Neurology Asia 2022; 27(4) : 909 – 929

Dengue virus encephalitis: A systematic review and critique

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Abstract

Background & Objective: Neurological manifestations were reported in dengue fever. We hypothesize that the diagnosis of DEN virus (DENV) encephalitis based on current clinical diagnostic criteria has been made too liberally. We conducted a systematic review of the literature to clarify this, and to characterize DENV encephalitis better. *Methods:* We systematically review the literature using MEDLINE and PubMed databases papers published from 1st January 1960 to 31st March 2020. We grouped the cases into definite and probable DENV encephalitis. Definite DENV encephalitis required demonstration of DENV (live virus, viral antigens, RNA) in the central nervous system or CSF whereas probable DENV encephalitis required positive anti-DENV IgM in the CSF and evidence of virus or anti-DENV IgM in the serum. *Results:* Of the 230 publications obtained from literature search, 121 case reports and series appeared relevant to DENV encephalitis. After applying our inclusion criteria, only 42 cases (34.7%) were acceptable as DENV encephalitis: 30 (24.8%) definite and 12 (9.9%) probable cases. Our findings suggest that DENV encephalitis is more uncommon than previously thought. Part of the reason for non-inclusion may be because many studies relied solely on DENV serology for diagnosis. Cross-reactivities of anti-DENV with anti-JE virus immunoglobulins in serum or CSF occur and this limits their usefulness to distinguish DENV encephalitis from JE.
Conclusions: The majority of DENV encephalitis cases reported in the literature did not satisfy a more stringent inclusion criteria. Further investigations including autopsy studies are warranted to better characterize and to understand the pathology and pathogenesis of DENV encephalitis.

Keywords: Dengue, encephalitis, diagnostic criteria, neurology.

INTRODUCTION

Dengue virus (DENV) is a single-stranded RNA virus belonging to the *Flavivirus* genus in the *Flaviviridae* family. It is transmitted by the mosquitoes, *Aedes aegypti* and *Aedes albopictus*. The 4 distinct DENV serotypes are all capable of causing human infections. It is estimated that there are 390 million cases of dengue (DEN) infection per year[1] with more than 100 endemic countries, Latin America, Southeast Asia and Western Pacific regions being the most seriously affected.

In 2009, the World Health Organisation (WHO) reclassified DEN infection into 2 main categories: DEN fever with or without warning signs and severe DEN.[2] DEN infection usually manifests initially with high grade fever, headache, retro-orbital pain, myalgia, arthralgia, nausea, vomiting and abdominal pain. As the infection progresses through the critical phase, patients can either recover uneventfully or progress into potentially fatal severe DEN which involves severe organ involvement or plasma leakage or bleeding, leading to shock. Impaired consciousness, which could be due to encephalitis or encephalopathy, is one of the defining features of severe DEN with central nervous system (CNS) involvement.

Neurological manifestations has been reported in 0.5% to 48.8% of patients with DEN fever[3-5] but its exact incidence as part of severe DEN is unknown. This is partly because the neuropathogenesis of DENV encephalitis and indeed, its very definition is unclear. The diagnosis of DENV encephalitis in the literature is mainly based on clinical features with/without the demonstration of DENV in the cerebrospinal fluid (CSF) or brain tissue. Soares *et al*.[6] and Carod-Artal *et al*.[7] had earlier proposed definitions and criteria for DENV encephalitis (Table 1).

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Date of Submission: 25 October 2022, Date of Acceptance: 26 October 2022

https://doi.org/10.54029/2022zzy

Table 1: DENV encephalitis definition criteria by Soares *et al*. and Carod-Artal *et al*.

Soares *et al*. 2011[8]	Carod-Artal *et al*. 2013[9]
1. Presence of fever 2. Acute signs of cerebral involvement such as altered consciousness or personality and/or seizures, and/or focal neurological signs 3. Reactive IgM DEN antibody, NS1 antigen or positive DEN PCR on serum and/or CSF 4. Exclusion of other causes of viral encephalitis and encephalopathy	Dengue diagnostic tests were highly suggestive of or confirming acute DENV infection as recommended by WHO[a] for clinical category of DEN encephalitis, and to fulfil all of the following: 1. CNS involvement[b] 2. Presence of DEN virus RNA, or NS1 antigen in CSF 3. CSF pleocytosis without other neuroinvasive pathogens

[a.] Highly suggestive DEN is defined as: IgM-positive in one serum sample; or IgG-positive in one serum sample with haemagglutination inhibition titre of 1280 or greater. Confirmed DEN is defined by one of the following: PCR-positive; virus culture-positive; IgM seroconversion in paired serum samples; IgM seroconversion in paired serum samples or four-times IgG titre increase in paired serum samples.

[b.] CNS involvement: At least one of the following: impaired conscious (for children < 6 years, Blantyre coma score ≤ 4; for those older than 5 years, GCS ≤14), neck stiffness, focal neurological signs or seizures.

Perhaps DENV encephalitis should best be defined as pathological demonstration of typical viral encephalitic changes in brain tissues such as perivascular cuffing and parenchymal infiltration by inflammatory cells, oedema and necrosis together with *in situ* demonstration of viral antigens and/or viral genome. Other flaviviral encephalitides caused by such Japanese encephalitis virus (JEV)[10,11] West Nile virus[12], St Louis encephalitis virus[13], tick-borne encephalitis virus[14], and Murray Valley encephalitis virus[15] have indeed been pathologically confirmed to be encephalitic and neurotropic. In our opinion and opinions from other authors[16], typical acute encephalitic changes and cellular localisation of viral antigens and/or viral RNA have not been convincingly demonstrated in DEN studies despite some autopsy cases reported by Chimelli *et al*. and Miagostovich *et al*.[17,18] Nonetheless, an interesting case of chronic DEN encephalitis in which virus has been demonstrated in infected CNS tissues in an immunocompromised patient has been recently reported.[19]

We believe the existing clinical criteria for DENV encephalitis diagnosis, including those by Soares *et al*.[6] and Carod-Artal *et al*.[7] (Table 1) although generally adequate, may not have been sufficiently or properly applied. Although both criteria require the exclusion of other neuroinvasive pathogens, many investigators may not have paid sufficient attention to this caveat. Since there is a huge overlap of geographical distributions of DENV and JEV, exclusion of JE is important as it is the most common viral encephalitis. Moreover, DENV-JEV immunoglobulin cross-reactivity is well-known, so relying on it without other confirmatory tests for DENV infection could potentially lead to a wrong diagnosis. Thus, we hypothesize there could be many cases of DENV encephalitis reported in the literature that may be misdiagnosed and confused with JE or may be DEN encephalopathy rather than true encephalitis. If this hypothesis is correct, clinical and laboratory data derived from these reports may not be reliable. We conducted a comprehensive systematic review of the literature to clarify these issues.

