Sectional Chairpersons' Address – Section C

An insight into dengue and dengue hemorrhagic fevers (DF/ DHF) in Jaffna

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Introduction

Dengue is one of the most common vector-borne infectious diseases two-fifth at risk of dengue in the world's population. Worldwide it is estimated that 50-100 million clinically apparent dengue viral (DENV) infections occur annually. Of the clinically apparent DENV infections, 500,000 cases of dengue are life-threatening and called dengue haemorrhagic fever (DHF)/ dengue shock syndrome (DSS) causing over 22,000 annual deaths (WHO, 2012). Moreover, an average of 1 million dengue cases has been reported annually in more than 100 countries in tropical and subtropical regions (Mendez et al., 2010). Dengue is an African word meaning "bone breaking". There is no breaking of bones in the dengue fever (DF) patient but it means there is severe body aches and pain in DF affected patients.

Structure of DENV

DENV fall into 4 phylogenetically and genetically divergent serotypes classified as DENV-1, DENV-2, DENV-3 and DENV-4. These serotypes are antigenically related to each other. Infection with one serotype provides lifelong immunity to the same serotype only but it has been associated with increased risk of severe dengue illness when secondary infection occurs with a different serotype (Rothman, 2003).The DENV particle is composed of an icosahedral nucleocapsid encased by a lipid envelope.

Transmission of DENV

DENV are transmitted to humans through the bites mosquito vectors, principally *A. aegypti,* carrying the infectious virus. However, the virus also can also be transmitted by the *A. albopictus* and *A. polynesiensis* mosquitoes. *A. aegypti* has been considered to be the principal vector and A. albopictus has been considered to be the secondary vector in the DENV transmission. Female mosquitoes can acquire the virus while feeding in viraemic humans. Viraemia in humans lasts approximately for 5 days and takes place after an incubation period of 6-8 days.

The spread of DENV is attributed to multiple factors including urbanization, global travel and the expanding distribution of mosquito vectors. With the changing climate, virus and vector adaptations to different environments, dengue has become the most important viral infection transmitted by mosquitoes in the world.

A. aegypti is a highly domesticated mosquito that breeds in artificial containers such as water storage tanks, subterranean pits, flower pot trays, discarded tyres, buckets, other ornamental containers and they also breed in natural sites such as tree holes and discarded coconut shells. The vector prefers to rest indoor, although studies have shown that they may seek oviposition outdoors. They are day-active and the peak biting activity is in the early morning or late afternoon. The multiple feeding behavior of *A. aegypti* and its preference for human hosts are assumed to

contribute to the explosive spread of DENV, even in the presence of a low *A. aegypti* population (Scott et al., 1997).

