bacteria, mycobacteria or fungi. However, low CSF glucose levels are occasionally observed in case of meningoencephalitis caused by mumps, enteroviruses, lymphocytic choriomeningitis, herpes simplex and varicella zoster virus.

Alteration of CSF protein concentration in CNS infections is the most common and the least specific diagnostic marker, since it is observed in a wide variety of infectious and non-infectious conditions. CSF protein levels may be normal or slightly elevated in viral encephalitis, and moderately to significantly elevated in bacterial and tuberculous meningitis.

**Laboratory Tests for Detection of AES pathogens**

**Sample Collection and Storage**

The success in identifying the aetiological agent in AES largely depends on the collection of appropriate specimens, prompt transport and storage, and the choice of the diagnostic tests used. CSF sample should be collected from all the patients with AES, unless contraindicated. CSF should be collected in sterile, screw capped containers. It should be immediately stored at 2-8°C until testing. If a delay in testing is expected, CSF samples must be frozen at -20°C or preferably at -80°C. If workup for bacterial pathogens has to be done, the CSF must not be refrigerated, but processed immediately for staining and culture. Blood (serum) sample should be collected in all cases of AES. The sample should be obtained within 4 days after the onset of illness for isolation of virus and at least 5 days after the onset of illness for detection of IgM antibodies. A second, convalescent sample should be collected at least 10-14 days after the first sample for serology. The sample should be kept at room temperature until complete clot retraction, after which serum should be separated and stored in a refrigerator at 2-8°C until testing, for not more than a week. If a delay in testing is expected, sera must be frozen at -20°C or preferably at -80°C. However repeated freeze-thawing should be avoided since the stability of IgM antibodies may be affected. If virus isolation has to be done, an aliquot of the serum sample should be frozen at -80°C, immediately after separation. Other specimens which may be collected depending on the clinical presentation and suspected aetiology of AES are nasopharyngeal/throat swabs, swabs from vesicles, rectal swabs/stool specimens, urine and brain biopsy.
Laboratory tests for Viral Pathogens (Table- 2)

The laboratory confirmation of a viral agent causing CNS infections is based on the following four principles:

(i) Isolation and identification of viruses from clinical samples,

(ii) Detection of virus specific components such as viral proteins or antigens,

(iii) Detection of virus specific antibodies in the patient’s serum and/or cerebrospinal fluid,

(iv) Detection of viral nucleic acid

A summary of laboratory tests currently in use for common pathogens causing AES in India is given in table 2.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Pathogen</th>
<th>Recommended specimen and test</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VIRAL PATHOGENS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>Flaviviridae</em> (JEV, West Nile, dengue)</td>
<td>IgM antibodies in serum/ CSF, virus isolation/ detection of viral RNA by PCR in serum/ CSF</td>
<td>Detection of IgM antibodies mainstay of diagnosis, PCR sensitive for detection in early phase of illness</td>
</tr>
<tr>
<td>2</td>
<td>Chikungunya</td>
<td>IgM antibodies in serum/ CSF, virus isolation/ detection of viral RNA by RT-PCR in serum/ CSF</td>
<td>Detection of IgM antibodies mainstay of diagnosis, PCR sensitive for detection in early phase of illness</td>
</tr>
<tr>
<td>3</td>
<td>Enteroviruses</td>
<td>Isolation of the virus/ Detection of viral RNA by RT-PCR from skin vesicle fluid, throat swabs and rectal swabs/ faecal specimen</td>
<td>Detection of viral RNA/ isolation from CSF is less sensitive, but more specific to prove AES aetiology</td>
</tr>
<tr>
<td>4</td>
<td>Chandipura</td>
<td>Detection of viral RNA by RT-PCR in serum/ CSF, IgM antibodies in serum by ELISA</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Rabies</td>
<td>Detection of viral RNA by RT-PCR in CSF, saliva, nuchal skin biopsy, neutralizing antibodies in CSF/ serum</td>
<td>FAT on brain tissue is the ‘Gold standard’ for diagnosis of rabies encephalitis</td>
</tr>
<tr>
<td>6</td>
<td><em>Paramyxoviridae</em> (mumps, measles, Nipah)</td>
<td>Detection of IgM/ IgG antibodies in serum/ CSF, viral RNA detection (RT-PCR) and virus isolation from serum/ CSF/ nasopharyngeal swabs/ throat swabs</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><em>Herpesviridae</em> (HSV 1 and 2, CMV, VZV, EBV, HHV 6)</td>
<td>PCR in CSF, virus isolation using cell culture, antigen detection</td>
<td>PCR has replaced culture as the ‘Gold standard.’</td>
</tr>
<tr>
<td><strong>BACTERIAL PATHOGENS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>S.pneumoniae, N.meningitidis, H.influenzae</td>
<td>CSF PCR, Gram’s stain, culture, Latex agglutination test (LAT) for antigen detection in CSF</td>
<td>Culture is ‘Gold standard’ but has low sensitivity. PCR can be performed on small sample volume with higher sensitivity</td>
</tr>
</tbody>
</table>
O. tsutsugamushi (Scrub typhus CNS involvement) | Detection of IgM antibodies in serum/ Weil- Felix test on serum/ PCR on blood or eschar material | Serological testing using IgM ELISA mainstay of diagnosis. Weil– Felix test is inexpensive and easy to perform, but lacks sensitivity and specificity.

