Chapter

The Global Distribution and Burden of Dengue and Japanese Encephalitis Co-Infection in Acute Encephalitis Syndrome

Shailendra K. Saxena, Swatantra Kumar, Vimal K. Maurya and Madan L.B. Bhatt

Abstract

Dengue is widespread throughout the tropics globally in more than hundred countries and coincides with various climatic factors for co-infection with other flaviviral infections of the central nervous system (CNS). Dengue and Japanese encephalitis virus co-infection are highly prevalent, with diagnosis dilemma including significant mortality and morbidity in Southeast Asia. Both dengue and Japanese encephalitis transmissions intensify during the rainy season, during which the vector population increases. CNS involvement during dengue and Japanese encephalitis co-infection-associated acute encephalitis syndrome (AES) is still poorly understood, and therefore, there is a desperate need to understand the etiology, therapeutics, clinical management, and prevention of these tropically neglected diseases. AES can be differentiated from other etiologies of encephalopathy through considering its essential features: sudden onset of fever, cerebrospinal fluid (CSF) comprising inflammatory cells, magnetic resonance imaging (MRI)-based confirmation, and presence of pathogen or pathogen-specific antibodies. Complementary and alternative medicine is progressively being used globally and can be effective for the overall management of this co-infection.

Keywords: dengue, Japanese encephalitis, co-infection, encephalitis, acute encephalitis syndrome (AES), differential diagnosis, treatment, management, prevention, complementary and alternative medicine

1. Introduction

Neglected tropical diseases (NTDs) are the diverse group of communicable diseases which exist in tropical and subtropical settings affecting more than one billion people worldwide [1]. Populations inhabiting places with poor sanitation are in close contact with infected vectors, and domestic animals are principally affected. Arthropod-borne or arboviruses such as dengue, Zika, and Chikungunya have been recently included in the list of NTDs by the World Health Organization [2].

Human infection of flavivirus may cause severe clinical manifestations and can be broadly subdivided into two groups as neurological diseases caused by Japanese encephalitis virus (JEV), West Nile virus, and Zika virus (ZIKV) and hemorrhagic and viscerotropic diseases caused by dengue virus (DENV), ZIKV, and yellow fever

virus (YFV) [3]. More than half of the global population is now at the risk of getting flavivirus infections where the majority of areas are endemic for more than one flaviviruses which results in the phenomenon of co-infection [4]. The worldwide incident of dengue has extensively grown in few decades [5]. Majority of the dengue cases are asymptomatic, and therefore, it is hard to anticipate the accurate burden of the disease. The rise in number of cases from 2.2 million in the year 2010 to 3.34 million cases in 2016 suggests the sharp increase in the disease burden. The 2016 year is characterized as the largest outbreak for dengue where 2.38 million cases were reported from the region of the Americas where 1.5 million cases were contributed by Brazil alone. Currently 3.9 billion in 128 countries people are at risk of DENV infection [6]. Unlike dengue, Japanese encephalitis (JE) is confined to Southeast Asia and Western Pacific regions. Approximately 68,000 clinical cases of JE are reported annually with 13,000 to 24,000 deaths. Currently more than 3 billion in 24 countries are at risk of JEV infection [7]. The epidemiology of dengue and JE has been depicted in **Figure 1**.

DENV and JEV belongs to the *Flaviviridae* family, which consists of more than 70 viruses, comprising of single-stranded positive-sense RNA genome protected by envelope protein [8]. Viruses from this family belong to the genus *Flavivirus*, which are transmitted by mosquitoes or ticks and are characterized as arthropod-borne infections. The transmission cycle of *Flavivirus* involves animals including human which are considered to be the dead-end hosts [9]. Hematophagous mosquitoes are the transmission vector for these diseases. *Aedes albopictus* and *Aedes aegypti* mosquitoes are known to transmit the dengue virus, whereas *Culex tritaeniorhynchus* is predominantly involve in the transmission of JEV [10]. These viruses have been shown to be transmitted via transplacental route as well [11].

Pathogen-associated acute encephalitis syndrome (PA-AES) may result from diverse pathogenic infections including DENV and JEV. PA-AES shows a wide range of symptoms including headache, vomiting and severe illness, reduced consciousness, altered sensorium, convulsions, and tremors [12]. Flaviviruses share substantial sequence similarities to stimulate sero-cross-reactivity which results in the antibody-dependent enhancement (ADE) of infection with other flaviviruses [13].