METHODS

Search strategy

We conducted a systematic literature review using databases including MEDLINE and PubMed, Scopus from 1960 to 1st March 2020. We used the terms of ("dengue") AND ("neurolog*" OR "encephal" OR "neurolog manifestations") in our search strategy.

Inclusion and exclusion criteria

We considered original studies published in peer-reviewed journals in English. We included all case reports and case series (with and without autopsy findings) were reviewed for their clinical presentations, including CSF, magnetic resonance imaging (MRI) and electroencephalograph (EEG) findings. Evidence of DEN infection based on available laboratory diagnostic tests was carefully evaluated. We excluded animal studies, cases in case reports or case series with incomplete confirmatory diagnostic tests for DEN encephalitis or encephalopathy, other DEN-related neurological disorders (myositis, acute demyelinating encephalomyelitis, Guillain

Barre' Syndrome, transverse myelitis, ocular complications, peripheral neuropathy).

Laboratory diagnostic tests were grouped into two categories: tests that demonstrated evidence of DENV in the CNS (CSF and/or brain tissue) and tests that showed evidence of recent systemic DENV infection. Evidence of DENV in the CNS was defined as presence of one or more of the following: NS1 antigens, viral RNA (detected by polymerase chain reaction (PCR)), viral antigens (detected in tissues by immunohistochemistry) and positive viral isolation. Recent evidence of systemic DENV infection was defined as presence in the serum of one or more of the following: NS1 antigens, viral RNA or positive viral isolation. DEN IgM alone either in the CSF or serum without excluding the possibility of cross-reacting JEV IgM is not accepted as evidence of DENV infection. Using our inclusion criteria, acceptable cases of DENV encephalitis were grouped into either definite or probable DENV encephalitis (Table 2). Although CSF pleocytosis it is a very good indication of an inflammatory process in meninges, brain parenchyma or both, its absence does not exclude the diagnosis of encephalitis. [20] Therefore, CSF pleocytosis was not required in our inclusion criteria.

Operational definition of DENV encephalitis and encephalopathy

Definite DENV encephalitis

We defined definite DENV encephalitis as cases with the clinical evidence of encephalitis and demonstration of virus particle in CSF or brain. (Table 3) However, in severe DEN with plasma leakage, where leakage of viral particles into CSF is possible, CSF pleocytosis and higher CSF DENV IgM titer (compared to serum) needed to be fulfilled for definite DENV encephalitis.

Probable DENV encephalitis

We defined probable DENV encephalitis as cases with the clinical evidence of encephalitis and demonstration of virus particle demonstration of anti-DENV IgM in the CSF and detectable serum NS1 antigen or DENV PCR or viruses by viral culture. (Table 3)

Dengue encephalopathy

We defined dengue encephalopathy as cases with altered sensorium, clinical and serological evidence of DEN infection without CSF serological evidence and viral particles in CSF or brain tissues. (Table 2)

Study selection

The PRISMA guidelines for conducting systematic reviews were followed. All potentially eligible studies were reviewed by two independent reviewers (SL, KT, CT) using the inclusion and exclusion criteria. We first screened the all titles and abstracts from the search followed by screening and selection of the full-texted articles. (Figure 1)

Table 2: Inclusion criteria for definite and probable dengue virus (DENV) encephalitis

Definite DENV encephalitis (all items to be satisfied)
1. Encephalitic symptoms and signs¥
2. Detection of DENV in CSF and/or brain tissue by one or more of the following:
- NS1antigen
- RNA by PCR
- virus isolation by culture
- viral antigens by immunohistochemistry
Probable DENV encephalitis (all items to be satisfied)
1. Encephalitic symptoms and signs
2. Evidence of anti-DENV IgMϒ in the CSF AND presence of one or more of the following in the serum:
- NS-1 antigen
- RNA by PCR
- virus isolation by culture
- anti-DENV IgM

¥, Altered sensorium or change in behaviour or mental status; ϒ, In endemic areas for Japanese encephalitis, concurrent CSF/serum anti-JEV IgM should be tested and titers compared with CSF/serum anti-DENV IgM. The higher IgM titer obtained should be considered as the infecting virus; CSF, cerebrospinal fluid; NS1 antigen, nonstructural-1 antigen; RNA, ribonucleic acid; PCR, polymerase chain reaction.

Table 3: Definite and probable dengue (DENV) encephalitis based on our criteria

No	Author, Year of Publication (Country)	Included number of cases using our criteria	Clinical features	Evidence of DENV in CNS			DENV lab tests					JEV IgM	Remarks
				CSF PCR	CSF viral isolation	Brain PCR	CSF NS1	Serum NS1	Serum PCR	Serum IgM	CSF IgM		
Definite DENV encephalitis													
1	Kyaw *et al.*, 2019 (Myanmar)[45]	1	Fever Altered sensorium Seizures	na	+ (n=1)	na	na	na	na	na	na	- (n=1) (CSF)	One of 123 cases of acute encephalitic syndrome included as DENV encephalitis.
2	Vasanthapuram *et al.*, 2019 (India)[46]	6	Fever Altered sensorium Seizures	+ (n=6)	na	na	na	- (n=3)	- (n=3)	na	na	- (n=6) CSF	Six of 359 cases of acute encephalitic syndrome included as DENV encephalitis. All 6 cases had negative CSF JEV IgM.
3	Bastos *et al.*, 2019 (Brazil)[26]	4	Fever Altered sensorium Headache	+ (n=4)	na	na	na	na	na	na	- (n=4) Single¥	na	Four of 700 cases with viral infection of the central nervous system and neurological signs included as DENV encephalitis.
4	Jois *et al.*, 2018 (India)[21]	3	Fever Altered sensorium Unstable gait Seizures Vomiting Giddiness	na	na	na	+ (n=3)	+ (n=3)	na	na	na	na	All 3 cases included as DENV encephalitis.
5	Vieira *et al.*, 2018 (Brazil)[52]	2	Encephalitis (not otherwise specified)	+ (n=2)	na	na	na	na	+ (n=1)	+ (n=1)	- (n=2)	na	Two of 4 cases with encephalitis included as DENV encephalitis.