Clinical features of dengue

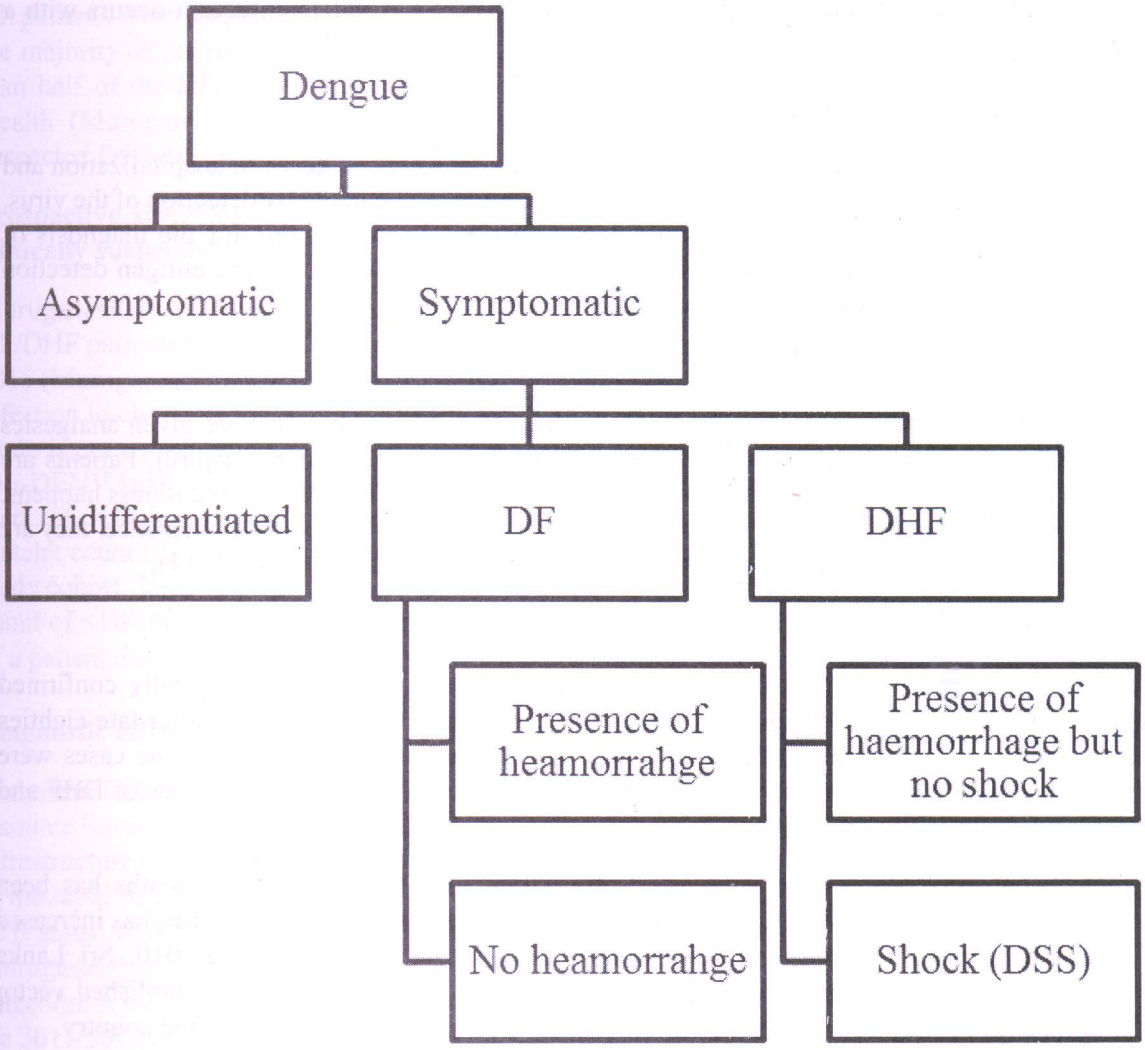

Figure 1 Clinical categorization of dengue (Adapted from Coffey et al., 2009).

Two third of all dengue infected individuals are asymptomatic, that is, they have no clinical signs or symptoms of disease. DENV infected symptomatic patients can be with a spectrum of clinical manifestation ranging from mild infection to potentially lethal complications, DHF or DSS (Figure 1). DF is a flu-like illness characterized by high fever, severe headache, retro-orbital pain, muscle and joint pain, nausea, vomiting and rash lasting approximately two to seven days. DHF is defined by haemorrhagic manifestations, thrombocytopenia, often with hepatomegaly but rarely with splenomegaly and plasma leakage. DSS is a more severe complication where extensive plasma

leakage leads to prolonged shock and disseminated intravenous coagulation. The pathogenesis of severe dengue is not fully understood, but it is generally accepted that the complications are immune-mediated and virus induced.

Infection with one serotype provides lifelong immunity to the same serotype only but it has been associated with increased risk of severe dengue illness when secondary infection occurs with a different serotype.

Laboratory diagnosis of dengue

The morbidity and the mortality of DHF can be reduced by an early detection, hospitalization and symptomatic care. Laboratory diagnosis of DENV infection can be made by detection of the virus, DENV antigen and genomic RNA or antibodies. Currently used methods for the diagnosis of DENV infections include virus isolation, detection of the genomic RNA, NS1 antigen detection and DENV specific antibodies such as anti-DENV IgM / IgG.

Treatment and prevention of dengue

There is no specific antiviral treatment for dengue and the affected patients are given analgesics (pain relief) with acetaminophen such as panadol and paracetamol but not aspirin. Patients are advised to rest, drink plenty of fluids and seek medical advice if worsening of the illness happens. If they experience nausea and severe abdominal pain in the first 24 hours of defeversence, they are immediately hospitalized for further evaluation and monitoring (CDC online, 2014).

Dengue infection in Sri Lanka

Sri Lanka has been affected by DF for over three decades. Although serologically confirmed dengue has been an old disease in Sri Lanka dating back to early sixties, only after late eighties dengue became an important public health problem in the island. In 1962, dengue cases were serological confirmed. There was an island-wide epidemic of dengue with 51 cases of DHF and 15 deaths between 1965 and 1968

In 1989, the first larger dengue epidemic with a total of 203 cases and 20 deaths has been documented in Sri Lanka (Messer, 2012). Subsequently, the number of dengue cases has increased annually with increased severity of the clinical disease. In 2004 and 2009/2010, Sri Lanka experienced major dengue outbreaks. Currently DF/DHF has become a well-established vector borne viral disease causing significant morbidity and mortality in many parts of the country.

Dengue infection in Jaffna District

The dengue cases started to increase in the Jaffna District from mid-2009 and this time period correlated with the opening of major highways connecting the Jaffna peninsula to the rest of the country after 30 years. In that regard prior to 2009, only less than 50 dengue cases have been reported to the Epidemiology Unit and that dengue was not considered as a public health issue in the Northern Province. Thus there were no detailed epidemiological studies or diagnostic data available on DF/DHF in the Jaffna District. However, after mid-2009, Jaffna District has been experiencing massive dengue outbreaks affecting many causing morbidity and mortality in the region.