Mycobacterium tuberculosis (meningitis) | CSF Ziehl Neelsen (ZN) stain for acid fast bacilli/ Culture/ PCR | CSF ZN stain and culture have low sensitivity, PCR offers better sensitivity

Plasmodium falciparum (cerebral malaria) | Demonstration of asexual form of P. falciparum in peripheral blood smear, rapid test for antigen detection and PCR | Absence of parasites may be due to sequestration of parasitized RBCs or earlier treatment with antimalarial drugs. At least 3 smears examined 6 hours apart should be negative to exclude cerebral malaria

Table 2. Laboratory tests currently in use for common pathogens causing AES in India

**Isolation and Identification of Viruses**

Isolation and identification of viruses from clinical samples is the most definitive mode of diagnosis. However, the sensitivity of isolation from CSF samples is low for most viral infections of the CNS, because small amounts of virus are shed into the CSF. Inadequate sample volume, and improper storage and transport of specimens also contribute substantially to poor virus isolation rates. Enteroviruses are the viruses most frequently isolated from the CSF while HSV isolation has been most successful from brain tissue. Insect cell lines especially Aedes albopictus C6/36 cell line has proved invaluable in the isolation of Japanese encephalitis virus from CSF samples.

Recently two modifications to conventional cell culture methods have resulted in the increased yield of viruses from clinical specimens-

(i) The shell vial method wherein cell lines are grown on cover slips in a tube and the specimen is directly centrifuged onto these cover slips increasing chances of contact between virus infected cells and the cell culture.

(ii) Use of genetically engineered cell lines that enhance the sensitivity as well as rapidity of virus isolation. For instance L20B is a genetically engineered mouse epithelial cell line in which receptors for poliovirus are hyper- expressed and is highly sensitive for the isolation of polioviruses.

**Detection of viral antigen**

Detection of viral antigen in the CSF has proved useful for the rapid diagnosis of JE especially in the patients who present early in the illness and are negative for IgM antibodies to JEV. Two monoclonal antibody based methods have been described: Immunofluorescence assay for detecting cell bound antigen in cytocentrifuged CSF specimens and a Reverse Passive Haemagglutination Assay (RPHA) for detection of soluble antigen in the CSF. Despite reduced sensitivity as compared to antibody detection methods, antigen detection methods are more useful in clinical practice since a positive result can immediately influence patient management, especially in Herpes simplex encephalitis.

The advantages of antigen- detection techniques are rapidity, and lack of requirement for viral viability in the specimen, allowing greater flexibility in the handling and transport of specimens. However, viruses such as enteroviruses and rhinoviruses that have extensive antigenic heterogeneity and cross-reacting antigens are not suitable for antigen-detection techniques.

**Detection of anti- viral antibodies**

The detection of anti- viral antibodies is the most widely used method for the diagnosis of viral infections of CNS. However, it is time consuming since most assays that are used such as ELISA and neutralization test are based on the detection of IgG antibodies. The clinical utility of the test is limited as many neurotropic viruses cause inapparent systemic infections at a young age, which results in majority of serum samples being positive for antiviral antibodies. Therefore interpretation of serological results on a single specimen of serum is not possible. The detection of virus specific IgM on the other hand can provide diagnosis rapidly on a single specimen of CSF as this class of antibody is present in body fluids only for 1- 3 months and therefore has diagnostic utility. Viruses for which detection of virus- specific IgM antibodies are available include JEV, dengue, chikungunya, West Nile, measles, rubella, and mumps.
Detection of viral nucleic acid

PCR is an important laboratory tool in the diagnosis of various infections of CNS especially because existing diagnostic modalities are inadequate or slow or provide only indirect evidences. Sufficient data has been accumulated to conclusively demonstrate that detection of several viral pathogens in the CSF by PCR based assays has now become a first line diagnostic tool, for most pathogens causing AES.\(^{59}\) Polymerase Chain Reaction (PCR) is an enzyme mediated in vitro nucleic acid amplification technique that has a high throughput and can yield rapid results, along with high sensitivity and specificity.

By inclusion of a step employing the enzyme reverse transcriptase (RT), PCR analysis can be adapted to detect viral RNA (RT-PCR); multiple pathogens can be identified in a single sample (multiplex PCR). Since only a small volume of the sample is required, PCR is especially valuable for clinical specimens like CSF that are usually available in limited quantity. An additional advantage of PCR is its ability to detect the viral nucleic acids even when the viability of virus is lost, most often due to storage or transport at inappropriate temperatures.