No	Author, Year of Publication (Country)	Included number of cases using our criteria	Clinical features	Evidence of DENV in CNS				DENV lab tests				JEV IgM	Remarks
				CSF PCR	CSF viral isolation	Brain PCR	CSF NS1	Serum NS1	Serum PCR	Serum IgM	CSF IgM		
6	Tassara *et al.*, 2017 (Brazil)[38]	1	Altered sensorium	na	na	+ (n=1)	na	na	na	+ (n=1) Paired[1]	na	na	One of 498 cases of neurological dengue included as DENV encephalitis.
7	Horwood *et al.*, 2017 (Cambodia)[27]	13	Fever Altered sensorium Seizures	+ (n= 13)	na	na	na	na	+ (n=10)	na	na	na	Thirteen of 1160 cases with acute meningoencephalitis included as DENV encephalitis.
8	Acevedo *et al.*, 2017 (Ecuador)[28]	1	Altered sensorium Lethargy	+ (n=a1)	na	na	na	na	na	na	na	na	One of 3 encephalitis cases included as DENV encephalitis CSF was also positive for Chikungunya virus and Zika virus PCR.
9	Fong *et al*, 2016 (Malaysia)[51]	1	Fever Altered sensorium Bilateral opthal-moplegia	+ (n=1)	na	na	na	+ (n=1)	+ (n=1)	+ (n=1) Single	na	na	Single case report.
10	Oliveira *et al*, 2016 (Brazil)[40]	1	Fever Altered sensorium Seizure	+ (n=1)	na	na	na	na	na	na	na	na	Single case report.
11	Mathew *et al*, 2014 (India)[50]	1	Fever Altered sensorium Seizure	+ (n=1)	na	na	na	+ (n=1)	na	+ (n=1) Single	na	na	Single case report.

No	Author, Year of Publication (Country)	Included number of cases using our criteria	Clinical features	Evidence of DENV in CNS				DENV lab tests				JEV IgM	Remarks
				CSF PCR	CSF viral isolation	Brain PCR	CSF NS1	Serum NS1	Serum PCR	Serum IgM	CSF IgM		
12	Singh *et al.*, 2014 (India)[57]	4	Fever Altered sensorium Focal neurological deficit Seizure Headache	+ (n=4)	na	na	na	na	na	+ (n=4) Single	+ (n= 4)	+ (n=4) Serum and CSF	Four of 76 cases of suspected CNS infection and thrombocytopenia included as DENV encephalitis. All 4 cases had positive CSF and serum JEV IgM but no mention of titres compared to DENV titres Two cases also had positive CSF JEV PCR.
13	Hapuarachchi *et al.*, 2013 (Singapore)[39]	1	Fever Altered sensorium Seizure	+ (n=1)	+ (n=1)	na	na	+ (n=1)	na	-	-	- (n=1) Serum	Single case report. Serum JEV IgM was negative
14	Araujo *et al.*, 2012 (Brazil) [5]	25	Fever Altered sensorium Seizure Headache	+ (n=7)	+ (n=1)	na	+ (n=22)	na	+ (n=2)	- (n=13)	+ (n=3)	na	Twenty five of 84 cases of DENV infection with neurological manifestations included as DENV encephalitis.
15	Soares *et al.*, 2011 (Brazil)[8]	1	Fever Altered sensorium Headache	+ (n=1)	na	na	na	na	+ (n=1)	+ (n=1) Single	+ (n=1) Single	na	One of 17 cases of encephalitis included as DENV encephalitis.

No	Author, Year of Publication (Country)	Included number of cases using our criteria	Clinical features	Evidence of DENV in CNS				DENV lab tests				JEV IgM	Remarks
				CSF PCR	CSF viral isolation	Brain PCR	CSF NS1	Serum NS1	Serum PCR	Serum IgM	CSF IgM		
16	Tan *et al.*, 2010 (Vietnam)[47]	9	Fever Altered sensorium Limb weakness Seizure Neck stiffness	+ (n=4)	+ (n=6)	na	na	na	na	na	na	na	Nine of 194 children with encephalitis and presumed viral aetiology, included as DENV encephalitis.
17	Kumar *et al.*, 2008 (India)[37]	21	Fever Altered sensorium Cranial nerve palsies Seizure Hypertonia Meningeal signs Headache	+ (n=21)	na	na	na	na	+ (n=9)	+ (n= 21)	na	- (n=21) Serum	Twenty one of 39 cases with DENV infection included as DENV encephalitis. All 21 cases had negative serum JEV IgM.
18	Domingues *et al.*, 2008 (Brazil)[72]	7	Fever Altered sensorium Abnormal coordination Mild hemiparesis Neck stiffness Syncope	+ (n=7)	na	na	na	na	na	+ (n=7)	na	na	Seven of 18 cases of DENV infection with neurological manifestations included as DENV encephalitis.
19	Tahir *et al.*, 2006 (India)[41]	1	Fever Altered sensorium Headache	+ (n=1)	na	na	na	na	na	+ Paired	- (n=1)	na	Single case report.