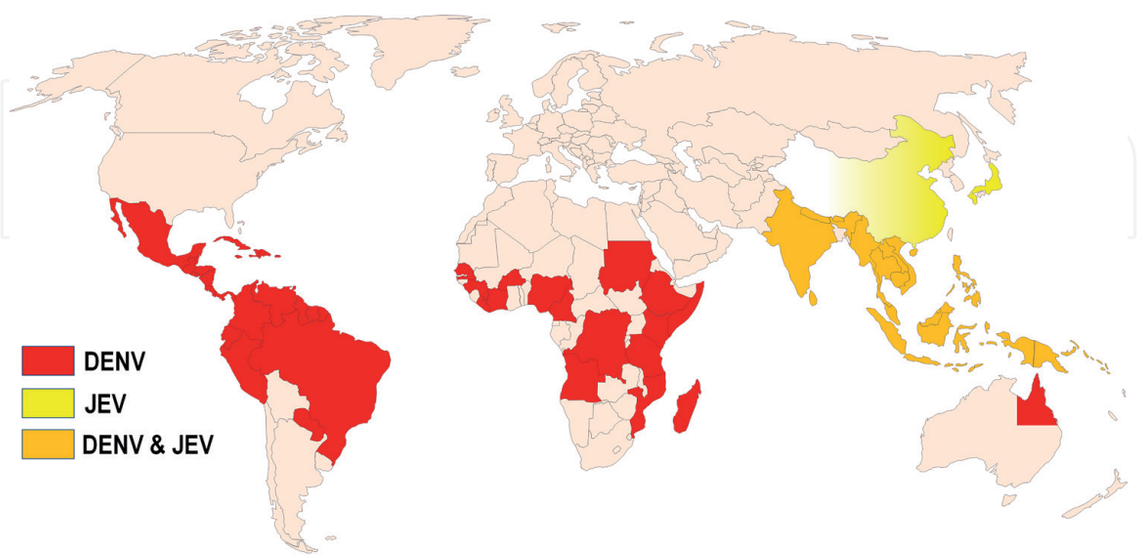


Figure 1.
Dengue and Japanese encephalitis epidemiology. The worldwide epidemiology of dengue and Japanese encephalitis has been depicted in this map. More than 100 countries are endemic for dengue where America, Western Pacific regions, and Southeast Asia are mostly affected. The Japanese encephalitis is confined to mostly in Southeast Asia and Western Pacific regions which include approximately 24 countries. The dengue-affected regions are highlighted in red color, JE-affected regions are highlighted in yellow color, and regions where both viruses are circulating have been highlighted in orange color.

Outbreaks of DENV co-infection are predominantly associated with the JEV endemic area or areas of JE immunization [14]. Although DENV-induced encephalitis is rare, the co-infection may increase the severity of encephalitis. DENV infection causes dengue hemorrhagic fever (DHF), whereas JEV infection may result in neurological complications [15]. Intermittently, DENV has been reported to cause encephalitis, and JEV infection may cause extraneural hemorrhage [16, 17]. However, this may not be the case in co-infection with both the flaviviruses. Simultaneous detection of both the viruses is not unusual; nevertheless we need to be attentive in the diagnosis of the etiology of PA-AES.

2. Molecular mechanism of DENV and JEV co-infection

Co-infection with both viruses may occur in the single-cell types or different cells in the infected individuals. Simultaneous infection of target cells or different cell types by DENV and JEV defines the phenomenon of co-infection [18]. Both the viruses share common cell surface receptors expressed on target cell types such as DC-SIGN, mannose receptors, and CLEC5A [19]. On the other hand, virus can be internalized in the presence of non-neutralizing antibodies via Fcγ receptor-mediated endocytosis [20]. Clathrin-mediated endocytosis has been shown to be involved in the internalization of flaviviruses [21]. After internalization, the virus from the endocytic vesicles is delivered to the early endosomes [22]. Acidification of the endosomal