Conventional PCR methods are now increasingly replaced by the real-time PCR techniques for rapid detection of many viruses. The ‘closed’ system ensures reduction in amplicon contamination. Moreover, since amplification is detected by fluorescent signals, no post-PCR processing is required, ensuring rapid results. The advances in the development of fluorophores, nucleotide labelling chemistries, and the novel applications of oligoprobe hybridization have provided real-time PCR technology with a broad enough base to ensure its acceptance.\(^{50,60}\)

The AES Chip: Syndrome Evaluation System

A large number of viral pathogens can cause AES, which can be clinically indistinguishable. Furthermore, the amount of CSF available from each patient is inadequate for sequential testing of so many viral pathogens. To address this concern, recently, a simple and robust Syndrome Evaluation System (SES), for the simultaneous identification of all the viral pathogens (11 RNA and 5 DNA viruses), that can cause AES has been developed in the authors’ laboratory in collaboration with XCyton Diagnostics Ltd, a Bangalore based Biotech Company. The assay uses multiplex PCR for amplification of all the 16 targets followed by a simple hybridization procedure using signature genes for detection of amplicons of a specific organism, in a macro array chip format for visual detection of PCR products. The limit of detection (LOD) of the assay ranged between 0.1 to 50 viral particles per ml of CSF for the various viruses. The AES-SES was validated using a panel of well characterized CSF samples obtained from AES cases and controls. Amongst them, the AES SES identified the correct pathogens in 36/41 CSF samples (87.8% obtained from laboratory proven AES cases, and in 73/145 CSF samples (50.3%) obtained from suspected AES cases all of which were negative for all the viruses by any of the conventional diagnostic methods. The positive results in AES-SES were further confirmed by sequencing. None of the 50 control CSF samples obtained from patients undergoing spinal anaesthesia for minor surgical problems were positive in the AES SES thereby indicating high specificity (unpublished data). This platform would be of tremendous importance in the rapid identification of viral pathogens in AES outbreaks and can also rule out other neurological conditions that mimic viral encephalitis.

Laboratory Tests for non-viral AES pathogens (Table-2)

Bacterial pathogens (Meningitis)

Conventional techniques like Gram’s stain and culture of CSF samples lack sensitivity for detection of S. pneumoniae, N. meningitidis and H.influenzae, the most common bacterial pathogens causing meningitis. Latex agglutination test for direct detection of bacterial antigens in CSF offers rapid results with reasonable sensitivity. Since the amount of CSF is limited and improper transport/storage of CSF may lead to loss of viability of bacteria, PCR is a sensitive and specific tool for detection as well as serotyping of this bacterial pathogens.\(^{51}\)

Cerebral malaria

Diagnosis of cerebral malaria requires demonstration of asexual form of P. falciparum in peripheral blood smear, in thick and thin blood smear films stained by Giemsa stain. Absence of parasites in some patients may be due to sequestration of parasitized RBCs in cerebral circulation or earlier treatment with
antimalarial drugs. In such a situation, at least 3
smears examined 6 hours apart should be negative
before excluding cerebral malaria. The rapid
diagnostic test for antigen detection and PCR are
additional tests, which can increase the sensitivity of
diagnosis. 62

Scrub Typhus (CNS manifestations)

The eschar, a pathognomonic clinical feature, is
present in less than 50% of the patients. The presence
of lymphocytic pleocytosis and increased CSF
protein in scrub typhus meningitis may mimic
findings in tuberculous meningitis. The commonly
available Weil–Felix test is inexpensive and easy to
perform, but lacks both sensitivity and specificity.
Serological testing using IgM ELISA continues to be
the mainstay in the laboratory diagnosis. Microimmunofluorescence, although considered the
test of choice, is not commonly used due to high cost

Conclusion

AES contributes to significant mortality and
morbidity in India. Though JE has been considered
the major cause of AES in India for several decades,
several recent studies have increasingly reported
non-JEV aetiologies, reflecting the changing
landscape of AES in India. Laboratory confirmation
of aetiological agents of AES using improved
diagnostic techniques will help explore alternative
strategies for prevention and control of AES. In order
to achieve this and to avoid duplication of efforts, an
algorithmic approach for laboratory investigations
and reporting of AES cases in India is essential using
the limited resources available. Such an algorithm is
depicted in figure 1.

Figure 1. Suggested algorithm for laboratory investigations and reporting of AES cases in India

References

1. WHO – recommended standards for surveillance
of selected vaccine-preventable diseases. Geneva:
WHO; 2003. Available at: http://whqlibdoc.who.int/hq/2003/who_v&b_03.01.pdf
2. Cassady KA, Whitley RJ. Pathogenesis and
Pathophysiology of Viral Infections of the central
nervous system. In: Scheld WM, Whitley RJ,
50. Guidelines on establishment of Virology Laboratory in Developing Countries, World Health Organization 2008; Available at: http://apps.searo.who.int/PDS_DOCS/B4249.pdf
52. Ravi V, Vanajakshi S, Gowda A, Chandramuki A. Laboratory diagnosis of Japanese encephalitis using monoclonal antibodies and correlation of