No	Author, Year of Publication (Country)	Included number of cases using our criteria	Clinical features	Evidence of DENV in CNS				DENV lab tests				JEV IgM	Remarks
				CSF PCR	CSF viral isolation	Brain PCR	CSF NS1	Serum NS1	Serum PCR	Serum IgM	CSF IgM		
20	Nogueira *et al.*, 2005 (Brazil)[23]	1	Fever Headache	+ (n=1)	na	+ (n=3)	na	na	+ (n=15)	+ (n=1) Single	na	na	One of 40 cases of fatal DEN cases included as DENV encephalitis
21	Nogueira *et al.*, 2002 (Brazil)[22]	1	Fever	-	+[β] (n=1)	na	na	na	na	- (n=1)	na	na	Single case report of sudden death; no CNS symptoms mentioned. This case is included as DENV encephalitis based on viral culture derivefrom a combined brain, liver and lymph node homogenate.
22	Srey *et al.*, 2002 (Cambodia)[55]	3	Fever Altered sensorium Motor weakness/ paralysis Hyperreflexia Tremors Choreiform movements Dysphasia, Dysphonia Seizures	+ (n=3)	na	na	na	na	+ (n= 3)	+ (n= 3) Paired	+ (n=1) Single, (n=1) Paired	- (n=3) CSF	Three of 99 cases with fever or history of fever and clinical encephalitic syndrome included as DENV encephalitis. All 3 cases had negative CSF JEV IgM.
23	Cam *et al*, 2001 (Vietnam)[3]	1	Fever Altered sensorium Seizures Hemiplegia	+ (n=1)	na	na	na	na	na	na	na	na	One of 27 cases of dengue hemorrhagic fever with severe CNS symptoms included as DENV encephalitis.

No	Author, Year of Publication (Country)	Included number of cases using our criteria	Clinical features	Evidence of DENV in CNS				DENV lab tests				JEV IgM	Remarks
				CSF PCR	CSF viral isolation	Brain PCR	CSF NS1	Serum NS1	Serum PCR	Serum IgM	CSF IgM		
24	Kankirawatna *et al.*, 2000 (Thailand)[53]	1	Fever Altered sensorium Facial nerve palsy	+ (n=1)	na	na	na	na	na	na	na	na	One of 8 cases of DENV infection with encephalitic-like illness included as DENV encephalitis
25	Solomon *et al.*, 2000 (Vietnam)[60]	4	Fever Altered sensorium Seizures Neck stiffness Hyperreflexia Frontal release signs Headache	+ (n=3)	+ (n=2)	na	na	na	na	+ (n=3) Paired	+ (n=1)	na	Four of 21 cases of DENV infection and neurological manifestations included as DENV encephalitis.
26	Hommel *et al.*, 1998 (France)[48]	1	Fever Altered sensorium	+ (n=1)	+ (n=1)	na	na	na	+ (n=1)	- (n=1)	- (n=1)	na	Single case report.
27	Janssen *et al*, 1998 (Netherland) [25]	1	Fever Altered sensorium Headache	+ β (n=1)	+ (n=1)	- (n=1)	Na	Na	na	+ (n=1)	na	na	Single case report.
28	Ramos *et al.*, 1998 (Mexico)[24]	1	Fever Altered sensorium Seizure	+ β (n=1)	na	na	na	na	na	na	na	na	Single case report.
29	Lum *et al.*, 1996 (Malaysia)[42]	5	Fever Altered sensorium	+ (n=2)	+ (n=4)	na	na	na	+ (n=2)	+ (n=4) Single	- (n=5)	na	Five of 6 cases of DENV infection with neurological manifestations included as DENV encephalitis.

No	Author, Year of Publication (Country)	Included number of cases using our criteria	Clinical features	Evidence of DENV in CNS				DENV lab tests				JEV IgM	Remarks
				CSF PCR	CSF viral isolation	Brain PCR	CSF NS1	Serum NS1	Serum PCR	Serum IgM	CSF IgM		
30	Miagostovich *et al.*, 1997 (Brazil)[17]	3	Fever Altered sensorium Neck stiffness Hemiparesis Headache	na	+ [β] (n=3)	na	na	na	na	+ (n=3)	na	na	Miagostovich et al.'s paper was based on Chimelli et al. (1990)'s cases. Three of the 5 cases were found to have DENV antigens on immunohistochemistry and included as definite DENV encephalitis.
Probable DENV encephalitis													
1	Chatur *et al.*, 2019 (India)[29]	2	Fever Altered sensorium Seizure Headache	na	na	na	na	+ (n=1)	na	+ (n=2) Single	+ (n=2) Single	- (n=2) CSF	All 2 cases included as DENV encephalitis. Both cases had negative CSF JEV IgM.
2	Weerasinghe *et al*, 2019 (India)[44]	1	Fever Altered sensorium Seizure	na	na	na	na	+ (n=1)	na	+ (n=1)	- (n=1)[θ]	- (n=1) CSF	Single case report. CSF JEV IgM negative.
3	Bhoi *et al.*, 2014 (India)[31]	21	Fever Altered sensorium Seizure	na	na	na	na	+ (n=3)	na	+ (n=21) Single	+ (n=4) Single	Study excluded cases of JE	No available information on the tests performed to exclude JE.
4	Ranasinghe *et al.*, 2013 (Sri Lanka)[43]	1	Fever Altered sensorium Seizure	na	na	na	na	na	na	+ (n=1) Single	+ (n=1)	- (n=1) (CSF)	Single case report. CSFJEV IgM was negative.

No	Author, Year of Publication (Country)	Included number of cases using our criteria	Clinical features	Evidence of DENV in CNS				DENV lab tests				JEV IgM	Remarks
				CSF PCR	CSF viral isolation	Brain PCR	CSF NS1	Serum NS1	Serum PCR	Serum IgM	CSF IgM		
5	Araujo *et al.*, 2012 (Brazil)[5]	15	Fever Altered sensorium Seizure Headache	- (n=15)	- (n=15)	- (n=15)	- (n=15)	na	- (n=14)	+ (n=7)	+ (n=15)	na	Fifteen of 84 cases of DEN infection with neurological manifestations included as DENV encephalitis. Since Brazil is not endemic for JE, tests to exclude the possibility of JE IgM is not required
6	Verma *et al.*, 2011 (India)[35]	1	Fever Altered sensorium Seizure Rigidity and spasticity	na	na	na	na	na	na	+ (n=1) Paired: Rising titer	+ (n=1) Single	- (n=1) (CSF)	Single case report. CSF JEV IgM was negative.
7	Agarwal *et al.*, 2009 (India)[32]	1	Fever Altered sensorium Seizure	na	na	na	na	na	na	+ (n=1) Paired: No titer	+ (n=1) (Single)	- (n=1) (CSF)	Single case report. CSF JEV IgM was negative.
8	Puccioni *et al.*, 2009 (Brazil)[54]	1	Fever Altered sensorium	na	na	na	na	na	na	+ (n=1) Paired: No titer	+ (n=1) (Single)	na	One of 10 cases of DEN infection with neurological manifestations included as DENV encephalitis. Since Brazil is not endemic for JE, tests to exclude the possibility of JE IgM is not required