Sectional Chairpersons' Address – Section C

Retrospective analysis of demographic and clinical features of suspected dengue from 2009-2010 in the Jaffna peninsula

In 2014 a retrospective study investigated 1085 individual patient's clinical and diagnostic notes from BHTs at THJ by Murugananthan et al. The major drawbacks observed in this study included the absence of DENV specific laboratory data to confirm the clinically diagnosed DF/DHF cases. All patients were diagnosed using the clinical criteria based on the National dengue guidelines and the majority of the patients were admitted 8 days after the onset of symptoms. Conversely, more than half of the DF/DHF cases were not notified to the Epidemiology Unit of the Ministry of Health (Murugananthan et al., 2014). In the retrospective study, incidence of clinically suspected DF/DHF cases clearly showed a seasonal trend in the distribution.

Prospective study on Clinical, non-specific and specific virological laboratory profiles of clinically suspected dengue in the Jaffna peninsula from 2009-2012

Murugananthan et al conducted a prospective study using a sample of clinically suspected DF/DHF patients to assess the clinical, non-specific and specific virological laboratory profiles in 2015 (Murugananthan et al 2013). This study provides valuable insights for the diagnosis of DENV infection in clinically suspected DF/DHF patients in the study area. Detection of anti-DENV IgM alone is not sufficient for confirming a more recent DENV infection. Detecting DENV NS1 and anti-DENV IgM together improves the detection of clinically apparent DENV infection in more than 2/3 of the study cohort. Moreover combining DENV NS1 antigen, anti-DENV IgM and the platelet count of <100,000 together for screening improves the detection by more than 90% in the study cohort. Thus a combined evaluation of DENV NS1 antigen, anti-DENV IgM and the platelet count of <100,000 will help the clinicians to fine tune the hospital admission during the first visit of a patient during an outbreak.

Diagnostic efficiency of a rapid ICT assay in the diagnosis of DENV infections

Molecular / ELISA based diagnosis for the detection of DENV markers is not widely available in resource limited countries like Sri Lanka. Since the rapid ICT assays do not require any laboratory infrastructure or expertise, their usage in developing countries becoming very popular. Accuracy of the rapid ICT assays in the diagnosis of DENV infection is less studied in Sri Lanka. Thus the ICT assay (Cortez, USA) which was widely available in the study area was validated by Murugananthan et al in 2017 using a standard ELISA (Pan Bio, Australia) for anti-DENV IgM/IgG detection. A total of 765 patients' samples collected from clinically suspected dengue patients from the 2011/2012 outbreak were tested for anti-DENV IgM and IgG using a rapid ICT assay and anti-DENV IgM and IgG ELISA.

Sensitivity and specificity for the anti-DENV IgM detection by a rapid ICT assay was moderate and for the anti-DENV IgG detection by the same rapid ICT assay was relatively high (Murugananthan et al 2017).

Molecular characterization of DENV in the Jaffna District from 2009 to 2012

Since the RT-PCR is known to be sensitive and specific for the detection of the genomic identity of DENV and thus acute DENV infections, Murugananthan et al in 2015 used RT-PCR for identification and DENV typing of DENV derived from 765 patients to study the molecular epidemiology of DENV infections from 2009 to 2012 in the Jaffna District.

Murugananthan et al described the first report on DENV typing of in the Jaffna District from 2009 to 2012. In the 2009/2010 outbreak, DENV-2 and DENV-3 were the predominant serotype identified as responsible for the outbreak. Moreover, co-infections were identified with DENV-2 and DENV-3 in 2009/2010. Whereas in the 2011/12 outbreak, DENV-1 was the predominantly identified DENV type and this type had co-infection with DENV-2 and DENV-3. The same trend was observed in Western and Central provinces during the same time period (Senaratne et al., 2016; Srisena and Noordeen unpublished data).

Culture free NGS and phylogenetic analysis of DENV-1serotype from Jaffna isolate

The whole genome analysis of DENV-1 isolated from the Jaffna District detected the similarities and differences of the isolate with DENV-1 isolated from other provinces (Murugananthan et al 2015). The DENV nucleic acid from several patients was transferred to University of Calgary, Canada using a cost effective way of DENV cDNA preservation and transport. Moreover, the current study also used a novel culture free technique for the acquisition of DENV cDNA for NGS band this is the first time a culture free acquisition of DENV cDNA was done. NGS has produced a high quality, consensus sequence of a DENV-1 isolate from a patient's sample without the use of virus culture. The whole genome sequence of this DENV was deposited in the NCBI GenBank (Accession No: KP398852). Phylogenetic analysis of the sequenced DENV-1 isolated from the current study closely associates with the DENV-1 isolates derived from other parts of Sri Lanka indicating a common ancestry and a single introduction event around 2007 (2007-2008) to the island.

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