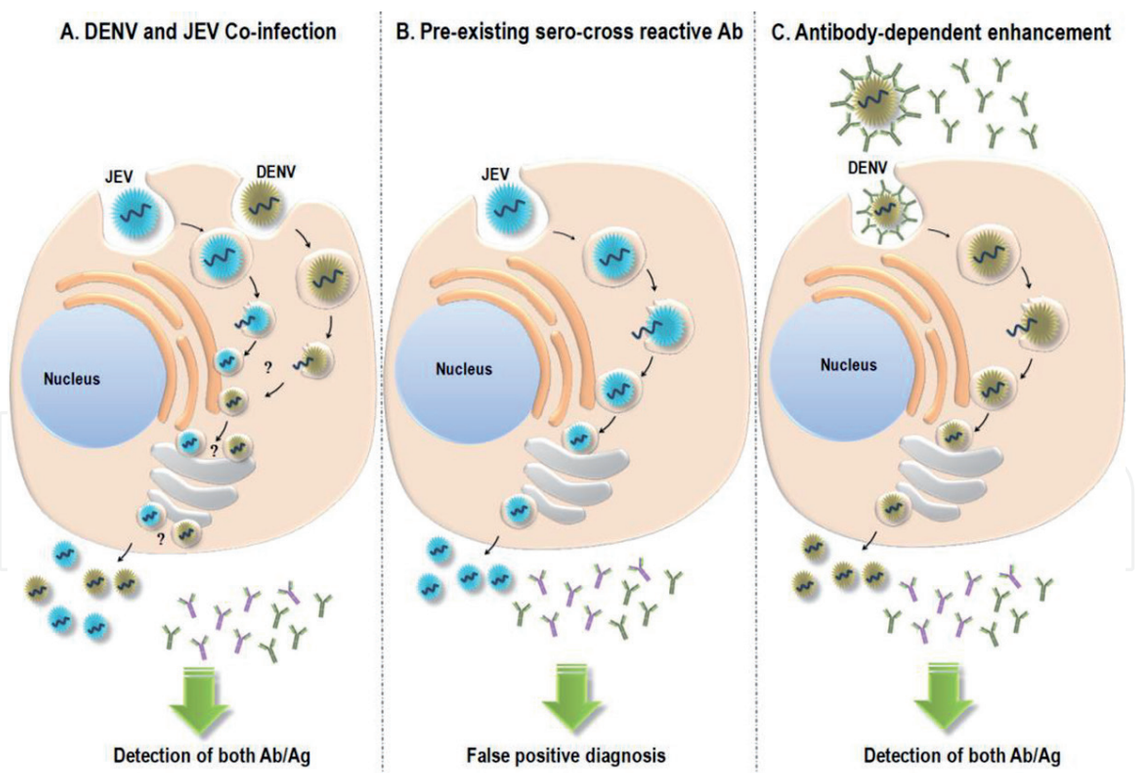


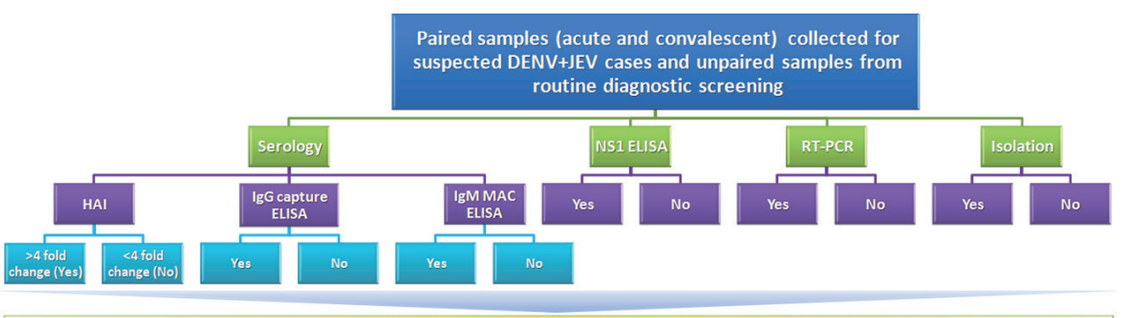
Figure 2.
DENV and JEV co-infection. The mechanism of co-infection has not been completely understood. Co-infection with DENV and JEV may occur in the single-cell types or different cells in the infected individuals. Co-infection results in the generation of antibodies against both viruses. However, detection of nucleic acids and antigens may confirm the phenomenon of co-infection (A). Upon infection, JEV is internalized by receptor-mediated endocytosis. The decrease in pH causes the fusion of viral and endosomal membrane which results in the release of viral RNA into the cytosol. The released viral genome is then translated prior to the commencement of replication. Virus maturation occurs at the Golgi complex and mature virus is released via the egress process. JEV infection in the hyperendemic area may result in false-positive diagnosis due to presence of pre-existing sero-cross-reactive antibody (B). Pre-existing sero-cross-reactive antibodies may bind to the viral particles upon infection which results in antibody-dependent enhancement of infection (C).

compartments causes trimerization of the envelope protein that results in the fusion of endosomes to the viral membrane and release of nucleocapsid in the cytosol [23].