No	Author, Year of Publication (Country)	Included number of cases using our criteria	Clinical features	Evidence of DENV in CNS				DENV lab tests				JEV IgM	Remarks
				CSF PCR	CSF viral isolation	Brain PCR	CSF NS1	Serum NS1	Serum PCR	Serum IgM	CSF IgM		
9	Misra *et al.*, 2006 (India)[49]	3	Fever Altered sensorium	na	na	na	na	na	na	+ (n=11)	+ (n=3) Single	Study excluded JE cases	Three of 11 cases of febrile encephalopathy included as DENV encephalitis No available information on the tests performed to exclude JE.
10	Soares *et al.*, 2006 (Brazil)[36]	2	Fever Altered sensorium	na	na	na	na	na	na	+ (n=2) Single	+ (n=2)	na	Two of 13 cases of acute DEN with neurological signs included as DENV encephalitis. Since Brazil is not endemic for JE, tests to exclude the possibility of JE IgM is not required
11	Witayathawornwong *et al.*, 2005 (Thailand)[33]	1	Fever Altered sensorium Seizure	na	na	na	na	na	na	+ (n=1) Single	+ (n=1) Single	+ (n=1) (CSF)	Single case report. CSF JEV IgM was positive at lower titers than DENV IgM
12	Cam *et al*, 2001 (Vietnam)[3]	7	Fever Altered sensorium Seizures Hemiplegia	na	na	na	na	na	na	+ (n=14)	+ (n=14) Single	+ (n=7)	Seven of 27 cases of DEN hemorrhagic fever with severe CNS symptoms included as DENV encephalitis. All 7 cases had positive CSF JEV IgM at lower titers than DENV IgM

CNS, central nervous system; JEV, Japanese encephalitis virus; CSF, cerebrospinal fluid; PCR, polymerase chain reaction; NS-1, nonstructural-1 antigen; na, not available ; +, presence of clinical sign or positive diagnostic test; -, absence of clinical sign or negative investigation or negative diagnostic test; ¥ Single: single test with no repeat in the convalescent phase; paired: repeated tests in convalescent phase; ϒ, With autopsy findings; β, Brain tissue sample; θ, Positive for CSF Dengue IgG at 2 months post infection.

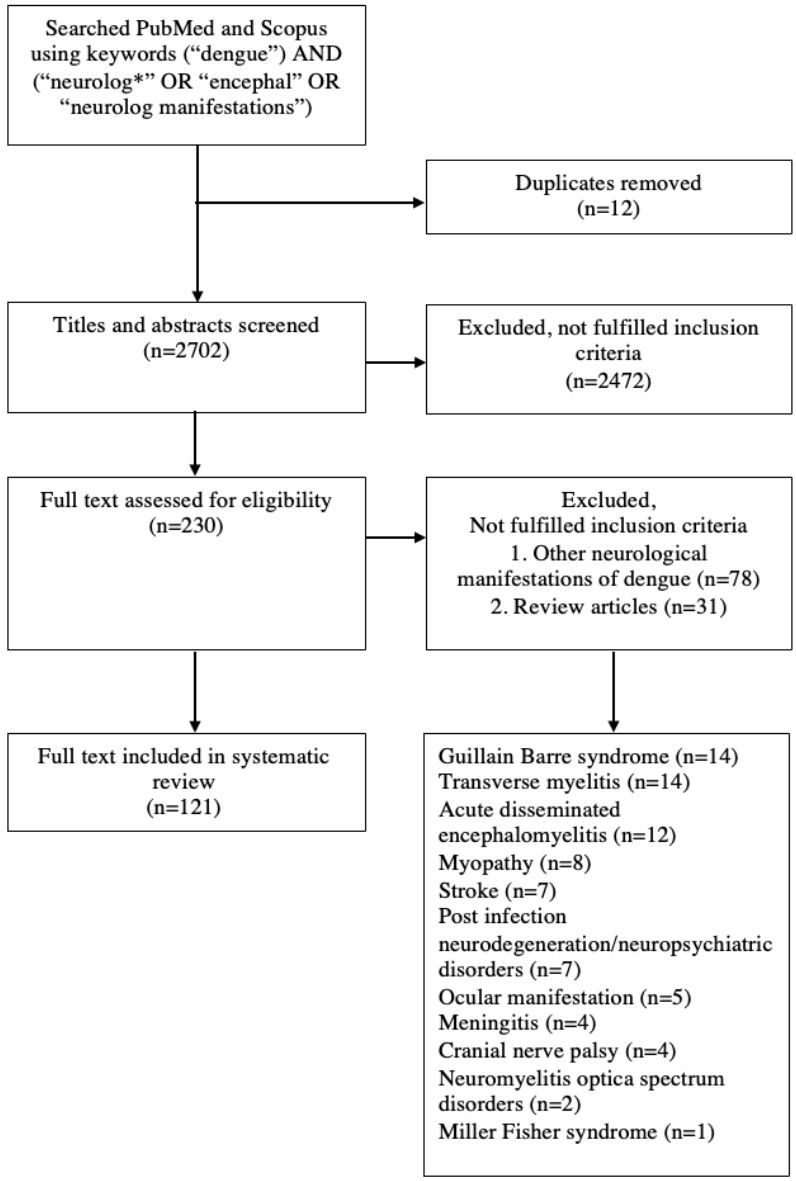

Figure 1. PRISMA flowchart for study selection.

RESULTS

Out of the 230 publications obtained from the databases using the keywords, a total of 121 articles (56 case series and 65 case reports) appeared relevant to DENV encephalitis or encephalopathy.