The released viral genome is then translated prior to the commencement of replication [24]. The backbone of flaviviral genome is invariable which are of ~10 kb that codes for three structural capsid (C), premembrane (prM), and envelope (E) and seven nonstructural proteins NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5. The viral genome encodes for 3400 amino acid long polyprotein which is arranged in the lumen endoplasmic reticulum where the replication takes place by the RNA-dependent RNA polymerase [25]. Replicated copies of the genome interact with viral proteins to form nucleocapsid. Immature virions enter into the secretary pathway where furin-mediated cleavage of prM results in the maturation of virus [26]. Mature virus is then released from the infected cells via the egress process [27]. The conventional mechanism of pathogenesis and viral replication may not be followed in case of co-infection and may involve distinct process. In order to effectively control and treat the co-infection of DENV and JEV, we need to first understand the immunopathogenesis of dual infection in various cell and animal models. The probable phenomenon of DENV and JEV co-infection has been demonstrated in **Figure 2**.

3. Diagnostic schemes for dengue and Japanese encephalitis co-infection

JEV is the most documented causative agent of acute encephalitis syndrome (AES) [28]. DENV has also been considered among the top three etiological agents causing



INTERPRETATION														
DENV					JEV					Co-infection (DENV + JEV)				
Virus Isolation	RT-PCR	NS1	Serology	Diagnosis	Virus Isolation	RT-PCR	NS1	Serology	Diagnosis	Virus Isolation	RT-PCR	NS1	Serology	Diagnosis
Y	?	?	?	Confirmed DENV	Y	?	?	?	Confirmed JEV	Y	?	?	?	Confirmed DENV+JEV
Y	Y	?	?	Confirmed DENV	Y	Y	?	?	Confirmed JEV	Y	Y	?	?	Confirmed DENV+JEV
Y	?	Y	?	Confirmed DENV	Y	?	Y	?	Confirmed JEV	Y	?	Y	?	Confirmed DENV+JEV
Y	?	?	Y	Confirmed DENV	Y	?	?	Y	Confirmed JEV	Y	?	?	Y	Confirmed DENV+JEV
?	Y	Y	?	Confirmed DENV	?	Y	Y	?	Confirmed JEV	?	Y	Y	?	Confirmed DENV+JEV
?	Y	?	Y	Confirmed DENV	?	Y	?	Y	Confirmed JEV	?	Y	?	Y	Confirmed DENV+JEV
?	?	Y	Y	Confirmed DENV	?	?	Y	Y	Confirmed JEV	?	?	Y	Y	Confirmed DENV+JEV
N	Y	N	N	Not DENV	N	Y	N	N	Not JEV	N	Y	N	N	Not DENV+JEV
N	N	Y	N	Not DENV	N	N	Y	N	Not JEV	N	N	Y	N	Not DENV+JEV
N	N	N	Y	Not DENV	N	N	N	Y	Not JEV	N	N	N	Y	Not DENV+JEV
N	N	N	N	Not DENV	N	N	N	N	Not JEV	N	N	N	N	Not DENV+JEV

Figure 3.
Laboratory diagnostic algorithms during DENV and JEV co-infection. Suspected cases of DENV and JEV co-infection can be decided by the above laboratory diagnostic algorithms of co-infection. Detection of IgG antibodies against both viruses may be considered as the previous exposure of the viruses, whereas detection of IgM or NS1 antigen suggests the recent or current infectious conditions. However, this should be further validated based on the nucleic acid-based tests such as RT-PCR and isolation of virus which may define the co-infectious condition in addition to the clinical correlation. "Y" indicates positive, "N" indicates negative, and "?" indicates inconclusive test report.

AES [29]. Broad spectrum of clinical features in the endemic areas of both viruses may dilute the encephalitis-like clinical presentation. Therefore, laboratory based diagnosis and discrimination of pathogenesis is crucial to understand. Detection of IgG antibodies against both viruses may be considered as the previous exposure of the viruses, whereas detection of IgM suggests the recent or current infectious conditions. However, this should be further validated based on the nucleic acid-based tests where detection of DENV and JEV nucleic acids defines the co-infectious condition.