Upon application of our stringent inclusion criteria, 42 out of 121 publications (34.7%) were accepted as DENV encephalitis (definite or probable); eight of these publications had apparent autopsy findings.[5,17,18,21-25] We excluded 79 publications for further analysis. The reasons for exclusion were:

1. Only positive CSF DENV IgM available but no JEV IgM for comparison for cases reported in endemic regions (n=20).
2. No CSF examination for DENV or DENV IgM, only serum findings (n=38).
3. CSF available for examination, but negative for DENV or DENV IgM (n= 9).
4. CSF DENV or DENV IgM available for examination, but no serum findings of DENV or DENV IgM (n=3).
5. Concurrent positivity of CSF DENV IgM and JEV IgM but no information on the titers for comparison (n=2).
6. Autopsy studies with no evidence of viral antigens in the CNS available (n=7).

Based on these 42 publications, a final tally of 181 DENV encephalitis cases comprising 125 cases of definite and 56 cases of probable DENV encephalitis was recorded (Table 3). Not surprisingly, most of the accepted cases were from India and Sri Lanka, with fewer cases from Brazil, Vietnam, Malaysia and Singapore. Overall, the clinical features of these definite and probable DENV encephalitis cases were rather non-specific, and appear to be common to other viral encephalitides. All of them had fever. Altered sensorium consisted of reduced consciousness level, altered behaviour, stupor, drowsiness, or coma. Other neurological signs reported were seizures, pyramidal tract signs, cranial nerve palsies and neck stiffness (Table 3).

A total of 97 cases had CSF analysis showing CSF pleocytosis (white blood cell counts ≥ 5 cells/mm^3) in 58.8% (57/97) of cases and raised protein in 56.7% (55/97).[21,26-37] Twenty two (22.7%) cases had normal protein and glucose[18,21,31,38-44] and in another 20 cases (20.6%) the protein or glucose results were not recorded.[4,45-48]

There were 42 cases with MRI findings in which 76.1% (32/42) had abnormalities detected by T2, fluid attenuated inversion recovery (FLAIR) or DWI (diffusion weighed imaging) (Table 4). The most common finding was cerebral edema (23.8%).[31,3,35,41,49] More interestingly, bilateral thalami hyperintensities were seen in 14.3% of cases.[21,28,29,31,50] The "double doughnut" sign in which bilateral thalami T2 and FLAIR hyperintensity, and restricted diffusion with blooming artefact on gradient echo sequence in the centres of the thalami, were described in 2 cases.[29] One of the cases with double doughnut signs was associated with lesions in the cerebellum and another case had lesions in the pons and midbrain.[29] Among the total of 16 cases with T2/FLAIR hyperintensities 43.8% (7/16) had solitary lesions and 56.2% (9/16) had multiple lesions.[21,28,29,31,44,49-51] There were total of 9.6% (4/42) cases with bilateral basal ganglia and lenticular nuclei changes.[28,31] Splenium involvement was detected in 7.0% of cases[31,51], isolated brainstem, cortical or subcortical white matter changes (corona radiata and internal capsule) were each reported in 2.4% of cases, respectively[31,50] (Table 4). Among the 6 cases with brain CT scans, 4 cases had isolated cerebral edema as the most common finding.[21,25,38,49] One case showed bilateral thalami changes with hemorrhagic transformation[21] and in another case, CT brain was reported as normal.[52]

Table 4. Summary of brain MRI findings in definite and probable DENV encephalitis cases (n=42)

MRI findings in specific brain regions	Percentage with detectable lesions (number of cases)	References
T2-weighted or FLAIR or DWI hyperintensities:		
Location:		
Bilateral thalami¥	14·3 (6)[e]	21,28,29,31,49
Bilateral basal ganglia	4·8 (2)	31
Lenticular nucleus	4·8 (2)	28,31
Splenium	7·1 (3)	31,50
Cortical region (not otherwise specified)	4·8(2)	31
Frontal lobe area	2·4 (1)	31
Parietal lobe area	2·4 (1)	31
Frontoparietal and temporal lobes area	2·4 (1)	44
Bilateral cerebellum	4·8 (2)	29,49
Pons	2·4 (1)	49
Midbrain	2·4 (1)	49
Subcortical white matter ϒ	9·5 (4)	31
Other MRI findings		
Cerebral edema only	23·8 (10)	31
Meningeal enhancement	9·5 (4)	31,44,49
Ischaemic encephalopathy	2·4 (1)	39
Normal MRI	23·8 (10)	3,35,41,51,55

MRI, magnetic resonant imaging; FLAIR, fluid attenuated inversion recovery; DWI, diffusion weighted image; ¥, "Double doughnut" sign was described in two cases reported by Chatur et al; ϒ, Subcortical white matter includes internal capsule, centrum semi-ovale, corona radiata.

EEG findings were reported in 38 cases.[28,31,35,40,42-44,49,55] The most common finding was diffuse slowing (71.1%)[31,43,44,49,55] but in 15.7% of cases, the findings were normal.[42,49] Focal and generalised spikes and waves were reported in 5.3% cases.[28,31,43,44,49,55]

DISCUSSION

Accurate case definitions are important in diagnosis, epidemiological and clinical research. However, case definitions can be problematic in diseases where the underlying pathology, preferably based on autopsies, is unknown or poorly characterized[52]. From the pathological perspective, DENV encephalitis should demonstrate typical features of acute viral encephalitis that includes perivascular cuffing and parenchymal infiltration by inflammatory cells, oedema and necrosis, accompanied by the presence of DENV demonstrable by electron microscopy, immunohistochemistry, in situ hybridization or other methods. This agrees with the consensus statement by the International Encephalitis Consortium that in confirmed viral encephalitis, there should be clear evidence of viral invasion into the CNS, viral replication and inflammation of the brain parenchyma.[20] However, antemortem pathological examination, considered as the "gold standard" diagnostic test for encephalitis, is rarely done due to potential risk of an invasive procedure that a biopsy requires.[20] Unfortunately, despite some autopsy reports[5,17,18,22-25], we and others[16] believe that acute DENV encephalitis in immunocompetent patients still has not been well characterised unlike the other flaviviral encephalitidies.[10-15] However, a recent autopsy report of chronic DEN panencephalitis in an immunocompromised patient demonstrated DENV in inflamed neuroglial tissues[19] confirming that DENV is neurotropic.

In lieu of a pathological diagnosis based on biopsy or autopsy, strictly speaking, clinical diagnosis of definite DENV encephalitis should be based on the presence of virus in the CSF or CNS tissues. This can be demonstrated by virus isolation, PCR detection of viral genome or detection of viral proteins/antigens. This is important since other flaviviral encephalitides especially JE, may show similar clinical, radiological and laboratory features that could complicate diagnosis. Thus, our inclusion criteria for this review emphasizes the need to demonstrate evidence of DENV (live virus, viral RNA, antigens in CSF or brain tissues) for confirmed DENV encephalitis. For probable DENV encephalitis, in the absence of direct evidence of DENV in the CNS, CSF anti-DENV IgM was required for this diagnosis after excluding cross-reacting JEV IgM.

The cross-reactivities of anti-DENV with anti-JEV IgMs and IgGs in serum or CSF limit their usefulness to distinguish DEN from JE, unless both tests are done in tandem.[3,5,29,32,33,35,43,44,49,55,57,58] Unfortunately, most publications overlooked this crucial point, so unless evidence of DENV is demonstrated, a positive DENV IgM or IgG as a standalone result or in the context of seroconversion, cannot be accepted. It was found that the co-detection rate for anti-DENV and anti-JEV IgM in the CSF ranged from 9.0% to as high as 50.0% in patients with encephalitis.[3,57,59,60] This observation may be due to serological cross-reactivity, or even sequential or co-infection by DENV and JEV.

In practice, the virus with a higher IgM titre in the CSF is considered as the infecting virus.[27,59-61] Detection of virus is largely dependent on the time of specimen collection during the short viraemic phase, and not after IgM seroconversion has occurred. In severe DEN, viral entry into the CNS is thought to result from increased vascular permeability at the blood brain barrier leading to leakage of virus and IgM into the CSF and brain parenchyma. IgM leakage could probably explain why CSF titres are lower compared to serum titres.[62,63] In the In view of possible viral particles leakage into the CSF in cases of severe DEN, CSF pleocytosis and higher CSF DENV IgM (compared to serum IgM) should be used to reinforce the evidence of encephalitis in the diagnosis of definite DENV encephalitis.

Based on our stringent inclusion criteria, we found only 34.7% or 181 (125 definite and 56 probable) of previously reported DENV encephalitis cases were acceptable cases. This suggests that the diagnosis of DENV encephalitis had been made too liberally and based on poor evidence of DENV in the CNS. Most of the rejected cases did not demonstrate the presence of DENV in the CNS either due to lack of CSF examination or negative DENV PCR or DENV IgM in the CSF.[64-71] We also did not accept cases with apparent positive CSF DENV IgM as probable DENV encephalitis if no JEV PCR or CSF IgM in the to exclude especially DENV and JEV endemic regions to address the issue of cross-reactivity between DENV IgM and JE IgM.[71]

While we accept the dangers of performing invasive procedure such as lumbar puncture on

patients with severe DEN due to thrombocytopenia or hemodynamic instability. in cases with no CSF DENV examination, the diagnosis of DENV encephalitis should be made with caution as the neurological symptoms might be due to other causes of encephalopathy. In the literature we reviewed, the term "encephalitis" and "encephalopathy" were often used interchangeably, leading to some confusion over the definitions of these 2 clinically similar but pathologically distinctive conditions. "Encephalopathy" refers to a clinical state of altered mental status manifesting as confusion, disorientation, behavioural changes or other cognitive impairments, with or without inflammation of brain tissue.[20] Encephalopathy in DEN infection could be attributed to metabolic complications (e.g. hepatic failure, renal failure, hyponatremia, metabolic acidosis), cerebral hypoperfusion due to hypovolaemic shock, intracranial haemorrhage or disseminated intravascular coagulopathy. In cases with altered sensorium, only serological evidence of DEN infection without the evidence of viral particles in the brain/ CSF or serological evidence of DENV in CSF would best be categorised as dengue encephalopathy as there we no direct evidence of CNS invasion.

Altered sensorium was included in our inclusion criteria for DENV encephalitis based on the criteria proposed by Soares *et al*.[6], Carod-Artal *et al*.[7] and the consensus statement of the International Encephalitis Consortium. Altered sensorium implied an acute change in behaviour or mental status. In the cases we included as definite and probable DENV encephalitis, all except two early fatal cases (one with sudden death after discharge from hospital and one with otherwise unspecified neurological manifestations)[22,23] had altered sensorium in the form of drowsiness, reduced level of consciousness or change in behaviour. We included the two cases without the usual altered sensorium as both cases showed evidence of DENV in CNS.[22,23] Other neurological signs reported in DENV encephalitis included in this review were bilateral opthalmoplegia, abnormal coordination, choreiform movements and frontal release signs.[51,58,63,72] These appeared to be non-specific to DENV encephalitis.

As fever is a non-specific finding in viral encephalitis[20] , we did not specify fever in our inclusion criteria for DENV encephalitis. Fever was included in the definition criteria proposed by Soares *et al*.[6] but not in the criteria proposed by Carod-Artal *et al*.[7] Nevertheless, all the cases in Table 3 had fever, but we need to be reminded that immunosuppressed patients may not be able to mount a febrile response to infection.

CSF pleocytosis was required in the diagnostic criteria proposed by Carod-Artal *et al*.[9] However, CSF pleocytosis may also be a non-specific finding in viral encephalitis. While it indicates an inflammatory process in meninges and/or brain parenchyma, absence of pleocytosis does not exclude encephalitis.[20]

In fact, only 61.9% of the included cases (both confirmed and probable DENV encephalitis) had CSF findings that reported pleocytosis. However, in severe DEN it is possible that plasma leakage may facilitate virus or IgM leakage into the CSF, so under these circumstances, additional evidence of CSF pleocytosis might be helpful to strengthen the evidence of CNS inflammation.