Nucleic acid-based detection by RT-PCR for other probable arboviral infections such as Zika and Chikungunya can also be included which may overlap with the clinical manifestations of dengue or JE [30]. Although the clinical manifestations presented in the individual cases of DENV and JEV infections are distinct, the co-infection might be a complex situation to diagnose. Co-detection of serum antibodies against both viruses may not be the evidence of co-infection. Surveillance programs conducted in the hyperendemic areas of flaviviruses have reported at least 9% of co-infections cases [31]. Moreover, the MRI findings of DENV-infected patients may show the characteristic features of encephalitis as in case of JEV where the basal ganglia, thalamus, and midbrain are predominantly affected [32]. Considering the sero-cross-reactivity among flaviviruses, the dependence of serum-based diagnosis may give false-positive results. However, simultaneous detection of nucleic acid or viral-specific antigens in blood or CSF samples may define the incident of co-infection. The laboratory diagnostic algorithms during DENV and JEV co-infection have been depicted in **Figure 3**.

4. Treatments regimes for dengue and Japanese encephalitis co-infection

Viral infections have always been a global threat to mankind due to scarcity of effective antiviral drugs. Flaviviral infections are not the exception since there is no specific treatment available for both DENV and JEV [33]. The overall treatment relies on the symptomatic relief of the patients. Due to the complex and unclear pathogenesis of dual infection, the potential candidate drugs may not effective. Therefore, we need to look for the complementary and alternative medicine (CAM) in alliance with conventional medicines as the choice of treatment during co-infection.

In case of DENV infection, various forms of CAM have been used such as *Carica papaya* leaf extracts is the most accepted one. Platelet-activating factor receptor (PTAFR) gene has been shown to be upregulated upon consumption of *Carica papaya* leaf extracts or its juices [34]. In case of sever dengue infection, maintenance of body fluids volume of the patients is critical [35]. However, this may or may not be effective in case of co-infections. Recently, andrographolide has been shown to exhibit anti-DENV activity [36]. *Eupatorium perfoliatum* which is a homeopathic medicine has been shown to exhibit anti-DENV activity [37]. Luteolin has been shown to be effective during JEV infection which also exhibits direct virucidal activity [38]. Similarly, belladonna has been shown to be effective in chick embryos infected with JEV [39]. Several of the CAM-based therapies have been shown to be effective in case of JEV infection, but these have to be validated in case of co-infection.

5. Preventive strategies for dengue and Japanese encephalitis co-infection

To prevent the worldwide burden of DENV infection, the WHO has recently approved a tetravalent vaccine, Dengvaxia (CYD-TDV) in 20 countries. This has

been designed by using the yellow fever vaccine backbone expressing the prM and envelope protein of DENV 1–4 serotypes [40]. In case of JEV, a live attenuated vaccine based on SA 14–14-2 has been used in China, India, Sri Lanka, Republic of Korea, and Thailand. Due to higher sero-cross-reactivity, the one vaccine may induce the other infection due to the antibody-dependent enhancement [41]. Therefore, to design an effective vaccine for co-infection, there is a need to understand the mechanism and probability of sero-cross-reactivity among the viruses. Apart from the vaccination, the personal preventive measures are always the paramount to prevent any vector-borne infections [42]. To prevent the mosquito biting, several protective measures include mosquito repellents, mosquito nets, and use of full sleeves cloths [43].

6. Conclusions

Genomic and proteomic sequence similarity among the flaviviruses causes the sero-cross-reactivity that leads to the phenomenon of antibody-dependent enhancement of infections. Incidence of DENV and JEV co-infection in the hyperendemic areas may be frequently reported. The diagnosis of co-infection should not rely on the presence of serum antibodies. However, simultaneous detection of nucleic acids or antigens may define the condition of co-infection. Clinical features may overlap in the patients infected with both viruses, and therefore we need to precisely distinguish the patients' clinical reports. Development of effective antivirals targeting both the viruses is the most imperative therapeutic strategy.

7. Future perspectives

Due to the similarity in the structural domains of the viral proteins, molecules may be designed to inhibit the action of viral proteins or enzymes. Similarly, vaccines may be designed to target the population living in the hyperendemic areas of flaviviruses. Peptide-based vaccines may be designed by using various immunoinformatics approaches by considering the consensus peptide sequences among the viruses. Apart from the vaccination, personal preventive measures are always recommended the best practice to reduce the chances of infections.

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Author details

Shailendra K. Saxena*, Swatantra Kumar, Vimal K. Maurya and Madan L.B. Bhatt
Center for Advanced Research (CFAR)-Stem Cell/Cell Culture Unit, Faculty of Medicine, King George's Medical University, Lucknow, India

*Address all correspondence to: myedrsaxena@gmail.com

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