Among the 8 publications that mentioned seizures, there was only a case of epilepsia partialis continua with EEG findings of right temporal focal spikes that showed good correlation.[35] Seizures in encephalitis may be focal or generalised[73], and the EEG findings were non-specific and not unique for DENV encephalitis. (Table 3) Similar EEGs were found in JEV encephalitis, including theta and delta slowing, alpha coma and some with epileptiform discharges.[74] In Murray Valley encephalitis, only diffuse slowing has been described.[72] All these findings may also occur in encephalopathy due to other causes.

Non-specific cerebral edema and normal findings in the brain MRI were reported in approximately equal frequencies and were apparently the most frequent findings in DENV encephalitis. T2 and FLAIR hyperintensities in bilateral thalami were one of the relatively more common abnormalities, being found in 14.3%. The double doughnut sign (bilateral thalami T2 and FLAIR hyperintensity with central blooming artefact) in the MRI brain as described in the 2 cases included as probable DENV encephalitis were interesting findings in DENV encephalitis.[29] The bilateral thalami changes with haemorrhage transformation as reported in the CT brain in one case may also be a possible double doughnut sign.[21] However, as this neuroimaging finding is not commonly reported, we were unsure its specificity in DENV encephalitis. Interestingly, bilateral thalamic lesions were also frequently seen in other flaviviral encephalitides, e.g. JE encephalitis[76-81], Murray valley virus encephalitis[79] and tick-borne encephalitis[80] and may suggest a common neuropathogenesis in flaviviral encephalitides. Early thalamic involvement in a footpad-infected JE mouse model had suggested

a role for sensory peripheral nervous system in neuroinvasion. Based on these findings it was postulated that following an infected mosquito bite, JEV travel up cutaneous sensory nerves to infect the thalami early and then spread to other parts of the human CNS.[81] Similarly, other flaviviruses including DENV, transmitted by mosquito or tick, may be able to use this route for neuroinvasion. Interestingly, it was suggested that hippocampal involvement seen in JE but apparently not in DENV encephalitis, might be a radiological clue to differentiate these 2 encephalitides.[77] Splenium involvement was described in some cases of definite DENV encephalitis but it is likely to be non-specific since reversible splenium lesions had been found in bacterial and viral CNS infections (including JE), metabolic and anti-epileptic drug induced encephalopathies.[82,83] Brainstem involvement seen in JE,[77] was described in one definite DENV encephalitis case.[19]

As alluded to earlier, we believe the pathological features of DENV encephalitis have so far not been well documented despite a few autopsy cases.[17,19] In a large series of autopsies, Bhoopat et al. described the presence of a few lymphocytes seen around vessels in the brain and reported DENV antigens in multiple brain regions and cells, including neurons in cerebral cortex, Purkinje and granular cells in the cerebellum, astrocytes, microglial, epithelial lining and endothelial cells in the choroid plexus. However, no neuronal necrosis was reported and illustrations not presented.[84] Chimelli *et al*. described 5 fatal DEN cases with neurological symptoms that had perivascular mononuclear cells (lymphocytes and macrophages) cuffing, non-specific edema, focal hemorrhage and perivenous demyelination but no evidence of cell necrosis. [18] They suggested the findings could represent acute disseminated encephalitis. The subsequent follow up study of these same cases by Miagostovich *et al*. was apparently able to find DENV antigen-positive cells located within Virchow Robin spaces in the white and grey matter with "cytopathic features" in adjacent neurons.[17] Unfortunately, the illustrations in this paper are unconvincing.

The subsequent few cases with autopsies also did not show histological evidence of encephalitis despite of detectable DENV or viral antigen in the brain tissues by PCR, immunohistochemistry staining or viral culture. One case reported by Janssen et al. found DENV 2 via PCR in brain tissue and immunoperoxidase stain for DENV IgM in the capillaries without signs of neuronal necrosis, viral inclusions or meningo-encephalitis changes.[25] Another case reported in the same year by Ramos et al. also had positive DENV 4 detected via PCR and DENV IgM via immunohistochemistry staining in the inferior olivary nucleus of medulla and granular layer of cerebellum. Again, there were no inflammatory perivascular cuffs or neural death with neurophagia and activated microglia seen to suggest encephalitis.[24] In the case reported by Noguiera et al. with DENV virus cultured from brain tissue with no mononuclear cells infiltration seen in the autopsy.[23] There were also other cases of DENV infection with neurological signs were reported as DENV encephalitis based on autopsy finding which were compatible with viral encephalitis but without further description of the microscopic findings. We did not include these cases in our results although the CSF had detectable DENV IgM, as there JE was not excluded from the cases and the evidence of encephalitis were unclear.[85,86]

We believe the only case with convincing evidence of DENV CNS infection to date was a case of chronic DEN panencephalitis in an immunocompromised patient who presented with dementia in which microglial nodules, predominant T cell infiltrates including CD8 cells were demonstrated in the CNS. Immunostaining showed extensive viral envelope proteins in cerebral vasculatures, neurons and some glial cells throughout the brain including, hippocampus, basal ganglia and cerebellum. Viral RNA was also detected in the same regions of the brain.[19]

Thus, unlike other acute flaviviral encephalitides such as JE, tick-borne encephalitis, West Nile encephalitis and Murray Valley virus encephalitis in which the viruses were found to be unequivocally neuronotropic (i.e. has a predilection for neurons), we believe CNS localization of viral antigens/RNA in acute DENV encephalitis in an immunocompetent patient has not been convincingly demonstrated. Further investigations are needed to confirm and to advance our knowledge of DENV encephalitis pathology and pathogenesis and to investigate if all flaviviral encephalitides share similar clinicopathological features. More post-mortem studies are needed in order to determine the neuropathogenesis and to provide more pathological evidence of DENV encephalitis in the human CNS.

In conclusion, in this review, we found only 34.7% of previously reported DENV encephalitis cases were definite or probable DENV encephalitis. We believe, when the diagnosis

is made it is critical to exclude confounding infections, especially JE. In this way, a better understanding of clinico-pathological features of DENV encephalitis, and estimation of disease burden and its impact on the population could also be done more accurately.

DISCLOSURE

Financial support: None

Conflict of interest: none

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